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Research Article

VARIATION IN LEAF ARCHITECTURE OF *VIGNA UNGUICULATA* (L.) WALP. CV. COFC-8 INDUCED BY SUPPLEMENTARY UV-B EXPOSURE

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ABSTRACT
The main cause of the current global warming trend is human expansion of the 'greenhouse effect' - warming those results when the atmosphere traps heat radiating from earth toward space. These green house gases accumulating around the earth increases in thickness and the heat that normally would leave the troposphere and get into the stratosphere no longer does so, cooling the stratosphere. Colder temperature helps to deplete ozone layer, allowing enormous ultraviolet-B (UV-B) radiation into earth's surface. Increased ultraviolet-B radiation has a direct effect on the leaves which make the photosynthetic organ of the crops. This work is an attempt to evaluate the effects of ultraviolet-B (UV-B) radiation in the morphology, epidermis and the anatomy of *Vigna unguiculata* (L.) Walp. cv. COFC-8 leaf. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. COFC-8 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 43.47 and 15.15 % over control on adaxial and abaxial surfaces respectively. Same trend was noticed in stomatal indices of stressed plants which showed increases by 75.69 to 78.74 % on both the surfaces. 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 24.28 % and 40.10 % 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 50 % on adaxial and by 8.93 % on abaxial surfaces in UV-B exposed plants. On adaxial surface cuticle and epidermis were three to four times thicker after UV-B irradiation. The volume and thickness of mesophyll increased by 61.32 % and 54.05 % making the leaves thicker by 83.60 % under UV-B exposure.

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

INTRODUCTION
The ozone layer is a belt of naturally occurring ozone gas that sits 15 to 30 kilometers above earth and serves as a shield from the harmful ultraviolet- B radiation emitted by the sun. Earth is losing the protective layer either directly by ozone depleting substances or indirectly by global warming effects. As a result, the UV-B fluence is bound to increase, affecting plants, animals and human beings, and in the long run, the ecosystems too. An elevation in the flux of ultraviolet-B (UV-B) radiation (280-320 nm) is an important atmospheric stress and is detrimental to plant growth and development. At the metabolism level, 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. Plants receive major proportion of the ultraviolet radiation through the leaves and so react to prevent its entry into the internal

organs (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013). The present study is carried out to investigate the response of the leaves to UV-B stress in COFC-8 variety of *Vigna unguiculata* (L.) Walp.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. COFC-8 obtained from Tamil Nadu Agriculture University, were grown in pot culture in the naturally lit greenhouse (day temperature maximum 38 ± 2 ºC, night temperature minimum 18 ± 2 ºC, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 µmol m^{-2} 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 kJ m^{-2} d^{-1} equivalents to simulated 20 % ozone depletion at Pondicherry (12º2'N, India). The control plants, grown under natural solar radiation, received UV-B_{BE} 10 kJ m^{-2} 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. COFC-8 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. Stornatal 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 µm. 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

UV-B irradiation reduced the size of the leaves in *Vigna unguiculata* (L.) Walp. cv. COFC-8 which were wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface (Plate 1; Plate 2. Figure 1 to 2). Normal leaves on adaxial surface had costal cells which are uniformly similar in being axially elongated, thin and straight walled bearing unicellular thin walled trichomes. The costal cells and trichomes on adaxial surface are shorter in length than abaxial surface (Table 1). The intercostal epidermal cells are sinuous and thin walled with unicellular trichomes occurring intermittently both on abaxial and adaxial surfaces. The epidermal cells in control and in all the UV-B irradiated leaves had dense and deeply stained nuclei (Plate 2. Figure 5, 6). Epidermal cell frequency was higher over control on adaxial surface by 76 % in UV-B exposed leaves, which continued to increase further on the abaxial side by 79 % (Table 1). The thickness of cuticles and the epidermis in UV-B exposed leaves, on both sides, increased significantly over control (Plate 3). On adaxial surface the epidermis was multilayered and it showed manifold thickness after ultraviolet-B exposure (Plate 2. Figure 3; Plate 3). Under UV-B stress the thickness of leaf and mesophyll and the volume of mesophyll were also increased (Plate 3). Plants obstruct the UV-B transmission to the inner leaf tissues either by absorbing some of the damaging UV radiation and by strengthening the tissues through marked elongation of palisade cells alleviating some of the deleterious effects (Wellmann, 1976; Caldwell *et al.*, 1983; Bornman and Vogelmann, 1991; Rajendiran, 2001). The thickness of leaf 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 only (Bornman and Vogelmann, 1991). Kokilavani and Rajendiran (2013), Kokilavani *et al.*, (2013) and

Kokilavani and Rajendiran (2014a) opined that 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. UV-B exposure also increased trichome frequency on adaxial (50 %) and on abaxial (89 %) surfaces compared with control samples (Table 1). Shorter trichomes occurred on adaxial side (24.28 %) as well as on abaxial side (39.43 %) in UV-B irradiated leaves along with few broken trichomes on the adaxial side (Table 1; Plate 2. Figure 4). The trichomes create a mechanical barrier against biotic attack (Johnson, 1975; Woodman and Fernandez, 1991), provide resistance to the diffusion of water vapour from the leaf interior to the atmosphere (Nobel, 1983) and behave as a reflector reducing the radiant energy absorbed by the leaves (Ehleringer, 1984; Rajendiran, 2001; Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b).

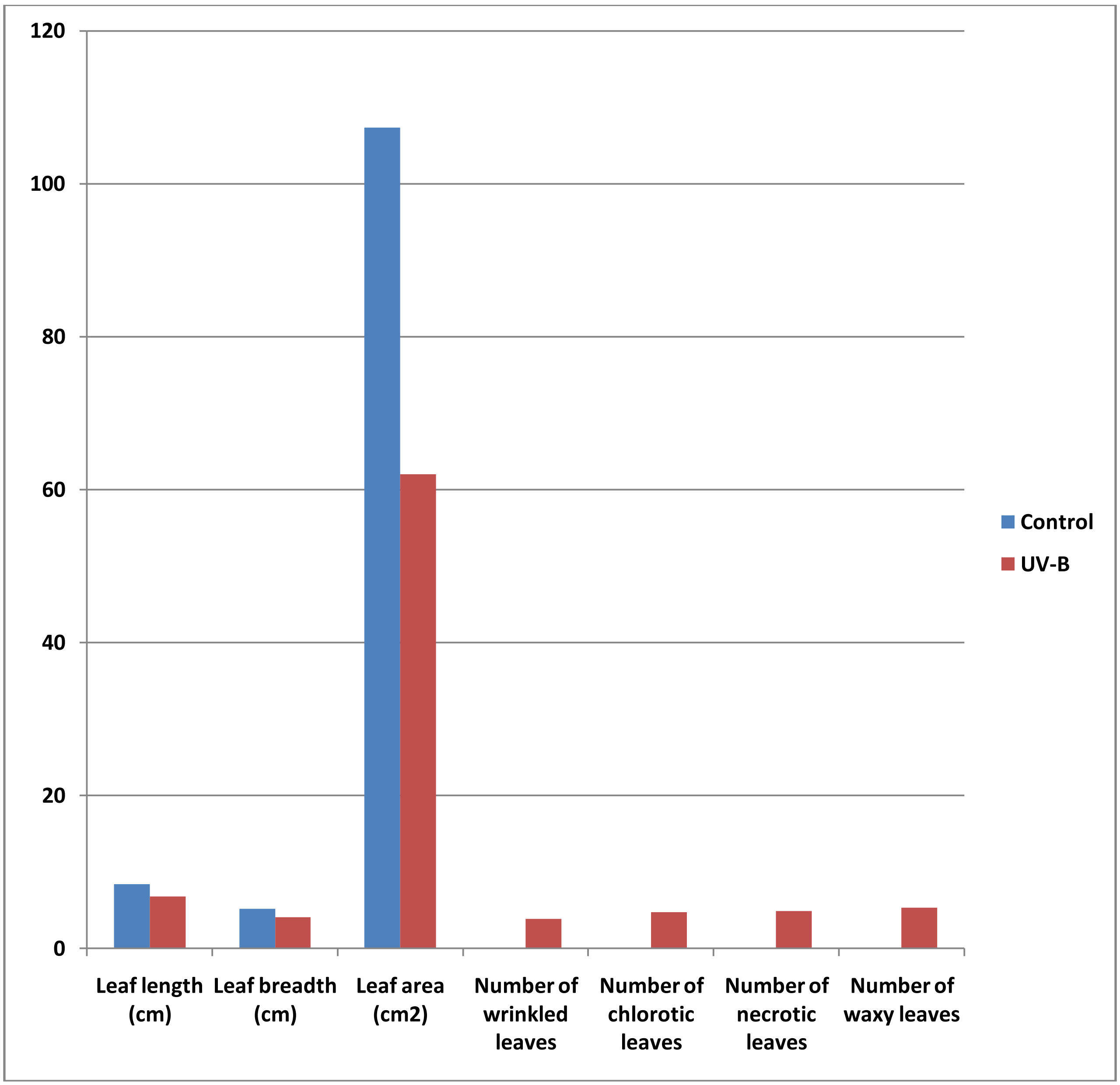

Plate 1: Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* alp. cv. COFC-8 exposed to supplementary UV-B radiation

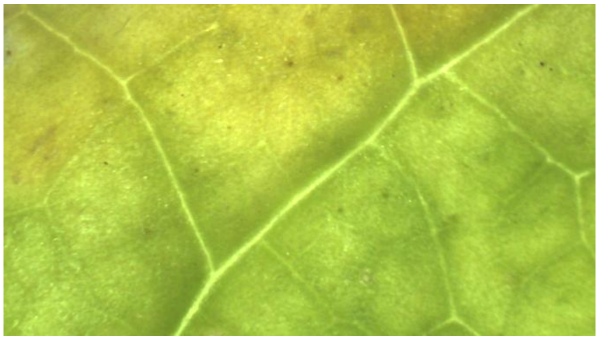

Figure 1: Shiny adaxial surface under UV-B

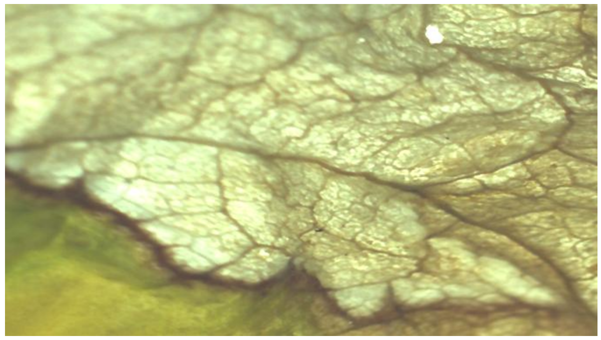

Figure 2: UV-B adaxial - Brittle and dead

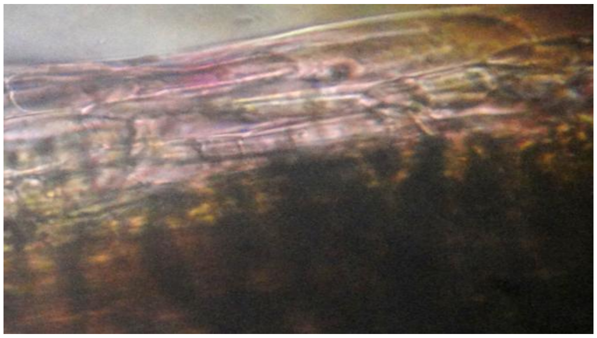

Figure 3: UV-B adaxial - Multiseriate epidermis

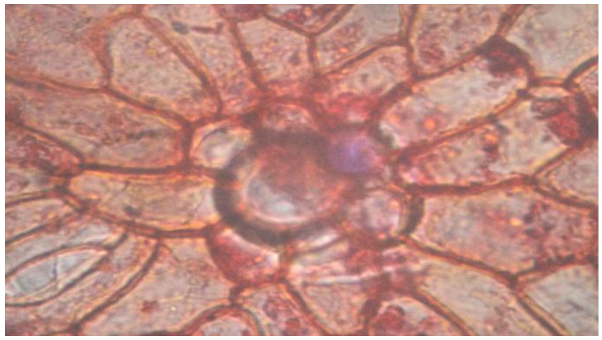

Figure 4: UV-B adaxial - Broken trichome

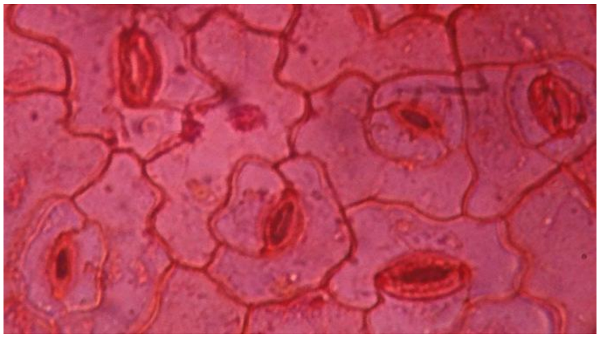

Figure 5: Control adaxial - Normal stomata

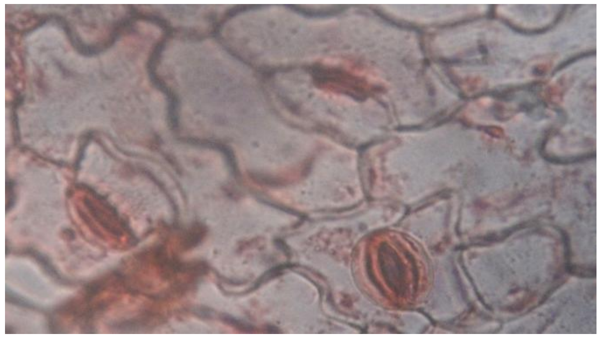

Figure 6: UV-B adaxial - Abnormal stomata

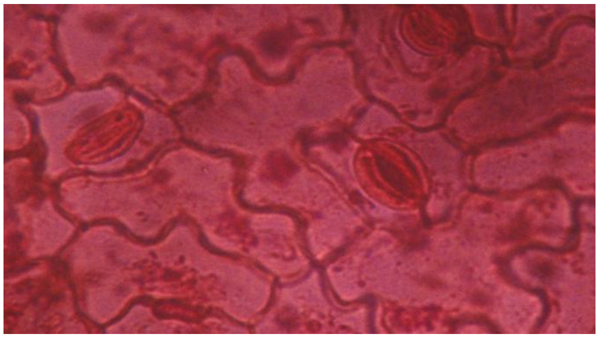

Figure 7: Control abaxial - Normal stomata

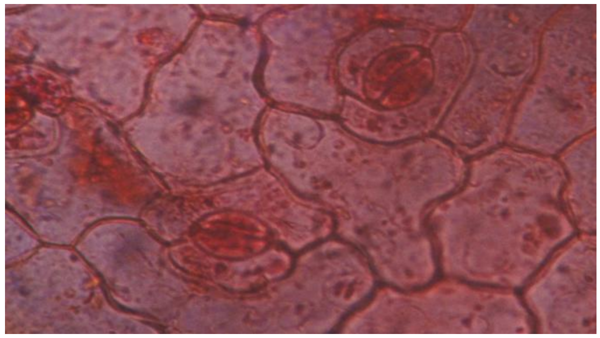

Figure 8: UV-B abaxial - Abnormal stomata

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

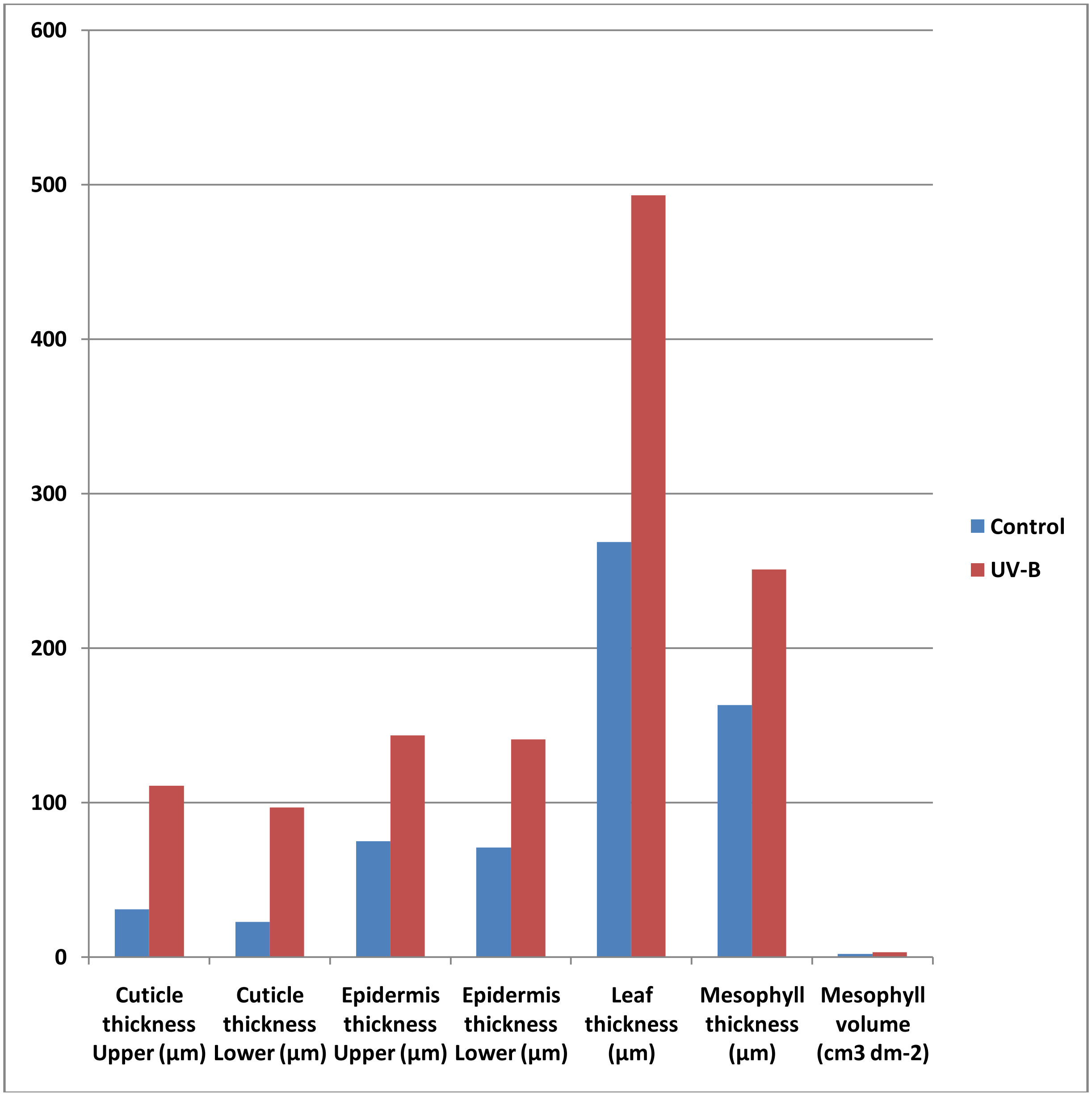

Plate 3: Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. COFC-8 exposed to supplementary UV-B radiation

The increased trichome frequency which could have been an adaptive feature to UV-B treatment (Kokilavani and Rajendiran 2014c) differs from the reduced frequency observed by Karabourniotis *et al*. (1995). In crops suffering heavily under UV-B stress, the epidermal cells were dead and collapsed taking on very deep stain on both the foliar surfaces (Table 1; Plate 2. Figure 6, 8).

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).

Size of epidermal cells (5 to 28%) and stomata (46 %) were decreased below control after UV-B exposure (Table 1; Plate 2. Figure 6 to 8). Stomata frequency (43.47 %) and stomatal indices were increased significantly (75 to 78 %) above control with low S/E ratio (19 to 37 %) on both sides under UV-B exposure (Table 1).

Table 1: Changes in the epidermal characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. COFC-8 exposed to elevated UV-B radiation

Parameter		Control		UV-B	
		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency mm^{-2}		101.2±1.02	119.4±0.11	145.2±0.76	137.5±0.52
Epidermal cell frequency mm^{-2}		237.6±0.29	221.1±1.19	418.2±1.93	396.0±0.71
Stomatal index		23.86±1.26	22.21±0.10	41.92±0.90	39.70±1.27
S/E ratio		0.42	0.54	0.34	0.34
Frequency of abnormal stomata mm^{-2}		-	-	26.4±0.73	31.7±0.80
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	88.3±1.90	81.8±0.74
Frequency of trichome mm^{-2}		17.6±0.79	14.9±0.53	26.4±1.36	28.3±0.64
Stomatal size	Length (µm)	44.3±3.12	41.1±0.93	23.3±0.32	27.7±1.57
	Breadth(µm)	22.7±0.43	17.8±1.26	12.2±2.58	14.2±0.41
Epidermal cell size	Length(µm)	78.8±1.05	56.5±3.96	52.5±1.46	52.8±0.52
	Breadth(µm)	41.4±0.23	47.0±0.27	39.1±2.11	45.7±0.17
Trichome length (µm)		73.3±0.43	78.8±0.24	55.5±0.18	47.2±0.32

According to Nogues *et al.* 1998, pea plants responding to UV-B treatment had higher stomatal frequency on the adaxial surface. Smaller stomata accompanied with severe abnormalities were more on both surfaces of UV-B irradiated crops with the maximum being on the adaxial surface (Table 1; Plate 2. Figure 6, 8). 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) reported similar results on the adaxial side of UV-B irradiated leaves. Abnormalities like stomata with single guard cell and collapsed stomata were seen in UV-B exposure leaves (Plate 2. Figure 6, 8), but no such aberrations were recorded in control leaves (Table 1; Plate 2. Figure 5, 7). The COFC-8 variety of cowpea is highly sensitive to UV-B radiation, as the leaves developed several modifications to combat the stress.

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