MODIFICATIONS IN LEAF ARCHITECTURE OF VIGNA UNGUICULATA (L.) WALP. CV. COVU-2 TO DEFEND FROM ULTRAVIOLET-B RADIATION

Kokilavani V. and *Rajendiran K.
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry – 605 008
*Author for Correspondence

ABSTRACT
Gases like CFC-11, CFC-12 and N₂O make a significant contribution to stratospheric ozone depletion, followed by numerous additional CFC’s, HCFC’s and other halogenated gases. Most of the gases that are responsible for depleting stratospheric ozone are anthropogenic, but some, such as CH₃Br and CH₂Cl, have natural contributions as well. Depleted ozone layer allows more ultraviolet-B (UV-B) rays into the surface of the earth. As increased ultraviolet-B radiation has a direct effect on the leaves of crop plants, an evaluation of the effects of ultraviolet-B (UV-B) radiation in the morphology, epidermis and the anatomy of Vigna unguiculata (L.) Walp. cv. COVU-2 leaf was carried out. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) Vigna unguiculata (L.) Walp. cv. COVU-2 after exposure to supplementary UV-B radiation (2 hours daily @ 12.2 kJ m⁻² d⁻¹; ambient = 10 kJ m⁻² d⁻¹) were monitored. Various types of malformations in the leaf architecture were recorded after UV-B exposure along with several injuries which were not observed in unstressed crops. In response to UV-B the leaves were small, shiny and thick compared to broader, longer and thinner leaves of normal plants. Stomatal frequency in UV-B was increased by 33.33 and 24.81 % over control on adaxial and abaxial surfaces respectively. Same trend was noticed in stomatal indices of stressed plants which showed increases by 63.38 and 56.89 % on adaxial and abaxial surfaces respectively. Stomata bearing single guard cell were more in number together with dead and collapsed epidermal cells on the adaxial surface of UV-B stressed leaves. However, no stomatal aberrations were recorded in normal leaves. The UV-B induced structural changes in the leaves were to obstruct the radiation from penetrating into the inner region. The trichomes were shorter by 29.29 % and 13.68 % on adaxial and abaxial surfaces respectively and were also brittle in UV-B treated leaves compared to healthier ones in control. Frequency of trichome was increased by three times on both the surfaces in UV-B exposed plants. On adaxial surface, cuticle was twice thicker and epidermis was thicker by 7.30 % after UV-B irradiation. The volume of mesophyll increased by 86.33 % while the thickness of mesophyll decreased by 12.89 %. However the leaves were thicker by 53.93 % under UV-B exposure.

Keywords: Ultraviolet-B, Cowpea, Variety COVU-2, Leaf Morphology, Leaf Epidermis, Leaf Anatomy, Abnormal Stomata

INTRODUCTION
Concerns about climate change stem from the increasing concentration of greenhouse gases in the atmosphere. These gases keep heat from dissipating into space, leaving the stratosphere cooler. Colder than normal temperatures in stratosphere increases ozone depletion. As a result, the UV-B radiation is bound to increase affecting all living organisms and the ecosystems in total. Ultraviolet-B (UV-B) radiation (280-320 nm) is an important atmospheric stress as it severely inhibits photosynthesis (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and suppresses nodulation and nitrogen fixation (Rajendiran and Ramanujam, 2006; Rajendiran and Ramanujam, 2003; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014) in sensitive plants. The epidermis of the leaves constitutes a dynamic barrier between the plant's internal and external environment. It is impregnated with waxes and cutins on the exterior and possesses stomata to regulate the exchange of gases. The foliar surface is also provided with appendages like trichomes, hydathodes and scales. Leaves are the organs that receive major proportion of the
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ultraviolet radiation and hence always react immediately to prevent its entry into the internal organs (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013). The article discusses the modifications brought about by *Vigna unguiculata* (L.) Walp. cv. COVU-2 in leaf architecture in order to adapt to UV-B elevated condition.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. COVU-2 obtained from Tamil Nadu Agriculture University, were grown in pot culture in the naturally lit greenhouse (day temperature maximum 38 ± 2 °C, night temperature minimum 18 ± 2 °C, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\), photoperiod 12 to 14 h). Supplementary UV-B radiation was provided in UV garden by three UV-B lamps (*Philips TL20W/12 Sunlamps*, The Netherlands), which were suspended horizontally and wrapped with cellulose diacetate filters (0.076 mm) to filter UV-C radiation (< 280 nm). UV-B exposure was given for 2 h daily from 10:00 to 11:00 and 15:00 to 16:00 starting from the 5th day after sowing. Plants received a biologically effective UV-B dose (UV-B\(_{\text{BE}}\)) of 12.2 \(\text{kJ m}^{-2} \text{d}^{-1}\) equivalent to a simulated 20 % ozone depletion at Pondicherry (12°2′N, India). The control plants, grown under natural solar radiation, received UV-B\(_{\text{BE}}\) 10 \(\text{kJ m}^{-2} \text{d}^{-1}\). For studying the epidermal and the anatomical characters the fully developed third trifoliate leaf from the top was taken from the 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. COVU-2 plants. The size and number of epidermal cells, stomata and trichomes were recorded using a calibrated light microscope. Stomatal frequency was determined by examining the leaf impressions on polystyrene plastic film. The plastic medium (1g of polystyrene in 100 ml of xylol) was applied on the control and UV-B irradiated leaves uniformly as a thin layer. After drying, the material was carefully removed and observed under magnification. Stomatal counts were made randomly from ten regions on the adaxial / abaxial surfaces. Since the stomatal frequencies vary according to cell size, Salisbury (1928) recommended the 'stomatal index' (SI) which relates the number of stomata per unit leaf area to the number of epidermal cells in the same area. Stomatal index (SI) = S / S + E x 100 where, S = number of stomata per unit leaf area, E = number of epidermal cells per unit leaf area. Cuticle, mesophyll and leaf thickness were measured using stage and ocular micrometers and the values were expressed in \(\mu\text{m}\). Mesophyll thickness (mm) was multiplied by 100 to calculate the mesophyll volume in cm\(^3\) dm\(^{-2}\) of leaf area as recommended by Patterson *et al.*, (1978).

RESULTS AND DISCUSSION

*Vigna unguiculata* (L.) Walp. cv. COVU-2 responded with small, wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface due to UV-B irradiation (Plate 1; Plate 2. Figure 1 to 2). The costal cells on the adaxial surface of normal leaves are uniformly similar in being axially elongated, thin and straight walled and have unicellular thin walled trichomes. The intercostal epidermal cells both on abaxial and adaxial surfaces are sinuous and thin walled with unicellular trichomes occurring intermittently. The epidermal cells possessed dense, deeply stained nuclei in control and in all the UV-B irradiated leaves (Plate 2. Figure 3 to 6). Frequency of Epidermal cells was higher (63.63 %) over control in UV-B exposed leaves but the effect was subdued on the abaxial side (56.89 %) compared to adaxial surface (Table 1). Cuticles and epidermis in UV-B exposed leaves were thicker than control. The cuticle was twice thicker while the epidermis was thicker by just 7.30 % over normal leaves (Plate 2. Figure 7; Plate 3). Leaf were thicker by 53.93 %, mesophyll became thin by 12.89 % while mesophyll volume increased by 86.33 % (Plate 3). With the mesophyll becoming voluminous, a thicker leaf would result (Rajendiran, 2001). According to Wellmann (1976) and Caldwell *et al.*, (1983), plants obstruct the UV-B transmission to the inner leaf tissues either by absorbing some of the damaging UV radiation, or by strengthening the tissues through marked elongation of palisade cells. At the structural level, increased leaf and cuticle thickness reduces UV-B penetration to internal tissues (Bornman and Vogelmann, 1991; Rajendiran 2001) alleviating some of the deleterious effects. Leaf thickness increased in *Medicago sativa* due to addition of spongy mesophyll cells, whereas in *Brassica campestris* there was an increase in the
number of palisade cells (Bornman and Vogelmann, 1991). Kokilavani and Rajendiran (2013), Kokilavani et al., (2013), Kokilavani and Rajendiran (2014a), Kokilavani and Rajendiran (2014b) and Kokilavani and Rajendiran (2014c) opined that greater thickness increased the amount of scattered light which could be due to low chlorophyll content, increased number of intercellular air spaces, cytoplasmic changes or altered cellular arrangements like the palisade becoming wider and cell layers increasing in number. Unicellular trichomes were present in the costal as well as intercostal regions of both the surfaces, and their frequency was comparatively less on the abaxial side than the adaxial side. Trichome frequencies under UV-B exposure were three times more on both surfaces compared to control (Table 1). Shorter trichomes (29.29 %) along with more broken ones were observed on adaxial side of UV-B irradiated leaves (Table 1; Plate 2. Figure 8) with the abaxial side also bearing small trichomes (13.68 %) (Table 1). The trichomes serve several functions as a mechanical barrier against biotic attack (Johnson, 1975; Woodman and Fernandez, 1991), as an additional resistance to the diffusion of water vapour from the leaf interior to the atmosphere (Nobel, 1983) and as a reflector reducing the radiant energy absorbed by the leaf (Ehleringer, 1984; Rajendiran, 2001). These non-glandular hairs offer additional mechanical barrier to UV-B penetration by reflecting the radiant energy (Kokilavani and Rajendiran, 2013). The increased trichome frequency which could have been an adaptive feature to UV-B treatment is at variance from the reductions observed by Karabourniotis et al., (1995).

Plate 1: Changes in the morphological characteristics of leaves of 30 DAS Vigna unguiculata (L.) Walp. cv. COVU-2 exposed to supplementary UV-B radiation

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Plate 2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. COVU-2 under control condition and supplementary UV-B radiation exposure. (Figure 3 to 8: 400 x)
Plate 3: Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. COVU-2 exposed to supplementary UV-B radiation

Very deeply stained dead and collapsed epidermal cells were found in large numbers on both the leaf surfaces of UV-B stressed plants (Table 1; Plate 2. Figure 4, 6). Due to collapsed epidermal cells, the leaves became glazed showing signs of bronzing of tissue surfaces which have been attributed to oxidised phenolic compounds (Cline and Salisbury, 1966). This may in some cases also be followed by tissue degradation (Caldwell, 1971).

The epidermal cell (10.39 % to 25.70 %) and stomata (21.32 % to 52.60 %) were smaller after UV-B irradiation (Table 1; Plate 2. Figure 4, 6).

The leaves are amphistomatic and the stomata are oval in outline and distributed all over the surface except over coastal regions without any definite pattern or orientation. Mature stomata were mostly diacytic and paracytic.

Stomatal frequency (33.33 and 24.81 %) and stomatal indices were increased significantly (63.38 and 57.11 %) over control with S/E ratio exhibiting low values (19 to 20 %) under UV-B exposure on both the surfaces (Table 1). This is in accordance with pea plants which responding to UV-B treatment had higher stomatal frequency on the adaxial surface (Nogues *et al.*., 1998).
In UV-B irradiated plants the stomata were smaller than control on both surfaces of the foliage and the abnormal stomata were more frequent, the maximum being on the adaxial surface (Table 1; Plate 2. Figure 4, 6).

Table 1: Changes in the epidermal characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. COVU-2 exposed to elevated UV-B radiation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>UV-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adaxial</td>
<td>Abaxial</td>
</tr>
<tr>
<td>Stomatal frequency mm$^{-2}$</td>
<td>211.2±0.76</td>
<td>224.1±0.92</td>
</tr>
<tr>
<td>Epidermal cell frequency mm$^{-2}$</td>
<td>250.8±0.92</td>
<td>268.6±2.08</td>
</tr>
<tr>
<td>Stomatal index</td>
<td>25.18±0.15</td>
<td>26.96±1.25</td>
</tr>
<tr>
<td>S/E ratio</td>
<td>0.84</td>
<td>0.83</td>
</tr>
<tr>
<td>Frequency of abnormal stomata mm$^{-2}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Frequency of dead/collapsed epidermal cells mm$^{-2}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Frequency of trichome mm$^{-2}$</td>
<td>13.2±1.86</td>
<td>13.2±0.99</td>
</tr>
<tr>
<td>Stomatal size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (µm)</td>
<td>42.2±1.32</td>
<td>38.1±1.05</td>
</tr>
<tr>
<td>Breadth (µm)</td>
<td>23.3±0.79</td>
<td>15.1±0.38</td>
</tr>
<tr>
<td>Epidermal cell size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (µm)</td>
<td>63.2±0.17</td>
<td>66.6±4.62</td>
</tr>
<tr>
<td>Breadth (µm)</td>
<td>44.4±2.86</td>
<td>42.2±1.51</td>
</tr>
<tr>
<td>Trichome length (µm)</td>
<td>71.0±0.65</td>
<td>69.4±1.50</td>
</tr>
</tbody>
</table>

Similar results were reported by Wright and Murphy (1982), Kokilavani and Rajendiran (2013), Kokilavani *et al.*, (2013), Kokilavani *et al.*, (2014) and Kokilavani and Rajendiran (2014c) on the adaxial side of the leaves after exposure to UV-B radiation. UV-B irradiated leaves developed abnormalities like persistent stomatal initials, stomata with single guard cell and thickened pore and collapsed stomata (Table 1; Plate 2. Figure 4, 6).

No such abnormalities were recorded in the leaves of the crops grown in control conditions (Table 1; Plate 2. Figure 3, 5). *Vigna unguiculata* (L.) Walp. cv. COVU-2 responded to UV-B stress by reducing leaf size and area, and created leaves with thick cuticle, thicker epidermal layers, many trichomes and increased mesophyll volume to withstand UV-B irradiation.

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