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VARIATIONS IN FOLIAR MORPHOLOGY AND ANATOMY OF *VIGNA UNGUICULATA* (L.) WALP. CV. CO-3 AFTER SUPPLEMENTARY ULTRAVIOLET-B EXPOSURE

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ABSTRACT

The present study was an attempt to record the effects of ultraviolet-B (UV-B) radiation in the morphology, epidermis and the anatomy of *Vigna unguiculata* (L.) Walp. cv. CO-3 leaf. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. CO-3 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. The epidermis on the adaxial surface revealed varying trends under UV-B exposure. The cuticle on the adaxial epidermis was three times thicker under UV-B radiation and the mesophylls by 44 % compared to control plants. The mesophyll volume in UV-B stressed leaves also increased one and a half times more than normal plants, thus making the leaves twice thicker. Shorter and brittle trichomes on adaxial (23%) and abaxial surfaces (19%) were predominant in UV-B treated leaves which were longer and healthier in control. However the trichome frequency was increased by 55.55% on adaxial and by 60.79 % on abaxial surfaces in UV-B stressed plants. The leaves of crops in elevated UV-B environment were small, necrotic, and shiny compared to broader, longer and greener leaves of control plants. The stomatal frequency under UV-B was increased by 45.45 % on adaxial surface which continued by an increase of 73.14 % on the abaxial surface. The stomatal indices also showed rapid increases on adaxial (28%) as well as on abaxial (39%) surfaces of stressed crop compared to control. However, the stressed leaves had smaller stomata on both surfaces (23 to 60%). Similar trend of smaller epidermal cells (24 to 35%) and increased cell frequencies (28 to 39%) were recorded on both the surfaces of UV-B exposed leaves. Abnormal stomata like, stomata with single guard cell, reduced size, malformations were more along with dead epidermal cells on the adaxial surface of UV-B irradiated plants. Such aberrations were absent in leaves under control conditions. CO-3 variety of cowpea modified the foliar architecture to reduce UV-B penetration.

KEY WORDS: Ultraviolet-B, cowpea, leaf morphology, leaf epidermis, leaf anatomy, abnormal stomata.

INTRODUCTION

The depletion of ozone layer has become an invincible environmental problem in the recent past. 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 Sudha and Rajendiran 2013a, Sudaroli Sudha and Rajendiran 2013b, Arulmozhi and Rajendiran 2014, Vijayalakshmi and Rajendiran 2014) in several crops. A part of every landscape and the basis of the terrestrial food chain, plants are excellent indicators of changing environments. They possess leaves as the above-ground plant organs, made up of a collection of tissues in a regular organization. They are designed to carry out vital functions *viz.* photosynthesis and exchange of gases and transpiration based on the habitat the plants survive. Leaves are the organs that receive major proportion of the ultraviolet radiation and hence always act immediately to block its entry into the internal organs (Bornman and Vogelmann 1991, Rajendiran and Ramanujam 2000, Kokilavani and Rajendiran 2013). The present study estimates the extent of variations in leaf architecture in *Vigna unguiculata* (L.) Walp. cv. CO-3 in response to UV-B radiation.

MATERIALS & METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. CO-3 obtained from Tamil Nadu Agriculture University, were grown in pot culture in the naturally lit greenhouse (day temperature maximum 38± 2ºC, night temperature minimum 18 ± 2 ºC, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 μmol $m^{-2}s^{-1}$, photoperiod 12 to 14h). 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-3 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 & DISCUSSION

Suffering under UV-B irradiation, the leaves of *Vigna unguiculata* (L.) Walp. cv. CO-3 were small, wrinkled, chlorotic, necrotic, shiny and brittle (Plate 1; Plate 2. Fig. 1 to 2). The costal cells of normal leaves are uniformly similar and 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). The epidermal cells both on abaxial and adaxial surfaces are sinuous and thin walled with unicellular trichomes occurring intermittently in intercostals region. The epidermal cells with dense, deeply stained nuclei were observed in control and in all the UV-B irradiated leaves (Plate 2. Fig. 3 to 6). Epidermal cell frequency was higher (28 to 29 %) over control in UV-B exposed leaves on both the surfaces (Table 1). The thickness of cuticles and the epidermis in UV-B exposed leaves, on both sides, increased significantly over control. However the cuticle and multilayered epidermis were three times thicker on adaxial surface of stressed leaves (Plate 2. Fig. 3; Plate 3). Similar trend expressed in cuticle and epidermis thickness continued in leaf thickness, mesophyll thickness and volume also (Plate 3). 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).

TABLE 1. Changes in the epidermal characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CO-3 exposed to elevated UV-B radiation.

		Control		UV-B	
Parameter		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency (mm^{-2})		167.2±0.27	149.3±1.59	243.2±2.46	258.5±0.53
Epidermal cell frequency (mm^{-2})		334.3±0.54	319.0±2.03	428.0±0.23	444.1±0.67
Stomatal index		33.53±0.17	32.00±3.03	42.90±0.26	44.51±1.67
S/E ratio		0.50	0.46	0.56	0.58
Frequency of abnormal stomata (mm^{-2})		-	-	47.8±2.04	41.6±0.28
Frequency of dead/collapsed epidermal cells (mm^{-2})		-	-	84.8±0.36	80.5±1.17
Frequency of trichome (mm^{-2})		19.8±0.63	17.6±0.12	30.8±1.26	28.3±1.37
Stomatal size	Length (µm)	42.2±1.71	35.5±1.58	16.6±0.88	18.9±0.79
	Breadth (µm)	34.4±3.02	17.8±0.42	14.4±0.63	13.7±0.92
Epidermal cell size	Length (µm)	56.2±1.63	56.5±0.68	36.6±2.58	36.6±0.44
	Breadth (µm)	47.1±0.30	50.1±0.29	35.5±1.67	36.2±1.17
Trichome length (µm)		81.1±1.57	83.6±1.24	62.2±1.10	67.7±0.38

PLATE 1: Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. CO-3 exposed to supplementary UV-B radiation.

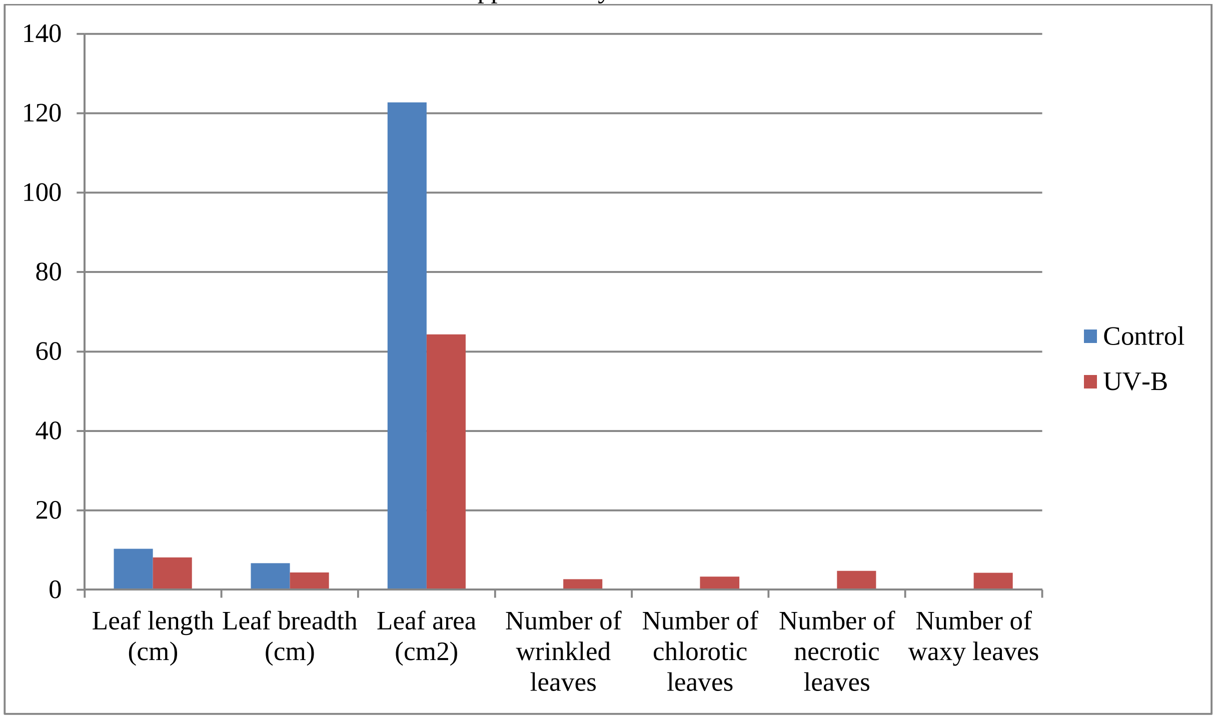


PLATE 2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. CO-3 under control condition and supplementary UV-B radiation exposure. (Fig. 3 to 8: 400 x)

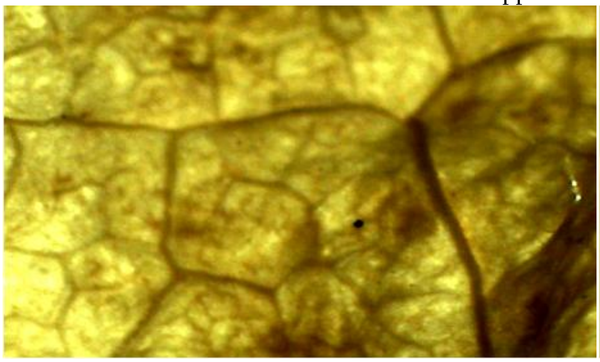


FIGURE 1: Shiny adaxial surface under UV-B

FIGURE 2: UV-B adaxial - Brittle and dead

FIGURE 3: UV-B adaxial - Multiseriate epidermis

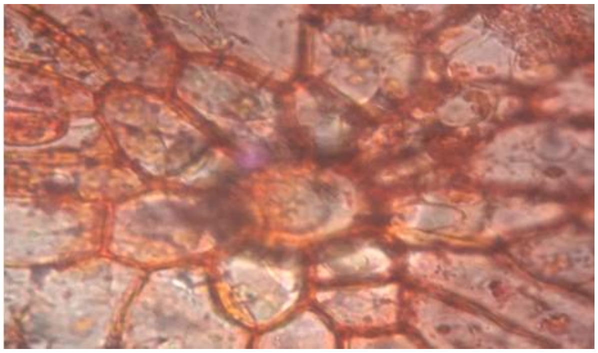


FIGURE 4: UV-B adaxial - Broken trichome

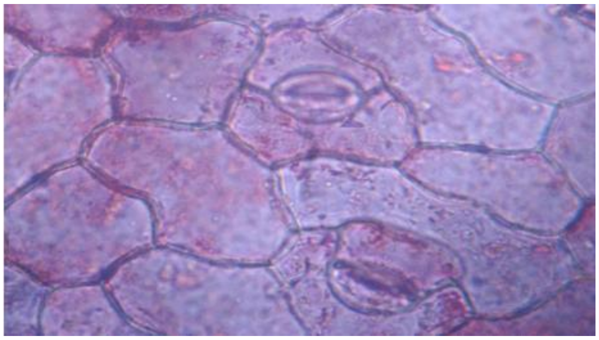


FIGURE 5: Control adaxial - Normal stomata

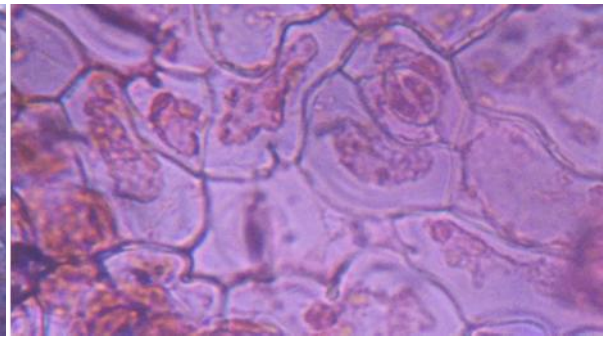


FIGURE 6: UV-B adaxial - Abnormal stomata

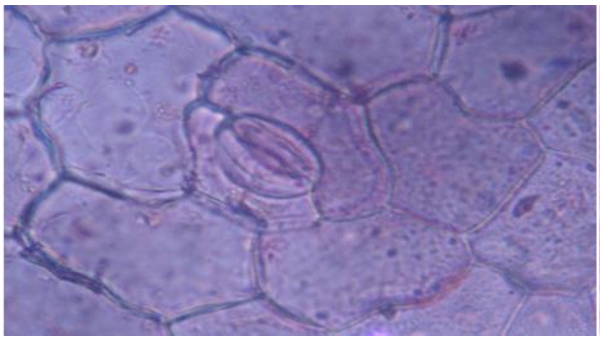


FIGURE 7: Control abaxial - Normal stomata

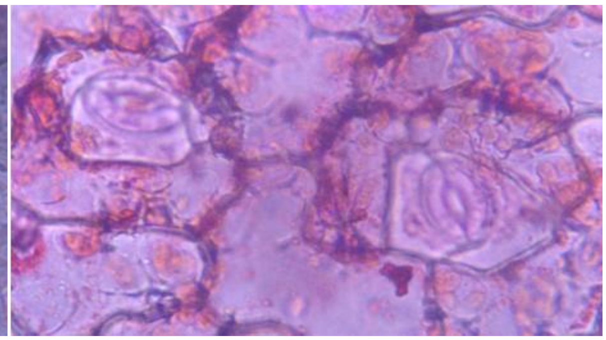
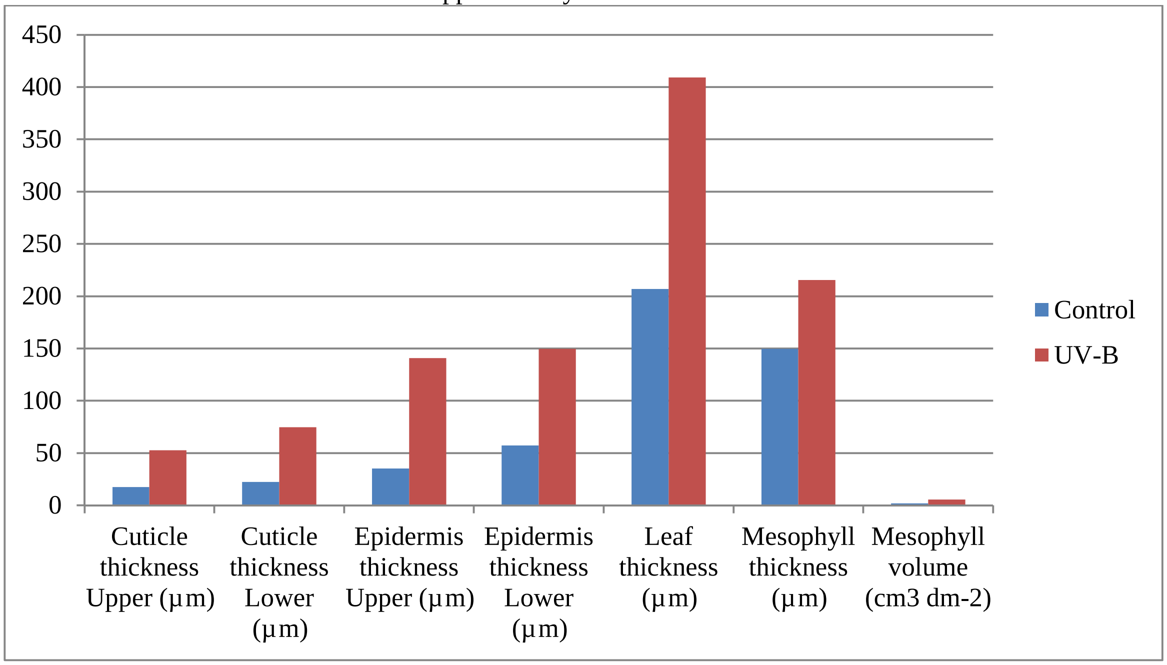


FIGURE 8: UV-B abaxial - Abnormal stomata

Bornman and Vogelmann (1991), Kokilavani and Rajendiran (2013), Kokilavani *et al.* (2013) and 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. In normal leaves the trichome frequency was comparatively more on the abaxial side than on the adaxial side. Trichome frequencies under UV-B exposure increased on adaxial (55.55 %) as well as on abaxial (60.79 %) surfaces compared to control leave (Table 1). Trichomes were broken and shorter (24.62 %) on the adaxial side of UV-B irradiated leaves (Table 1; Plate 2. Fig. 4). The trichomes act as mechanical barriers against biotic stress (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, 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), differ from the reductions observed by Karabourniotis *et al*. (1995). Very deeply stained dead, collapsed epidermal cells were frequently found only on both the leaf surfaces of UV-B stressed crops (Table 1; Plate 2. Fig. 6, 8). Adaxial epidermis showed damages in the form of collapsed cells and the leaves became glazed and showed signs of bronzing of tissue surfaces which have been attributed to oxidised phenolic compounds (Cline and Salisbury, 1966). Caldwell (1971) opined that this may in some cases also be followed by tissue degradation. The epidermal cell (24 to 35 %) and stomata (23 to 60 %) were smaller after UV-B irradiation (Table 1; Plate 2. Fig. 6, 8). The leaves are amphistomatic and the stomata are oval in outline and distributed all over the surface except over costal regions without any definite pattern or orientation. Mature stomata were mostly diacytic and paracytic. Stomatal frequency (45 to 73 %) and stomatal indices showed significant increases (28 to 29 %) over control with S/E ratio exhibiting very high value (12 to 26 %) under UV-B exposure on both the 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). 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. Fig. 6, 8). 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 in UV-B sensitive plants. UV-B irradiated leaves developed abnormalities on both sides, like persistent stomatal initials**,** stomata with single guard cell and thickened pore and collapsed stomata (Table 1; Plate 2. Fig. 6, 8). Leaves of cowpea crops cultivated under control conditions did not show any stomatal abnormalities (Table 1; Plate 2. Fig. 5, 7). The consequences of climatic change on leaf architecture as observed in *Vigna unguiculata* (L.) Walp. cv. CO-3 are immediate but temporary, however they can remain irreversible under prolonged UV-B exposure.

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



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