Exclusion of solar UV-B (280–315 nm) radiation on vegetative growth and photosynthetic activities in *Vigna unguiculata* L.

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**Abstract**

The impact of solar radiation with and without UV-B component was studied in a tropical legume *Vigna unguiculata* L. The average level of ambient UV-B was 10 kJ m$^{-2}$ day$^{-1}$. Exclusion of solar UV-B increased the seedling height, leaf area and fresh weight. On a unit fresh weight basis, a 50% increase in total chlorophyll and 47% decrease in flavonoids was noticed after 20 days of growth. Decrease in flavonoid content in UV-B filtered seedlings indicates the plant responsiveness to minimal UV-B radiation existing inside the filter cage. UV-B exclusion during the early phase of seedling growth also promoted photosynthetic activities, measured as O$_2$ evolution or fluorescence transients. Such an enhancement in activity was attributed to modification in thylakoid organization under UV-B exclusion. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** UV-B radiation; Exclusion; Growth; Photosynthesis; *Vigna unguiculata*

1. **Introduction**

Depletion of the stratospheric ozone layer causes an increase in ultraviolet-B (UV-B; 280–315 nm) radiation reaching the earth’s surface. This increase is likely to continue into the foreseeable future with potentially deleterious consequences for agriculture, horticulture and forestry. The impact of enhanced UV-B radiation on algae and higher plants is many. Several reports have come out emphasizing the potential effects of UV-B on vegetative growth and photosynthetic activities [1–3]. The molecular mechanism of UV-B damage was extensively reviewed by Jordan [4].

While studying the effects of enhanced UV-B radiation, it is essential to know the impacts of ambient UV on growth and other physiological processes. Most of the UV-B exclusion studies reported so far have been conducted in growth chambers. Hence, an attempt was made to evaluate the effects of UV-B exclusion from solar radiation on vegetative growth, pigment composition and photosynthetic activities in a tropical legume, *Vigna unguiculata* L.

2. **Materials and methods**

2.1. **Plant material and growth conditions**

Seedlings of *V. unguiculata* L. were grown in 1.5 dm$^3$ pots filled with garden soil and watered daily. Soon after germination, the seedlings were shifted outdoors and exposed to ambient (−15%) and UV-B filtered (−UV-B) solar radiation with an 11 h light/13 h dark photoperiod. Solar UV-B was effectively filtered by covering a cage (1×1 m) using a polyester transparent film (Mylar type). The control seedlings were exposed in a similar cage covered with polyethylene film. The UV–visible transmittance spectra of both the films are...
shown in Fig. 1. The filter cage was lifted from the ground by 20 cm to allow free air circulation.

2.2. Radiation measurements

Absolute solar irradiance with and without UV-B were measured using a monochromatic spectroradiometer (IL-700, International Lights, USA). The average level of ambient UV-B during the experimental period was 10 kJ m$^{-2}$ day$^{-1}$. Under –UV-B radiation condition the dose was 1.5 kJ m$^{-2}$ day$^{-1}$. The daily average PPFD inside the filter cages was 54 mol m$^{-2}$.

2.3. Growth analysis

Seedling weight, leaf area and shoot length were determined soon after harvest. Specific leaf weight (SLW) was defined as the fresh weight per unit leaf area. Only plant material above ground was considered for fresh and dry weights in which the latter involved drying the plant parts at 80°C for 48 h.

2.4. Pigment analysis

Fresh leaf tissue was extracted with 100% acetone for the complete release of chlorophyll and carotenoids. The amounts of chlorophyll and carotenoid were quantified using the formulae of Wellburn and Lichtenthaler [5]. The concentration of flavonoids was determined from the acidified methanolic leaf extracts following the method of Mirecki and Teramura [6].

2.5. PSII electron transport assay

PSII mediated O$_2$ evolution was continuously monitored at 25 ± 1°C from leaf discs using a leaf disc O$_2$ electrode (Hansatech, UK). The assay procedure was the same as that of Delieu and Walker [7]. The capillary matting used to floor the leaf discs was wetted in 1 M HCO$_3$ buffer. The leaf discs were exposed to a saturating light intensity of 600 μmol m$^{-2}$ s$^{-1}$.

2.6. Chl a fluorescence kinetics

In vivo Chl a fluorescence transients were followed in intact leaves after excitation with broad band blue light (400–620 nm, Corning, CS 4-96) at a photon flux density of 460 μmol m$^{-2}$ s$^{-1}$. Prior to the excitation, the leaves were incubated in darkness for 10 min to restore complete oxidation of Q$_A$. The experimental conditions were the same as that of Lingakumar and Kulandaivelu [8].

2.7. Low temperature fluorescence emission

Type II broken chloroplasts were isolated following the method of Reeves and Hall [9]. Freshly isolated chloroplasts were resuspended in 70% (v/v) glycerol medium containing 20 mM Tris–HCl (pH 7.5), 5 mM MgCl$_2$ and 5 mM NaCl. A specially constructed Dewar flask was used for measurements at 77 K. The F740/F686 ratios were calculated from the emission spectra obtained with a Hitachi spectrofluorimeter (MPF-4).

3. Results

3.1. Effect of UV-B filtered solar radiation on growth characteristics

To study the effects of solar radiation without the UV-B component, Vigna seedlings were raised inside a cage covered with UV-B cut-off transparent polyester (Mylar type) film. The control seedlings received the same amount of visible light along with 85% of ambient UV-B. The seedlings grown under UV-B filtered radiation showed a significant increase in various growth parameters as compared to the control. Fig. 2 shows the effect of UV-B exclusion on vegetative growth. Throughout the growth period a significant in-
crease in growth parameters was observed. A maximum of 50% increase in shoot length, 11% in shoot fresh weight, 30% in dry weight and 60% in leaf area was noticed in 20-day-old seedlings. On the contrary, a 40% decrease in SLW was found at this stage of growth. Below ground, a high amount of nodulation was noticed in UV-B filtered seedlings (data not shown) suggesting that the vegetative growth was very much influenced by the UV-B component of solar radiation.

3.2. Pigment analysis

Changes in the photosynthetic and non-photosynthetic pigment composition of Vigna seedlings grown under UV-B filtered radiation and ambient (−15%) light are given in Table 1. On a unit fresh weight basis, UV-B filtered radiation caused a 50% increase in total Chl, 47 and 58% increase in Chl a and Chl b, respectively, after 20 days of growth. Though marked changes were not observed in the Chl a/b ratio in the early growth stages, a slight decrease was observed after 20 days. A similar trend was observed in the carotenoid levels. In contrast to Chl, the flavonoid content was reduced to 47% under UV-B filtered radiation. Exclusion of UV-B slowed the synthesis of UV-B absorbing compounds from the onset of seedling growth.

3.3. Photosynthetic activities

The photosynthetic efficiency of seedlings grown under ambient (−15%) and −UV-B radiation was monitored using Chl a fluorescence induction. Typical fast and slow Chl a fluorescence transients recorded with intact Vigna leaves are shown in Fig. 3. The leaves of ambient (−15%) solar radiation grown Vigna seedlings exhibited a typical OIDPSMT fluorescence induction pattern [10]. The constant fluorescence (Fo) level remains unaffected in seedlings under UV-B radiation. A significant increase in variable fluorescence (Fv) with a fast OP rise was noticed in these seedlings. Since Fv/Fm is a measure of photochemical efficiency, quantification of the Fv/Fm ratios was done. A steady increase in Fv/Fm with increase in the growth period was observed (Table 2).

Under the −UV-B condition, seedlings showed prominent PS decline and a slow attainment of the T state. These changes in slow transients reflect the efficient functioning of PSII and the CO₂ fixation pathway. The functioning of PSII was also
Table 1
Changes in pigment composition of *Vigna* seedlings exposed to ambient (−15%) and UV-B filtered solar radiation

<table>
<thead>
<tr>
<th>Treatment period (days)</th>
<th>Chl <em>a</em> (mg g⁻¹ LFW)</th>
<th>Chl <em>b</em> (mg g⁻¹ LFW)</th>
<th>Chl <em>a/b</em></th>
<th>Total Chl (mg g⁻¹ LFW)</th>
<th>Carotenoids (mg g⁻¹ LFW)</th>
<th>Flavonoids (A₃₁₅ 100 mg⁻¹ LFW)</th>
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<tr>
<td>Ambient (−15%)</td>
<td>0.56 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>2.15</td>
<td>0.82 ± 0.03</td>
<td>0.14 ± 0.01</td>
<td>1.66 ± 0.08</td>
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<tr>
<td>−UV-B</td>
<td>0.64 ± 0.02</td>
<td>0.27 ± 0.04</td>
<td>2.37</td>
<td>0.91 ± 0.02</td>
<td>0.15 ± 0.04</td>
<td>1.07 ± 0.09</td>
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<tr>
<td>(−14)</td>
<td>(+ 4)</td>
<td>(+ 7)</td>
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<tr>
<td>Ambient (−15%)</td>
<td>0.79 ± 0.04</td>
<td>0.47 ± 0.05</td>
<td>1.69</td>
<td>1.26 ± 0.05</td>
<td>0.22 ± 0.02</td>
<td>2.20 ± 0.06</td>
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<tr>
<td>−UV-B</td>
<td>0.96 ± 0.05</td>
<td>0.48 ± 0.06</td>
<td>2.06</td>
<td>1.44 ± 0.06</td>
<td>0.25 ± 0.01</td>
<td>1.34 ± 0.08</td>
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<td>(−22)</td>
<td>(+ 2)</td>
<td>(+ 14)</td>
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<tr>
<td>Ambient (−15%)</td>
<td>0.98 ± 0.04</td>
<td>0.42 ± 0.06</td>
<td>2.33</td>
<td>1.40 ± 0.08</td>
<td>0.24 ± 0.02</td>
<td>2.56 ± 0.09</td>
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<tr>
<td>−UV-B</td>
<td>1.46 ± 0.01</td>
<td>0.61 ± 0.08</td>
<td>2.39</td>
<td>2.07 ± 0.09</td>
<td>0.35 ± 0.01</td>
<td>1.52 ± 0.01</td>
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<tr>
<td>Ambient (−15%)</td>
<td>1.15 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>2.40</td>
<td>1.63 ± 0.07</td>
<td>0.31 ± 0.02</td>
<td>2.94 ± 0.04</td>
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<tr>
<td>−UV-B</td>
<td>1.69 ± 0.04</td>
<td>0.76 ± 0.08</td>
<td>2.22</td>
<td>2.45 ± 0.11</td>
<td>0.41 ± 0.03</td>
<td>1.55 ± 0.09</td>
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<tr>
<td>(−47)</td>
<td>(+ 58)</td>
<td>(+ 32)</td>
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* Values are an average of five independent measurements. Mean ± S.E., n = 5. The figures in parentheses are percent changes with reference to respective controls.
analysed by measuring O$_2$ evolution rates using a leaf disc O$_2$ electrode. Table 2 provides the PSII mediated O$_2$ evolution rates. A maximum increase of 36% in PSII activity was noticed after 20 days of growth under UV-B filtered radiation. These changes show the efficient photosynthetic pathway in UV-B filtered *Vigna* seedlings.

Table 2

<table>
<thead>
<tr>
<th>Treatment period (days)</th>
<th>Ratio of variable to maximum fluorescence (Fv/Fm)</th>
<th>PS II activity (μmol mg$^{-1}$ Chl h$^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>5</td>
<td>Ambient (15%) 0.70 ± 0.02</td>
<td>6.40 ± 0.8</td>
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<td>- UV-B 0.77 ± 0.04</td>
<td>7.36 ± 0.6</td>
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<tr>
<td>10</td>
<td>Ambient (15%) 0.72 ± 0.05</td>
<td>8.40 ± 0.5</td>
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<td>- UV-B 0.80 ± 0.03</td>
<td>10.24 ± 0.8</td>
</tr>
<tr>
<td>15</td>
<td>Ambient (15%) 0.77 ± 0.05</td>
<td>10.52 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>- UV-B 0.82 ± 0.02</td>
<td>13.20 ± 0.8</td>
</tr>
<tr>
<td>20</td>
<td>Ambient (15%) 0.80 ± 0.06</td>
<td>11.90 ± 0.9</td>
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<tr>
<td></td>
<td>- UV-B 0.85 ± 0.05</td>
<td>16.22 ± 0.8</td>
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</table>

* The values are an average of 5 independent measurements. Mean ± S.E., n = 5. Both the measurements were performed in different leaf samples of the same age.

Though the room temperature fluorescence induction kinetics reveal the efficiency of PSII primary photochemistry, typical emission characteristics of individual Chl-protein complexes such as PSII, LHCP and PSI were followed at 77 K which reflects the energy transfer reactions between PSII and PSI [11]. At 77 K excitation with 435 nm radiation *Vigna* chloroplasts exhibited major peaks at 686 nm (F686) and 740 nm (F740) and a minor peak at 701 nm (Fig. 4). In both
ambient (-15%) and UV-B filtered radiation grown seedlings, the F740 level was less than that of F686. Qualitative changes in the level of F740/F686 as a function of days of illumination to ambient (-15%) and UV-B filtered radiation are presented in Table 3. The F740/F686 ratios were high under UV-B filtered radiation. A steady increase with respective controls in F740/F686 ratio was noticed under UV-B exclusion.

4. Discussion

Removal of UV-B in solar radiation caused a significant increase in the vegetative growth of Vigna seedlings. These results correspond to earlier field studies [12,13]. Among the parameters studied, leaf expansion was more prominent under filtered radiation. Furthermore, a remarkable increase in shoot length and root nodulation indicates that this species favours solar radiation without the UV-B component for better vegetative growth. It is well known that UV-B radiation inhibits vegetative growth which is attributed to the destruction of endogenous IAA [14,15] and induction of oxidative enzymes associated with growth responses [16]. Protection of apical meristems alone during UV-B irradiation was shown to be effective in relieving the UV-B induced changes [15]. Thus, the enhanced vegetative growth under UV-B filtered radiation is assigned to a high level of auxin synthesis or the low stimulation of oxidative enzymes of IAA metabolism.

The Chl and carotenoid content in seedlings grown under UV-B filtered radiation showed a significant increase. The increase in total Chl level was found to be due to the enhanced synthesis of Chl a than Chl b. Enhanced UV-B was shown to cause damage to Chl b rather than Chl a, which could be due to its direct absorption [17] or due to inhibition in the Chl biosynthesis [18]. In contrast to photosynthetic pigments, flavonoid content showed a large tendency to decrease under UV-B filtered radiation. Generally, accumulation of UV-B absorbing compounds, mainly flavonoids, in leaf epidermis was suggested to be a protective measure against UV-B effects. Thus, the extent of flavonoid reduction in seedlings grown under UV-B filtered radiation could be considered as a measure of responsiveness to solar UV-B.

Evidence has been presented on the changes induced by UV-B radiation on the photosynthetic electron transport chain [19]. Among the possible target sites, PSII has been considered as the most susceptible site to UV-B [19,20]. A significant increase (approximately 36%) in O2 evolution in seedlings grown under UV-B radiation suggests that the PSII remains intact. This could be due to either an increase in the number of PSII units/Chl or the efficient functioning of primary photochemistry. The evidence for such a conclusion was obtained from the fluorescence kinetics. A high Fv/Fm ratio found throughout the growth period in seedlings under UV-B filtered radiation is considered as a photosynthetic adaptive response. Enhanced solar UV-B radiation was shown to induce Chl synthesis during the early stages of growth but such enhancement was not noticed in the ambient light grown seedlings. Thus it is ascertained that UV-B filtered radiation could significantly induce photosynthetic activities through modification in thylakoid organization.

In the slow fluorescence kinetics, the secondary S–M rise was evident only in UV-B filtered seedlings with a sharp P–S decline. Since the fluorescence changes observed in the SMT sector are linked to the redistribution of excitation energy between PSII and PSI in which PSI is more favoured [21], the possibility that more excitons are transferred towards PSI in UV-B filtered seedlings holds good. Supporting evidence for the redistribution of excitation energy was obtained.
from the low temperature fluorescence emission spectra. The ratio F740/F686 is used to determine the fluorescence emission of PSI relative to that of PSII [22]. The F740/F686 values were found to be high after 20 days of growth under UV-B filtered radiation which reflects an altered stoichiometry of PSI/PSII. Similar alterations were reported under short-term UV-B irradiation [17] and BASF treatment of thylakoids [23].

Our results show that exclusion of the UV-B component from solar radiation brings about a significant increase in the vegetative growth as well as photosynthetic activities especially during the early phases of plant growth. This study also indicates the need to have a control (without UV-B) while studying the realistic impacts of solar UV radiation.

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References