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Research Article

ANATOMICAL AND EPIDERMAL ALTERATIONS IN THE LEAVES OF *VIGNA UNGUICULATA* (L.) WALP. CV. CO-6 DUE TO UV-B EXPOSURE

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

ABSTRACT
The small increase in stratospheric ozone in more than two decades was attributed to worldwide compliance with international treaties regarding the phase-out of ozone-depleting chemicals and to the upper stratospheric cooling because of increased carbon dioxide. The expected increases in ozone will be gradual, primarily because of the long residence times of CFCs and other halocarbons in the atmosphere. *Vigna unguiculata* (L.) Walp. cv. CO-6 was subjected to ultraviolet-B (UV-B) stress to study the changes in morphology, epidermis and anatomy of leaves. For the experiment, third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. CO-6 after UV-B irradiation (2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) were used. Several modifications along with few abnormalities were recorded in UV-B exposed foliage. UV-B altered the leaf size and appearances which were small, shiny and thick compared to broader, longer and thinner leaves of unstressed plants. Frequency of stomata in UV-B was increased by 29.41 and 24.93 % over control on adaxial and abaxial surfaces respectively. Same impact was recorded in stomatal indices of stressed plants which increased by 22.70 to 32.50 % on both sides. Abnormal stomata with single guard cell occurred with dead and collapsed epidermal cells on the adaxial side of UV-B irradiated leaves. Control foliage did not show any aberrant stomata. Shorter (26.19 % to 33.59 %) and brittle trichomes were recorded in UV-B treated leaves in contrast to healthier and longer trichomes in control. Frequency of trichome was increased by two times on both surfaces in UV-B exposed plants. Same trend continued in cuticle and epidermis as they were also twice thicker after UV-B irradiation. Mesophyll volume and thickness increased by 52.79 % and 38.23 % respectively resulting in thicker leaves (77.41 %) under UV-B stress.

Keywords: *Ultraviolet-B, Cowpea, Variety CO-6, Leaf Morphology, Leaf Epidermis, Leaf Anatomy, Abnormal Stomata*

INTRODUCTION
Scientific evidence indicates that stratospheric ozone is being destroyed by man-made chemicals, containing chlorine or bromine called "ozone-depleting substances" (ODS). Stability of ODS allows them to float up, intact, to the stratosphere where they are broken apart by the intense ultraviolet light, releasing chlorine and bromine. Chlorine and bromine demolish ozone at an alarming rate, by stripping an atom from the ozone molecule. A single molecule of chlorine can break apart thousands of molecules of ozone. The drop in ozone concentration increases UV-B rays affecting plants, animals including human beings and the ecosystems. Ultraviolet-B (UV-B) radiation (280-320 nm) is an important atmospheric stress and is detrimental to plant growth 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 crop plants. Foliar epidermis constitutes a dynamic barrier between the plant's internal and external environment. To serve this purpose it is impregnated with waxes and cutins on the exterior with stomata to regulate gaseous exchange. Leaves also possess appendages like trichomes, hydathodes and scales. Leaves receive major proportion of the 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 present study was carried out to document the response of *Vigna unguiculata* (L.) Walp. cv. CO-6 leaves to UV-B stress.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. CO-6 obtained from Saravanan Farms, Villupuram, Tamil Nadu, 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} equivalent to a 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. CO-6 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 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 exposure reduced the leaf size of *Vigna unguiculata* (L.) Walp. cv. CO-6 which appeared wrinkled, shiny and brittle with chlorotic and necrotic lesions over the adaxial surface (Plate 1; Plate 2: Figure 1 to 2). Leaves from control crops 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. Epidermal cells had dense, deeply stained nuclei both under control and UV-B stressed leaves. Epidermal cell were more in number (22.76 to 32.50 %) over control in UV-B exposed leaves on both the surfaces, the highest occurring on adaxial surface (Table 1). UV-B exposed leaves, on both the surfaces, developed cuticle and epidermis which were two times thicker than control (Plate 3). Double thickness of epidermis was due to multilayer formation on adaxial surface in UV-B irradiated leaves (Plate 2: Figure 3; Plate 3). Same trend continued in leaf thickness (77.41 %), mesophyll thickness (38.23 %) and mesophyll volume (52.79 %) under UV-B stress (Plate 3). Wellmann (1976), Caldwell *et al.*, (1983), Bornman and Vogelmann (1991) and (Rajendiran 2001) suggested 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. Thickness of leaves increased in *Medicago sativa* due to addition of spongy mesophyll cells, while 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) reported 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. Unicellular trichomes present in the costal as well as intercostal regions of both the surfaces were comparatively less in number on the abaxial side than the adaxial side (Table 1). UV-B exposure enhanced the number of trichomes on both the sides over the control (Table 1). Trichomes were smaller by 26.19 % on adaxial side and by 33.59 % 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).

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 was in accordance with the report of Kokilavani and Rajendiran (2014c) which differed from the reductions observed by Karabourniotis *et al.*, (1995). Dead and collapsed epidermal cells took very deep stain and were found in more numbers on both the leaf surfaces of UV-B stressed plants (Table 1; Plate 2: Figure 6, 8).

Collapsed epidermal cells and glazed leaf surfaces with signs of bronzing of tissue have been attributed to oxidised phenolic compounds (Cline and Salisbury, 1966) followed by tissue degradation (Caldwell, 1971). Epidermal cell (12.33 to 29.69 %) and stomata (22.93 to 47.84 %) were smaller in size 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 costal regions without any definite pattern or orientation. Frequency of stomata (29.41 %) and stomatal indices were increased significantly (22.70 to 32.40 %) above control with S/E ratio on adaxial side showing a low value (1.58 %) and on abaxial side showing more value (1.69 %) under UV-B exposure on the adaxial as well as abaxial surfaces (Table 1).

Table 1: Changes in the epidermal characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CO-6 exposed to elevated UV-B radiation

Parameter		Control		UV-B	
		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency (mm^{-2})		224.4±4.36	219.8±0.15	290.4±0.69	274.6±1.62
Epidermal cell frequency (mm^{-2})		352.0±3.47	368.1±0.55	466.4±1.39	451.9±0.62
Stomatal index		35.30±0.32	36.91±1.36	46.74±1.89	45.29±1.07
S/E ratio		0.63	0.59	0.62	0.60
Frequency of abnormal stomata (mm^{-2})		-	-	52.8±1.53	48.1±0.12
Frequency of dead/collapsed epidermal cells (mm^{-2})		-	-	86.9±0.87	80.2±2.72
Frequency of trichome mm^{-2}		14.9±0.73	13.2±1.47	36.4±0.85	30.8±0.25
Stomatal size	Length (µm)	42.2±1.30	38.8±2.65	31.0±0.41	29.9±0.24
	Breadth (µm)	25.5±0.92	17.8±1.47	13.3±1.67	13.3±0.36
Epidermal cell size	Length (µm)	74.6±1.26	78.8±0.44	65.4±1.58	55.4±1.71
	Breadth (µm)	47.2±5.01	45.4±0.83	44.4±1.50	41.3±0.39
Trichome length (µm)		79.4±1.24	76.2±0.57	58.6±1.47	50.6±1.12

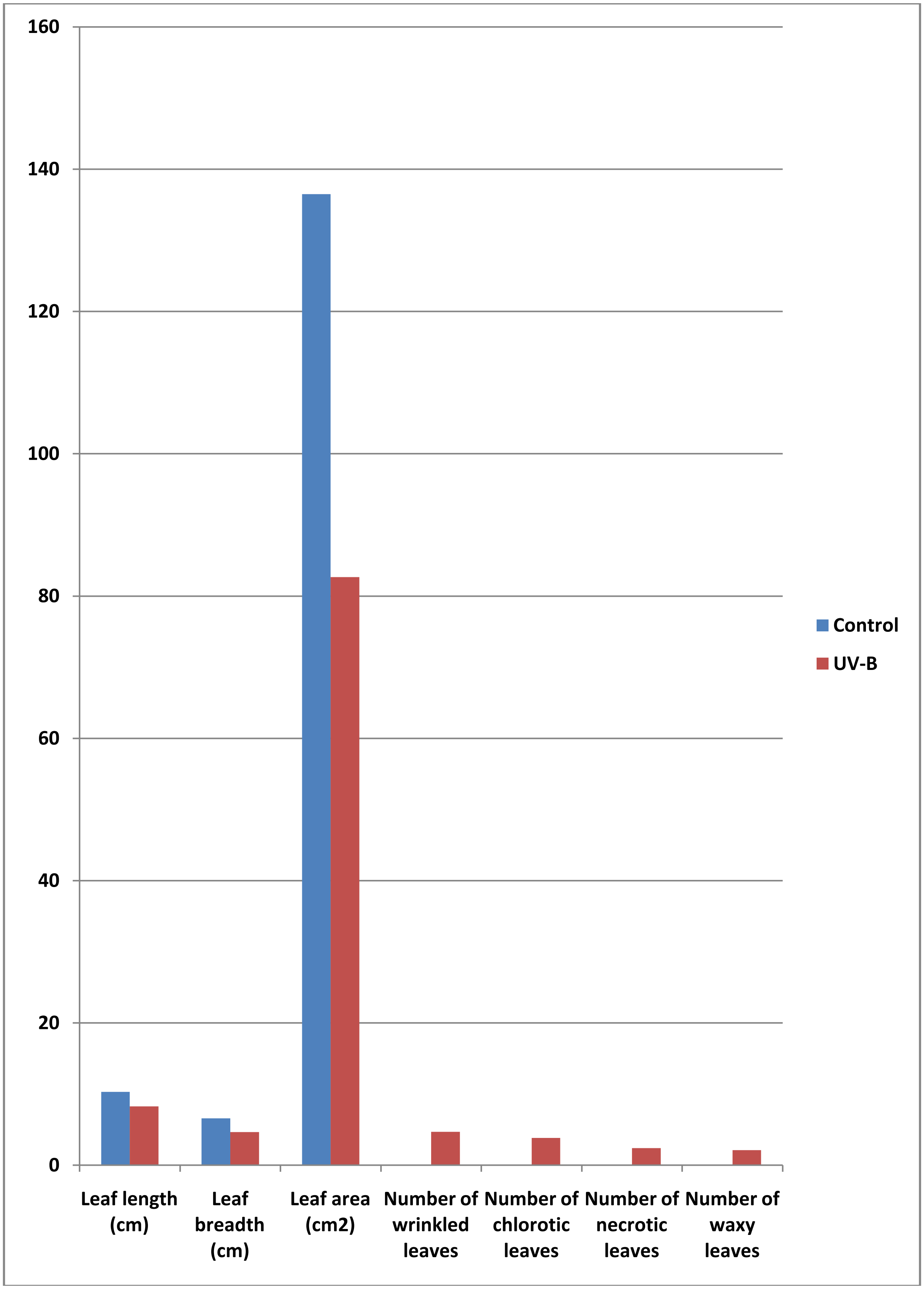

Plate 1: Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CO-6 exposed to supplementary UV-B radiation

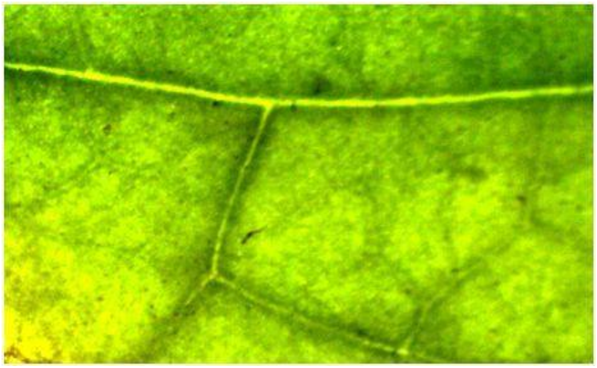

Figure 1: Shiny adaxial surface under UV-B

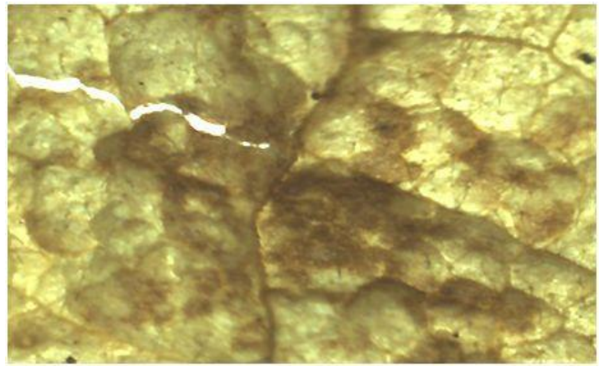

Figure 2: UV-B adaxial - Brittle and dead

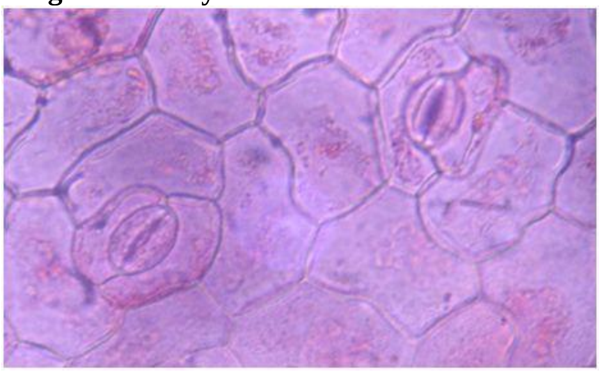

Figure 3: Control adaxial - Normal stomata

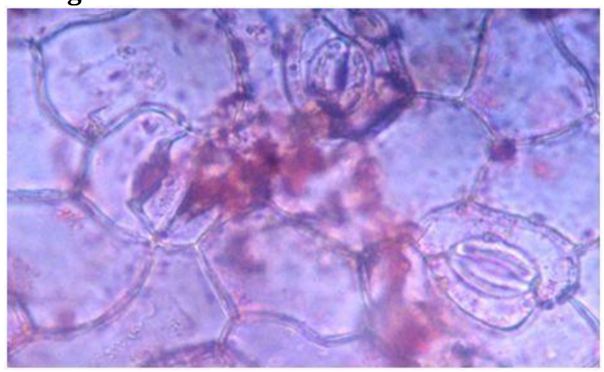

Figure 4: UV-B adaxial - Abnormal stomata

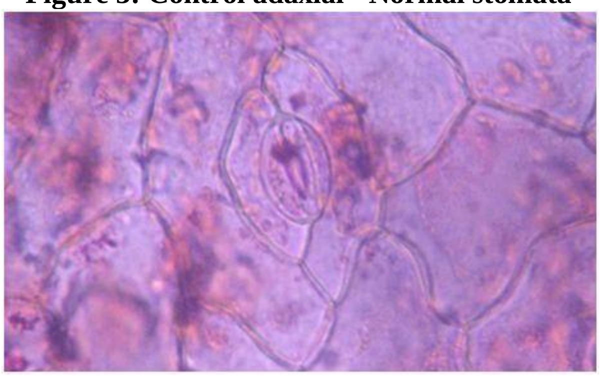

Figure 5: Control abaxial - Normal stomata

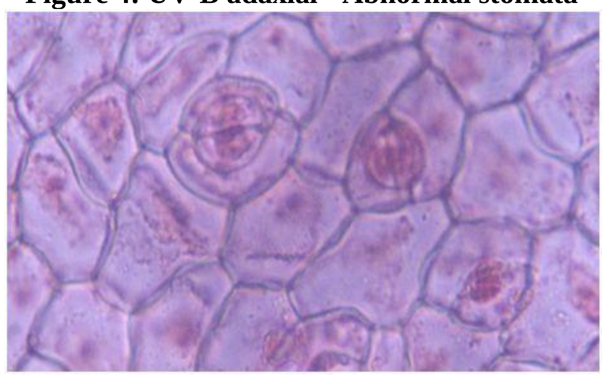

Figure 6: UV-B abaxial - Abnormal stomata

Figure 7: UV-B adaxial - Multiseriate epidermis

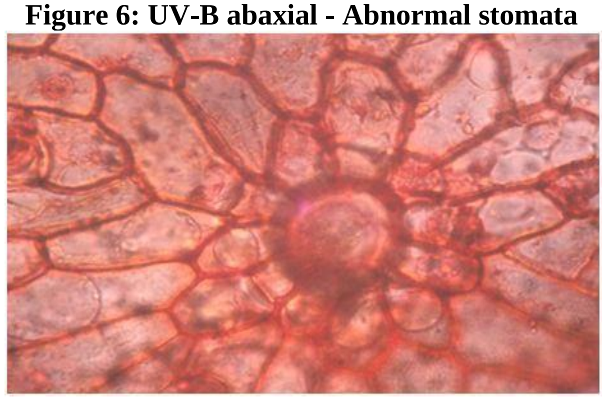

Figure 8: UV-B adaxial - Broken trichome

Plate 2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. CO-6 under control condition and supplementary UV-B radiation exposure (Figure 3 to 8: 400 x)

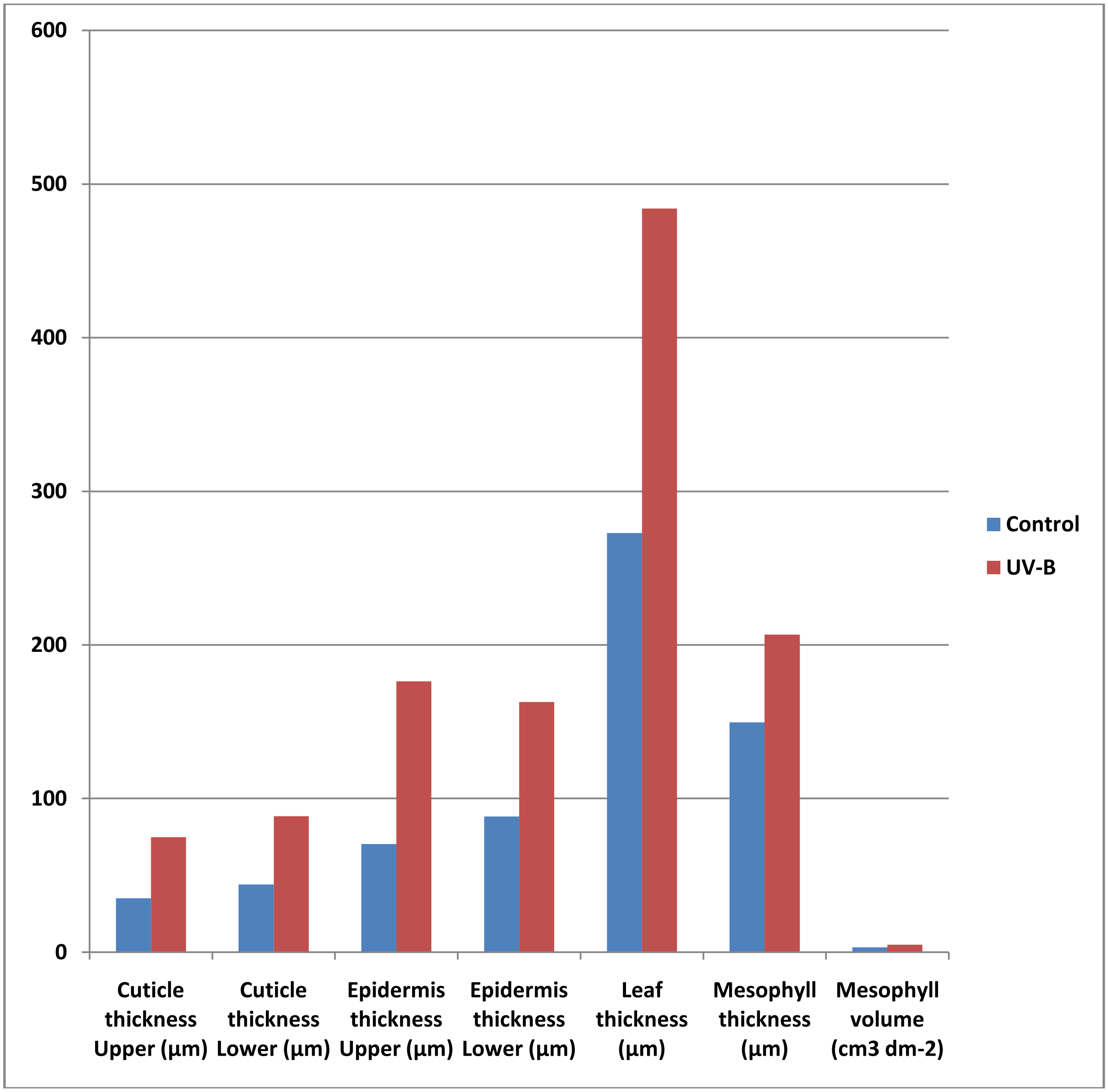

Plate 3: Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CO-6 exposed to supplementary UV-B radiation

Nogues *et al.*, (1998) reported that pea plants responding to UV-B treatment had higher stomatal frequency on the adaxial surface. Stomata were smaller than control on both surfaces of the foliage after UV-B irradiation and the maximum abnormalities were recorded on the adaxial surface (Table 1; Plate 2: Figure 6, 8). 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). 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) have reported similar stomatal abnormalities on the adaxial side of UV-B irradiated leaves. Such abnormalities were not recorded in the leaves of the crops grown in control environment (Table 1; Plate 2: Figure 5, 7). *Vigna unguiculata* (L.) Walp. cv. CO-6 leaves under UV-B impact developed maximum alterations in morphology and anatomy to adapt to the stress.

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