

EFFICACY OF *VIGNA UNGUICULATA* (L.) WALP. CV. VAMBAN LEAVES TO WITHSTAND SUPPLEMENTARY ULTRAVIOLET-B IRRADIATION

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ABSTRACT

The distribution of ozone in the troposphere and stratosphere depends on the changes in chemical composition of the atmospheric viz., changes in levels of carbon dioxide which affects temperatures in both the troposphere and stratosphere, methane which affects the levels of reactive hydrogen oxides in the troposphere and stratosphere that can react with ozone, and nitrous oxide which affects levels of nitrogen oxides in the stratosphere that can react with ozone. Any decline in the level of ozone increases the penetration of ultraviolet-B (UV-B) radiation which has a direct effect on the leaves that cooks food in the crops. The effects of ultraviolet-B radiation in the morphology, epidermis and the anatomy of *Vigna unguiculata* (L.) Walp. cv. VAMBAN leaf is evaluated. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. VAMBAN after exposure to supplementary UV-B radiation (2 hours daily @ $12.2 \text{ kJ m}^{-2} \text{ d}^{-1}$; ambient = $10 \text{ kJ m}^{-2} \text{ d}^{-1}$) and under normal condition were monitored. UV-B exposed leaves recorded various types of malformations and injuries which were not observed in control crops. Crops after UV-B stress had leaves which were small, shiny and thick compared to healthier and thin leaves of normal plants. Leaves receiving UV-B had increased stomatal frequency (54.79 and 35.49 %) over control on adaxial and abaxial surfaces. Similarly stomatal indices of stressed plants showed increases by 26.29 to 33.48 % on both the surfaces. More number of abnormal stomata in the form of single guard cell occurred along with dead and collapsed epidermal cells on the adaxial surface of UV-B irradiated leaves. Normal leaves had no stomatal aberrations. The trichomes were shorter by 30.48 % and 22.05 % on adaxial and abaxial surfaces respectively and were also brittle in UV-B exposed leaves compared to normal trichomes in control leaves. Frequency of trichome, thickness of cuticle and epidermis were two times more than that of control on both surfaces in UV-B treated plants. Under UV-B exposure the volume and thickness of mesophyll increased by 44.44 % and 51.97 % making the leaves thicker by 71.53 % over unstressed leaves.

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

INTRODUCTION

Stratospheric ozone filters out most of the sun's potentially harmful shortwave ultraviolet (UV) radiation. This ozone has become depleted, due to the release of such ozone-depleting substances as chlorofluorocarbons (CFCs). When stratospheric ozone is depleted, more UV rays reach the earth. Exposure to higher amounts of UV radiation could have serious impacts on human beings, animals and plants. At the metabolic level, elevated ultraviolet-B (UV-B) radiation (280-320 nm) 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. Leaves possess epidermis that constitutes a dynamic barrier between the plant's internal and external environment. Impregnated with waxes and cutins on the exterior for protection and with stomata to regulate the exchange of gases, the leaves are also provided with appendages like trichomes, hydathodes and scales. The foliage of crops receives major proportion of the ultraviolet radiation and hence always react in reflux to obstruct its penetration into the

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inner organs (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013). The modifications in leaf architecture developed by cowpea variety VAMBAN receiving UV-B stress was reported in this paper.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. VAMBAN obtained from Saravanan Farms, Villupuram, Tamil Nadu, 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) $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$, 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_{BE}) of $12.2 \text{ kJ m}^{-2} \text{d}^{-1}$ equivalent to a simulated 20 % ozone depletion at Pondicherry ($12^{\circ}2' \text{N}$, India). The control plants, grown under natural solar radiation, received UV-B_{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. VAMBAN 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) = $S / S + E \times 100$ 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 μm . Mesophyll thickness (mm) was multiplied by 100 to calculate the mesophyll volume in $\text{cm}^3 \text{dm}^{-2}$ of leaf area as recommended by Patterson *et al.*, (1978).

RESULTS AND DISCUSSION

The leaves of *Vigna unguiculata* (L.) Walp. cv. VAMBAN grown under UV-B rays were small, wrinkled, shiny and brittle with chlorotic and necrotic lesions over the adaxial surface (Plate 1; Plate 2. Figure 1 to 2). Unstressed leaves on the adaxial surface had uniformly similar costal cells which are axially elongated, thin and straight walled with unicellular thin walled trichomes. The costal cells and trichomes on adaxial surface differ from abaxial surface in being shorter in length (Table 1). Intercostal epidermal cells are sinuous and thin walled with unicellular trichomes occurring intermittently both on abaxial and adaxial surfaces. The epidermal cells with dense, deeply stained nuclei occurred in control and in all the UV-B irradiated foliage. The frequency of epidermal cells was higher by 26.36 to 33.57 % compared to control in UV-B exposed leaves on both the surfaces, the highest value being on adaxial side (Table 1). Cuticle and epidermis of UV-B exposed leaves were thicker than control on both surfaces (Plate 3). Formation of multiple layers on adaxial surface resulted in two times thicker epidermis in UV-B stressed leaves (Plate 2. Figure 3; Plate 3). The trend continued in leaf thickness (71.53 %), mesophyll thickness (44.44 %) and mesophyll volume (51.97 %) in crops receiving UV-B stress (Plate 3). Wellmann (1976), Caldwell *et al.*, (1983), Bornman and Vogelmann (1991) and Rajendiran (2001) suggested that leaves obstruct the UV-B penetration to the inner tissues by absorbing some of the damaging radiation and by strengthening the tissues through marked elongation of palisade cells to alleviate some of the deleterious effects. Bornman and Vogelmann (1991) reported that leaf thickness in *Medicago sativa* increased due to addition of spongy mesophyll cells, whereas there was an increase in the number of palisade cells only in *Brassica campestris*. According to Kokilavani and Rajendiran (2013), Kokilavani *et al.*, (2013) and

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Kokilavani and Rajendiran (2014a) leaf 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. Frequency of unicellular trichomes present in the costal as well as intercostal regions was comparatively more on the abaxial side than the adaxial side (Table 1). UV-B exposure increased frequency of trichome by two times over the control on both the surfaces (Table 1). Trichomes were shorter on adaxial side (30.48 %) as well as on abaxial side (22.05 %) in UV-B irradiated leaves (Table 1; Plate 2. Figure 4). The trichomes form a mechanical barrier against biotic attack (Johnson, 1975; Woodman and Fernandez, 1991), provide 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). The trichomes also make additional mechanical shield to UV-B penetration by reflecting the radiant energy (Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b). The increase in trichome frequency which could have been an adaptive character to UV-B treatment (Kokilavani and Rajendiran, 2014c) differs from the report of Karabourniotis *et al.*, (1995) who observed reductions in trichome number. Deeply stained dead and collapsed epidermal cells were found in more numbers on both the surfaces of UV-B stressed foliage (Table 1; Plate 2. Figure 6, 8). Damages in the form of collapsed cells and the leaves becoming glazed with signs of bronzing of tissue surfaces have been attributed to oxidised phenolic compounds (Cline and Salisbury, 1966) followed by tissue degradation (Caldwell, 1971).

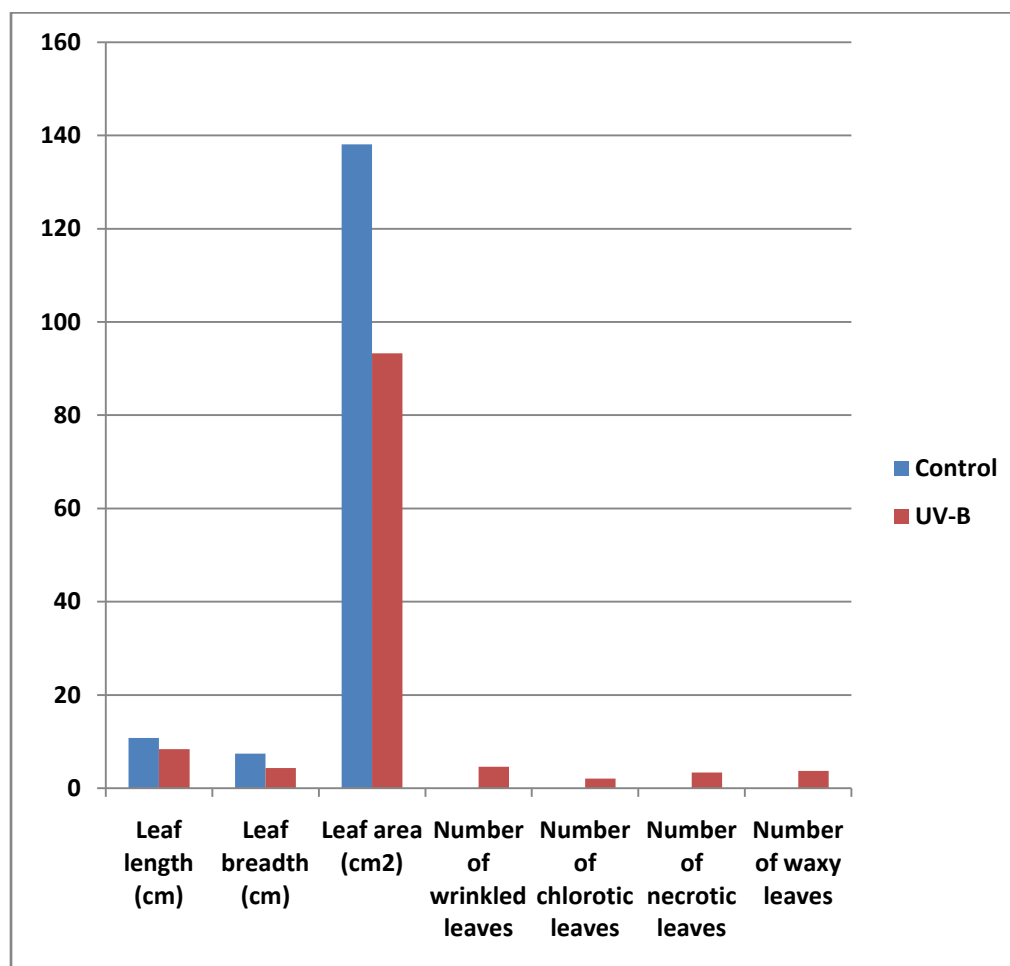


Plate 1: Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. VAMBAN exposed to supplementary UV-B radiation

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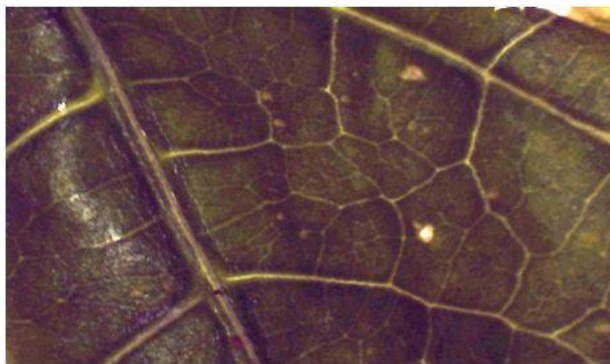


Figure 1: Shiny adaxial surface under UV-B

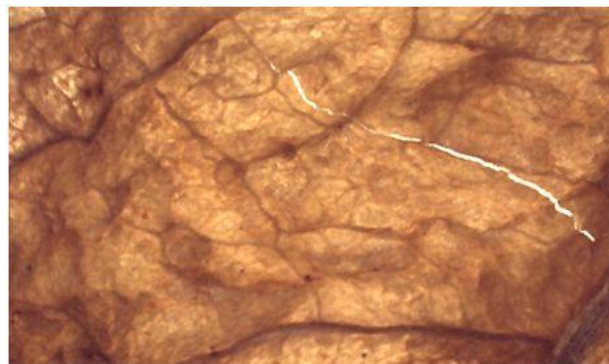


Figure 2: UV-B adaxial - Brittle and dead

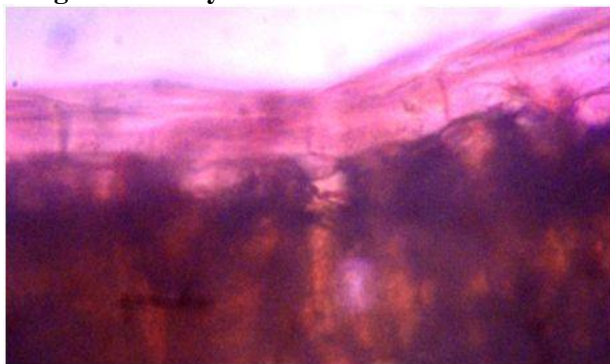


Figure 3: UV-B adaxial - Multiseriate epidermis

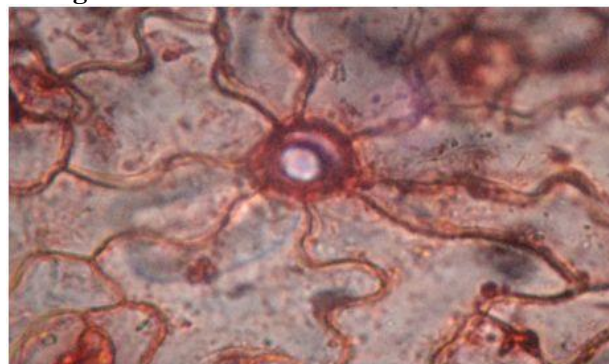


Figure 4: UV-B adaxial - Broken trichome

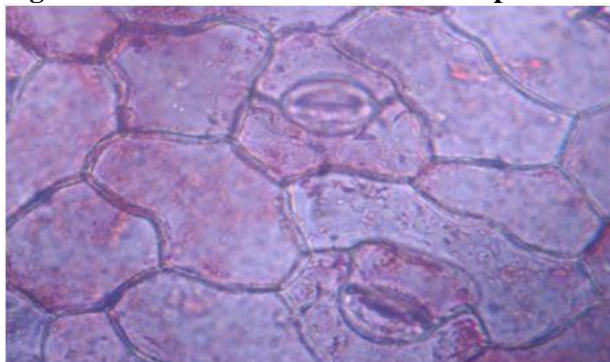


Figure 5: Control adaxial - Normal stomata

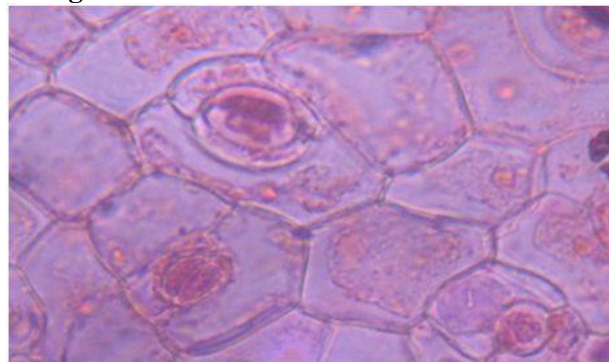


Figure 6: UV-B adaxial - Abnormal stomata

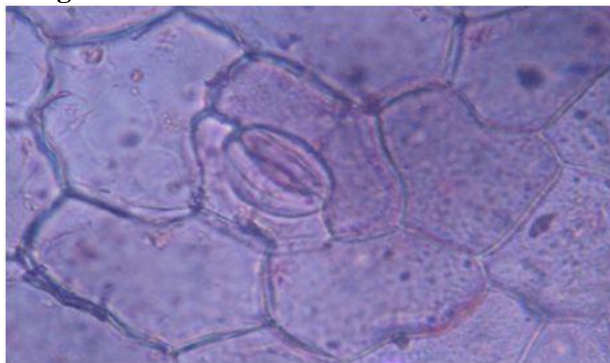


Figure 7: Control abaxial - Normal stomata

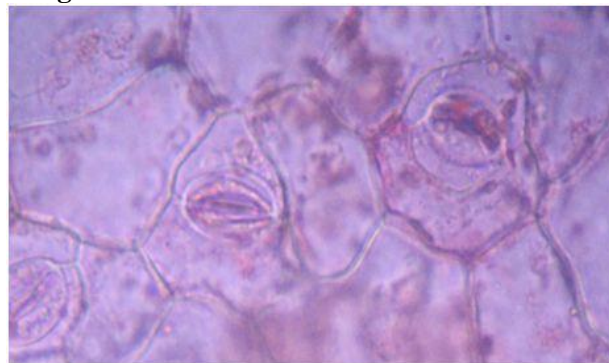


Figure 8: UV-B abaxial - Abnormal stomata

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

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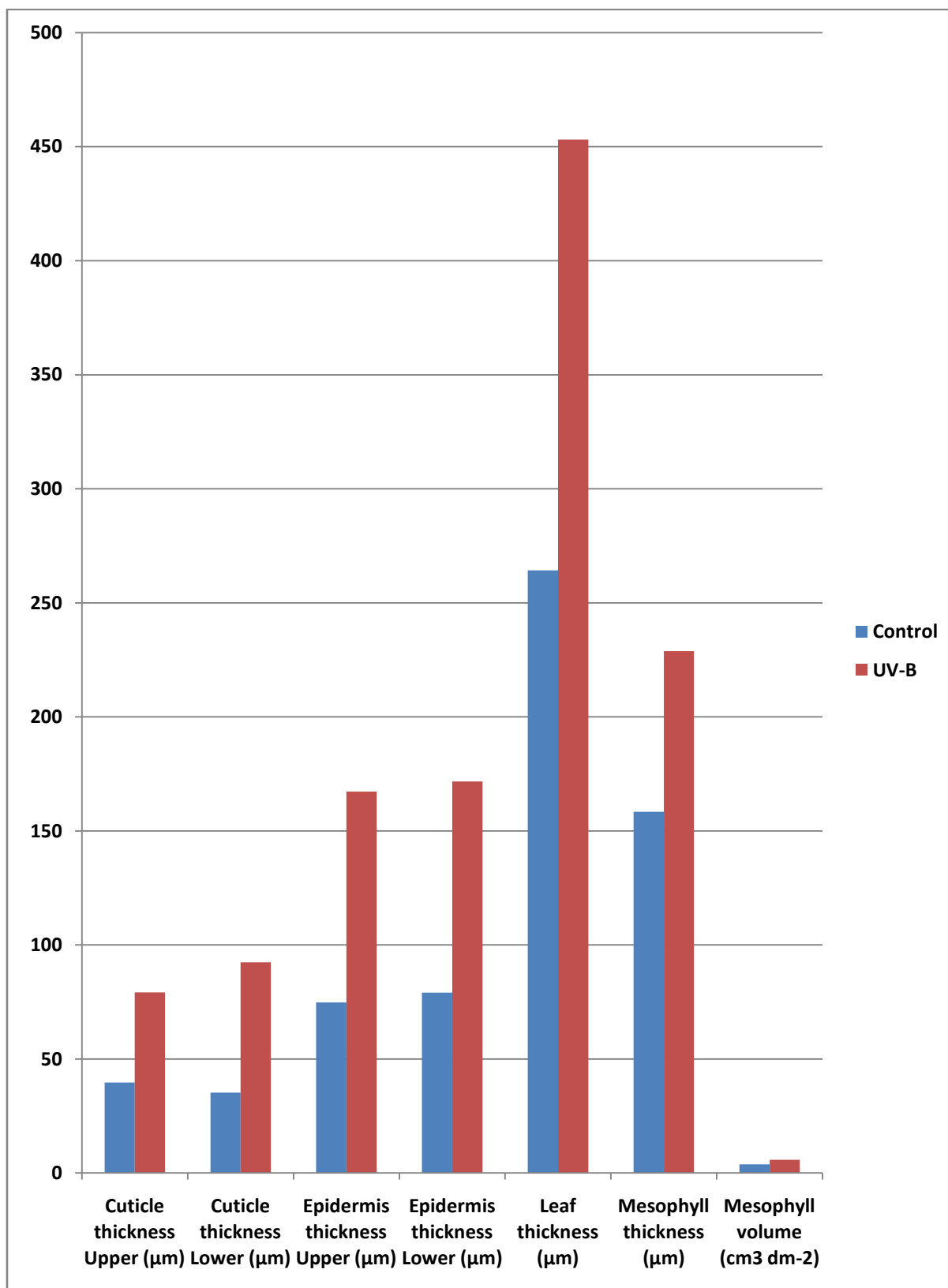


Plate 3: Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. VAMBAN exposed to supplementary UV-B radiation

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

Parameter	Control		UV-B	
	Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency (mm ⁻²)	162.8±2.41	175.8±0.74	252.0±1.31	238.2±0.61
Epidermal cell frequency (mm ⁻²)	360.4±0.48	372.4±1.08	481.4±1.82	470.6±0.19
Stomatal index	36.14±0.91	37.34±2.08	48.24±0.65	47.16±1.19
S/E ratio	0.45	0.47	0.52	0.50
Frequency of abnormal stomata (mm ⁻²)	-	-	57.2±0.38	60.4±1.99
Frequency of dead/collapsed epidermal cells (mm ⁻²)	-	-	71.6±0.16	68.9±0.21
Frequency of trichome (mm ⁻²)	13.2±1.86	14.9±1.71	39.6±0.52	36.2±0.29
Stomatal size	Length (µm)	37.7±1.33	34.4±0.58	17.8±0.69
	Breadth (µm)	20.0±0.42	17.8±1.34	12.1±0.51
Epidermal cell size	Length (µm)	69.9±0.66	56.6±1.28	36.6±0.21
	Breadth (µm)	48.6±2.37	47.2±0.25	43.2±0.86
Trichome length (µm)	85.3±0.58	83.9±0.68	59.3±0.44	65.4±0.41

Epidermal cell size (11.11 to 47.63 %) and stomata (19.10 to 52.78 %) were decreased below control after UV-B stress (Table 1; Plate 2. Figure 6 to 8). The leaves are amphistomatic and the stomata are diacytic and paracytic and distributed all over the surface except over costal regions without any definite pattern or orientation. Frequency of stomata (35.49 to 54.79 %) and stomatal indices (26.29 to 33.48 %) were increased significantly above control with S/E ratio on both sides showing more value (6.38 to 15.55 %) under UV-B exposure on the adaxial as well as abaxial surfaces (Table 1). Pea plants under UV-B treatment had higher stomatal frequency on the adaxial surface (Nogues *et al.*, 1998).

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Stomata were smaller than control on both surfaces of the foliage under UV-B and the abnormal stomata were more frequent with the maximum on the adaxial surface (Table 1; Plate 2. Figure 6, 8). Similar results were reported by Wright and Murphy (1982), Kokilavani and Rajendiran (2013), Kokilavani *et al.*, (2013), Kokilavani *et al.*, (2014), Kokilavani and Rajendiran (2014a) and Kokilavani and Rajendiran (2014b) on the adaxial side of UV-B irradiated leaves. Abnormalities like persistent stomatal initials, stomata with single guard cell and thickened pore and collapsed stomata were observed after UV-B exposure (Plate 2. Figure 6, 8). Stomatal abnormalities were not recorded in the leaves receiving sunlight only (Table 1; Plate 2. Figure 5, 7). *Vigna unguiculata* (L.) Walp. cv. VAMBAN leaves in response to supplementary ultraviolet-B radiation modified the leaf architecture to overcome the stress.

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