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Research Article

INFLUENCE OF ELEVATED ULTRAVIOLET-B RADIATION ON FOLIAR ORGANISATION IN *VIGNA UNGUICULATA* (L.) WALP. CV. CW-122

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ABSTRACT

Plants are unable to move away from unfavorable environmental conditions, but they have evolved a number of mechanisms to tide over them. In addition to manufacture of food, leaves act as a system to sense and react immediately in adverse conditions for survival, growth, and reproduction of the plants. In recent years the green house gases accumulating around the earth due to human activity, increases in thickness and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, leaving the stratosphere cooler. Colder than normal temperatures act to deplete ozone layer, allowing enormous ultraviolet-B (UV-B) radiation into earth's surface thereby affecting the ecosystems. Increased flux of ultraviolet-B radiation has a direct effect on the leaves of crop plants. The present study is to evaluate the effects of ultraviolet-B (UV-B) radiation in the morphology, epidermis and the anatomy of *Vigna unguiculata* (L.) Walp. cv. CW-122 leaves. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. CW-122 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. UV-B exposure induced various types of malformations in the leaf architecture and created several injuries which were not observed under control conditions. Structurally, the epidermis exhibited varying trends under UV-B irradiation. Cuticle and epidermis on adaxial surface were two to three times thicker under UV-B irradiation. The volume and thickness of mesophyll in UV-B exposed leaves increased by 77 % and 28 % making the leaves thicker by 51.61 % more than control leaves. The trichomes were shorter by 25 % on adaxial and by 34 % on abaxial surfaces but brittle in UV-B treated leaves which were shorter as well as healthier in control. The trichome frequency was also increased by 126 % on adaxial and by 75 % on abaxial surfaces in UV-B stressed plants. The small, shiny and thick leaves of crops in elevated UV-B environment compared poorly to broader, longer and thinner leaves of control plants. The stomatal frequency under UV-B was increased by 28 and 50 % above control on adaxial and abaxial surfaces respectively. Similar trend was seen in the stomatal indices which showed increases (28 to 50 %) on both the surfaces. Abnormal stomata like, stomata with single guard cell, reduced size and malformations were predominant along with dead and collapsed epidermal cells on the adaxial surface of UV-B irradiated crop. Such aberrations were absent in leaves under unstressed conditions. Sensing the stress under elevated UV-B habitat, CW-122 variety of cowpea reacted quickly with foliar modifications for survival.

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

INTRODUCTION

Ozone depletion is one of the man-made calamities that threatens to continue due to increases in thickness of green house gases around the earth and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, making the stratosphere cooler. Far below normal temperatures in this layer increases ozone depletion. As a result, the UV-B irradiation will increase, affecting all the components of the ecosystem. Elevated ultraviolet-B (UV-B) radiation (280-320 nm) is a dangerous atmospheric stress as it is detrimental to plant growth and development. UV-B severely suppresses photosynthesis (Rajendiran and Ramanujam 2003; Rajendiran and Ramanujam 2004) and inhibits nodulation and nitrogen metabolism (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. Foliar epidermis which is impregnated with waxes and cutins on the exterior and possessing stomata to regulate the exchange of gases forms a dynamic barrier between the plant's internal tissues and external habitat. Crop foliage receives major proportion of the ultraviolet radiation and hence always reacts immediately to prevent its entry into the internal organs (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b). The present work deals with the modifications brought about in the leaf of *Vigna unguiculata* (L.) Walp. cv. CW-122 in response to UV-B stress.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. CW-122 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. CW-122 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 exposed leaves of *Vigna unguiculata* (L.) Walp. cv. CW-122 was small, wrinkled, shiny and very brittle with chlorotic and necrotic lesions over the adaxial surface (Plate 1; Plate 2. Figure 1 to 2). Control 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. Epidermal cell frequency was higher (28.16 to 50.89 %) over control in UV-B exposed leaves on both the surfaces, the highest being on adaxial surface (Table 1). Cuticle and epidermis of UV-B exposed leaves, on both surfaces, were significantly thicker than control (Plate 3). Due to multilayer formation on adaxial surface, epidermis was two to three times thicker in stressed leaves (Plate 2. Figure 3; Plate 3). Similarly leaf thickness, mesophyll thickness and volume also increased under UV-B stress (Plate 3). Wellmann (1976), Caldwell *et al.*, (1983), Bornman and Vogelmann (1991) and (Rajendiran 2001) opined that 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. 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 only (Bornman and Vogelmann 1991). According to Kokilavani and Rajendiran (2013), Kokilavani *et al.*, (2013) and 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 of both the surfaces was comparatively less on the abaxial side than the adaxial side (Table 1). UV-B exposure increased trichome frequency on adaxial (126 %) as well as on abaxial (75 %) surfaces over control leaves (Table 1). Trichomes were shorter by 25 % on adaxial side and by 34 % on abaxial side 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).

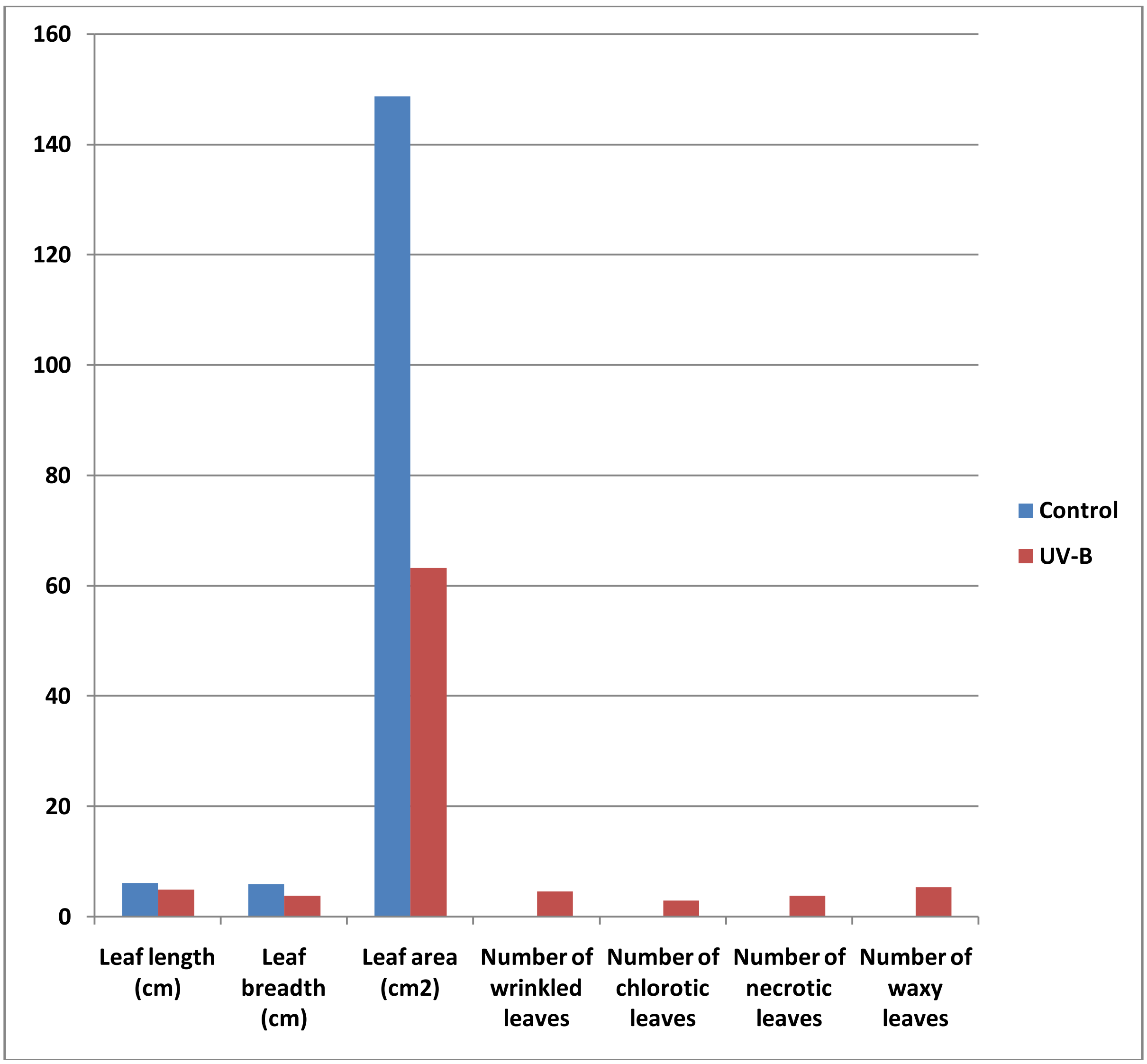

Plate 1: Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CW-122 exposed to elevated UV-B radiation

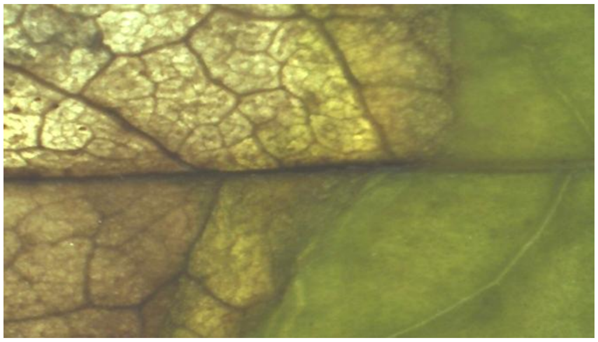

Figure 1: Shiny adaxial surface under UV-B

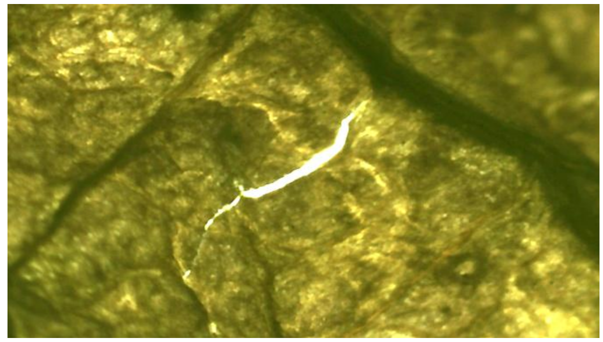

Figure 2: UV-B adaxial - Brittle and dead

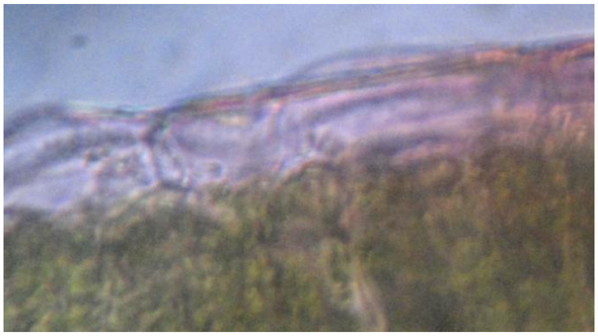

Figure 3: UV-B adaxial - Multiseriate epidermis

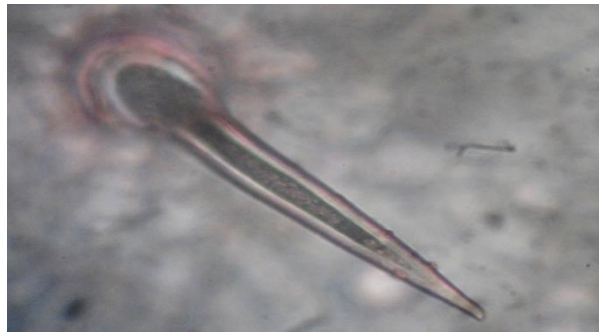

Figure 4: UV-B adaxial - Short trichome

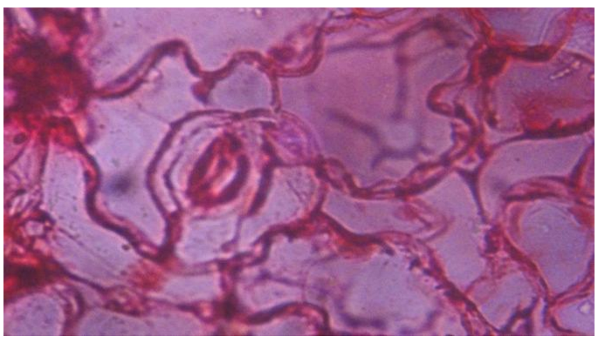

Figure 5: Control adaxial - Normal stomata
stomata

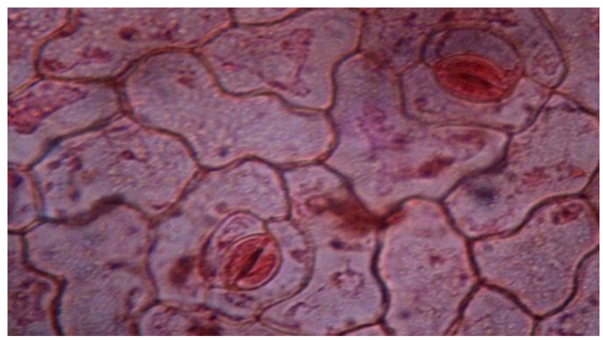

Figure 6: UV-B adaxial - Abnormal

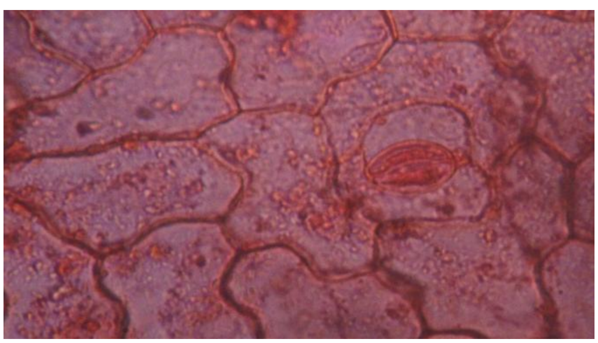

Figure 7: Control abaxial - Normal stomata
stomata

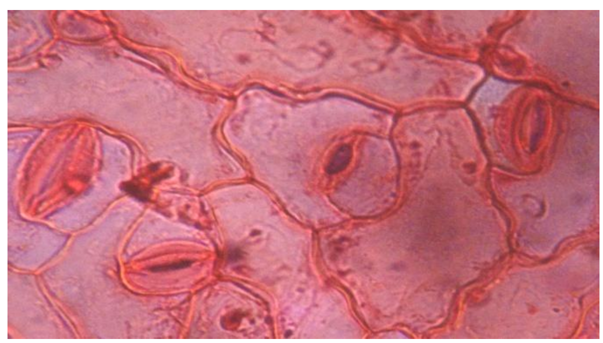

Figure 8: UV-B abaxial - Abnormal

Plate 2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. CW-122 under control condition and supplementary UV-B radiation exposure (Figure 3 to 8: 400 x)

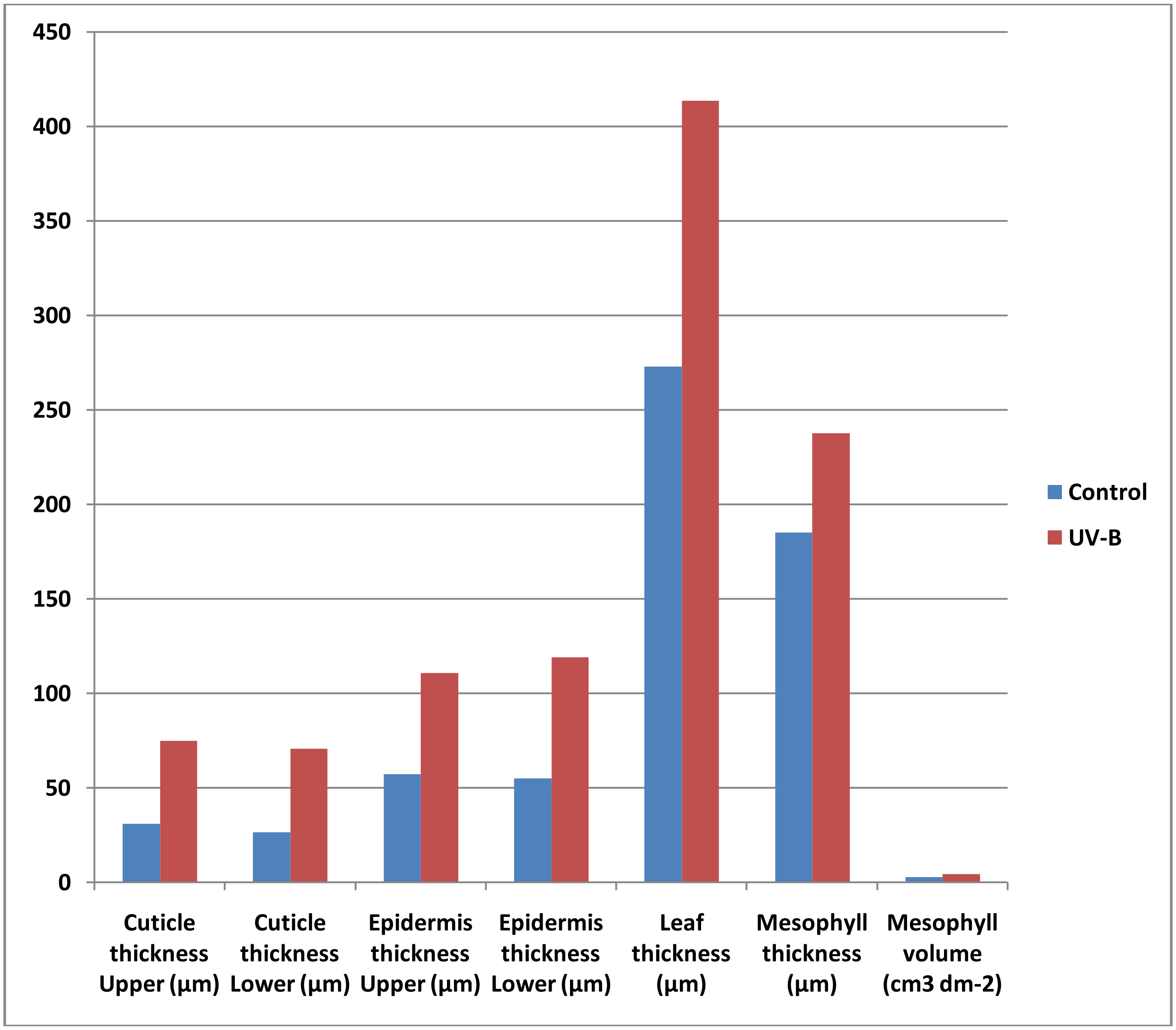

Plate 3: Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CW-122 exposed to supplementary UV-B radiation

These hairs form 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 increased trichome frequency which could have been an adaptive feature to UV-B treatment (Kokilavani and Rajendiran, 2014c) differs from the reductions observed by Karabourniotis *et al*., (1995). Dead and collapsed epidermal cells were very deeply stained and were found in more numbers on both the leaf surfaces of UV-B stressed plants (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). Size of epidermal cell (16 to 29 %) and stomatal (42 to 71 %) were decreased below normal due to UV-B irradiation (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 coastal regions without any definite pattern or orientation. Frequency of stomata (37 %) and stomatal indices were increased significantly (28 to 50 %) above control with S/E ratio on both sides showing more value (7.5 %) under UV-B exposure on the adaxial as well as abaxial surfaces (Table 1). On the contrary, pea plants responding to UV-B treatment had higher stomatal frequency on the adaxial surface

(Nogues *et al.*, 1998). 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).

Table 1: Changes in the epidermal characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CW-122 exposed to elevated UV-B radiation

Parameter		Control		UV-B	
		Adaxial	**Abaxial**	**Adaxial**	**Abaxial**
Stomatal frequency mm^{-2}		162.8±2.91	158.7±0.73	224.4±0.44	218.6±0.43
Epidermal cell frequency mm^{-2}		312.0±3.59	306.4±0.92	470.8±0.51	392.7±0.36
Stomatal index		31.30±0.93	30.74±1.03	47.18±1.21	39.37±0.62
S/E ratio		0.52	0.51	0.54	0.55
Frequency of abnormal stomata mm^{-2}		-	-	61.6±0.54	58.1±1.82
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	88.0±1.20	76.2±0.71
Frequency of trichome mm^{-2}		17.6±1.58	17.6±0.19	39.8±0.32	30.8±1.83
Stomatal size	Length (µm)	56.2±0.18	51.1±0.44	32.2±0.86	24.4±1.69
	Breadth(µm)	42.7±1.84	45.2±0.10	12.2±0.24	19.9±2.31
Epidermal cell size	Length(µm)	86.5±0.28	84.4±0.34	61.0±0.86	62.1±2.37
	Breadth(µm)	54.4±1.96	48.8±2.55	45.3±1.10	39.9±0.39
Trichome length (µm)		75.5±1.17	73.2±0.36	56.6±0.33	48.1±0.44

These results were in accordance with 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. Leaves receiving UV-B developed abnormalities like persistent stomatal initials**,** stomata with single guard cell and thickened pore and collapsed stomata (Plate 2. Figure 6, 8). Such abnormalities were not recorded in the leaves of the crops grown in normal conditions (Table 1; Plate 2. Figure 5, 7). From the results obtained it is evident that *Vigna unguiculata* (L.) Walp. cv. CW-122 responded quickly to supplementary ultraviolet-B radiation by modifying the foliar anatomy to withstand the stress.

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