INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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CHANGES IN LEAF ARCHITECTURE OF *VIGNA UNGUICULATA* (L.) WALP. CV. BCP-25 AFTER EXPOSURE TO ELEVATED ULTRAVIOLET-B RADIATION

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ABSTRACT
Present study is an attempt to assess the effects of ultraviolet-B (UV-B) radiation in the morphology, epidermis and the anatomy of *Vigna unguiculata* (L.) Walp. cv. BCP-25 leaf. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. BCP-25 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 epidermal characteristics exhibited varying trends in Ultraviolet-B treatments. The cuticles on the adaxial epidermis and the mesophylls were thicker under UV-B radiation by 40.22 and 14.65 % respectively compared to control plants. The mesophyll volume in UV-B stressed leaves also increased by 14.97 % making the leaves thicker by 40.83 % more than that of normal plants. The trichomes were longer by 7.26 % on adaxial and by 2.55 % 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 30.81 % on adaxial and by 32.54 % 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 reduced by 19.72 % on adaxial surface which was compensated by an increase of 40.86 % on the abaxial surface. Similar trend was seen in the stomatal indices which showed rapid decline on adaxial (25.13 %) but an increase on abaxial (70.76 %) surfaces of stressed crop compared to control. 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. The UV-B stressed cowpea plants modified the foliar architecture to ameliorate the impact for survival.

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

INTRODUCTION

The depletion of ozone layer has become an insurmountable environmental problem in the recent past. It threatens to continue so as the green house gases around the globe 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 in this layer enhances ozone depletion. 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 Sudha and Rajendiran 2013a, Sudaroli Sudha 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. Leaves are the organs that 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 records the modifications in leaf architecture developed in *Vigna unguiculata* (L.) Walp. cv. BCP-25 to survive under UV-B stress.

MATERIALS & METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. BCP-25 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 ($UV\text{-}B_{BE}$) of 12.2 kJ m^{-2} d^{-1} equivalents 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. BCP-25 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 & DISCUSSION

The leaves of *Vigna unguiculata* (L.) Walp. cv. BCP-25 was small, wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface due to UV-B irradiation (Plate 1; Plate 2. Fig. 1 to 4). On the adaxial surface of normal leaves the costal cells are uniformly similar in being axially elongated, thin and straight walled and have unicellular thin walled trichomes. The costal cells and trichomes on adaxial surface differ from abaxial surface in being shorter in length (Table 1).

TABLE 1. Changes in the epidermal characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. BCP-25 exposed to elevated UV-B radiation.

Parameter		Control		UV-B	
		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency mm^{-2}		167.24 ± 0.21	112.34 ± 0.23	134.25 ± 0.32	158.25 ± 0.27
Epidermal cell frequency mm^{-2}		136.25 ± 0.23	153.45 ± 0.25	237.14 ± 0.29	198.27 ± 0.21
Stomatal index		56.38 ± 0.32	43.27 ± 0.42	42.21 ± 1.32	52.21 ± 1.21
S/E ratio		1.22	0.73	0.56	0.80
Frequency of abnormal stomata mm^{-2}		-	-	28.74 ± 0.21	9.66 ± 0.23
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	23.43 ± 0.36	-
Frequency of trichome mm^{-2}		19.73 ± 0.22	12.32 ± 0.26	25.81 ± 0.12	16.33 ± 0.19
Stomatal size	Length (µm)	23.58 ± 0.17	22.46 ± 0.15	19.12 ± 0.21	21.24 ± 0.19
	Breadth(µm)	20.18 ± 0.16	13.32 ± 0.19	10.13 ± 0.23	13.47 ± 0.15
Epidermal cell size	Length(µm)	43.18 ± 0.14	73.11 ± 0.29	32.18 ± 0.23	56.71 ± 0.37
	Breadth(µm)	25.58 ± 0.24	22.15 ± 0.13	23.24 ± 0.17	18.78 ± 0.21
Trichome length (µm)		278.55 ± 6.83	264.53 ± 10.32	298.76 ±7.34	271.28 ±7.86

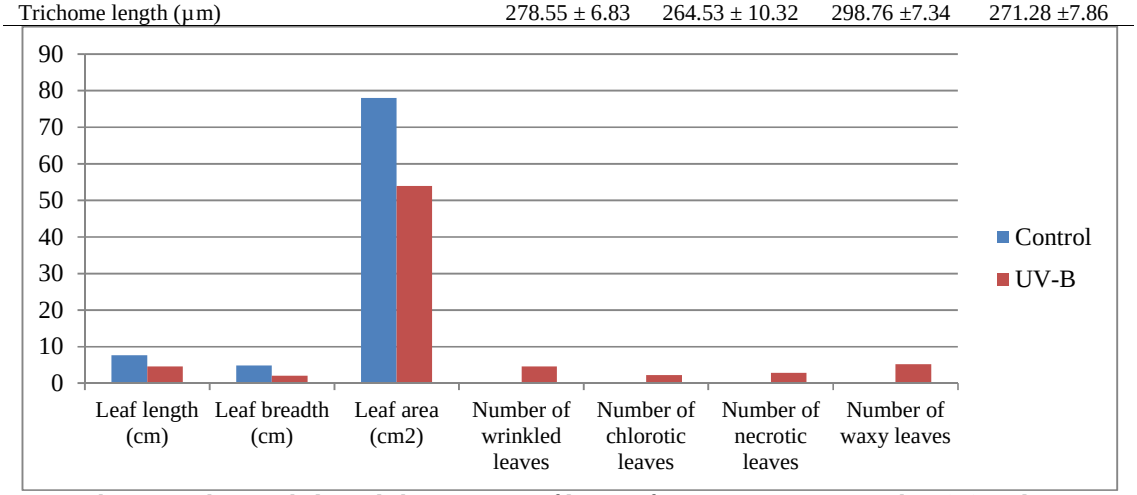

PLATE 1. Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. BCP-25 exposed to supplementary UV-B radiation

The intercostal epidermal cells both on abaxial and adaxial surfaces are sinuous and thin walled with unicellular trichomes occurring intermittently. The epidermal cells with dense, deeply stained nuclei were observed in control and in all the UV-B irradiated leaves (Plate 2. Fig. 5, 6). Epidermal cell frequency was higher (74.04 %) over control in UV-B exposed leaves but the effect was subdued on the abaxial side compared to adaxial surface (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 more thickened on adaxial surface of stressed leaves by 40.22 % and 46.48 % respectively over normal leaves (Table 2; Plate 2. Fig. 7).

TABLE 2. Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. BCP-25 exposed to elevated UV-B radiation.

Parameter		Control	UV-B
Cuticle	Adaxial (µm)	8.75 ± 0.23	12.27 ± 0.32
thickness	Abaxial (µm)	7.84 ± 0.21	10.34 ± 0.27
Epidermis thickness	Adaxial (µm)	17.23 ± 0.26	25.24 ± 0.46
	Abaxial (µm)	16.87 ± 1.32	19.46 ± 0.78
Leaf thickness (µm)		196.20 ± 2.48	276.31 ± 2.21
Mesophyll thickness (µm)		165.51 ± 2.43	189.76 ± 2.47
Mesophyll volume (cm^3 dm^{-2})		1.87 ± 0.23	2.15 ± 0.32

PLATE 2. Epidermal and anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. BCP-25 under control condition and supplementary UV-B radiation exposure. Ada: Adaxial surface, Aba: Abaxial surface (Fig. 2, 3, 5 to 8: 400 x)

FIGURE 1: Control and UV-B stressed leaves

FIGURE 2: Shiny adaxial surface under UV-B

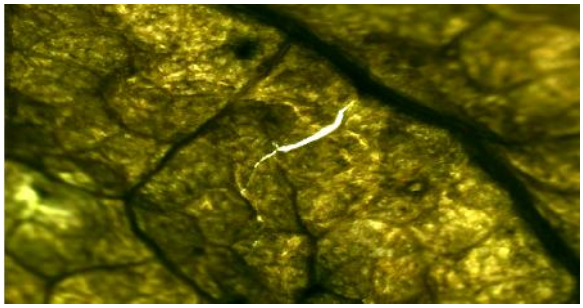

FIGURE 3: UV-B adaxial - Brittle and dead

FIGURE 4: UV-B - Chlorosis and necrosis

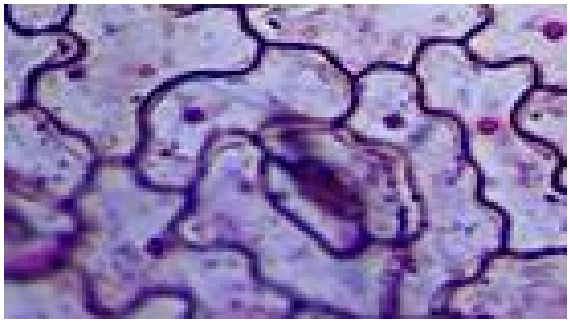

FIGURE 5: Control adaxial - Normal stomata

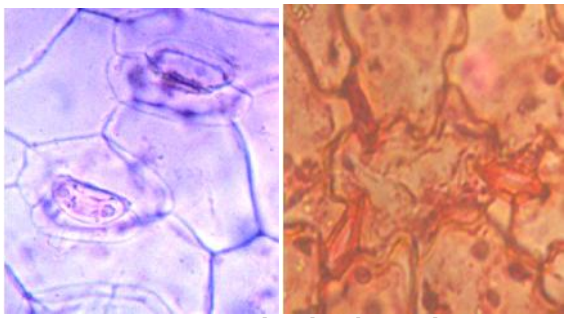

FIGURE 6: UV-B adaxial - Abnormal stomata

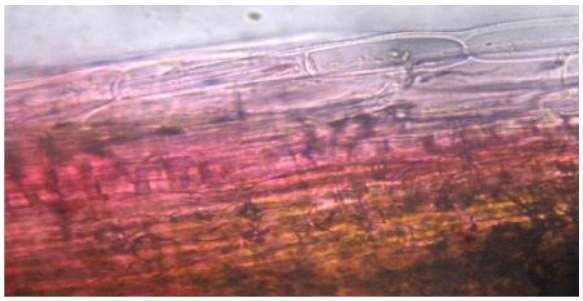

FIGURE 7: UV-B adaxial - Multilayered epidermis

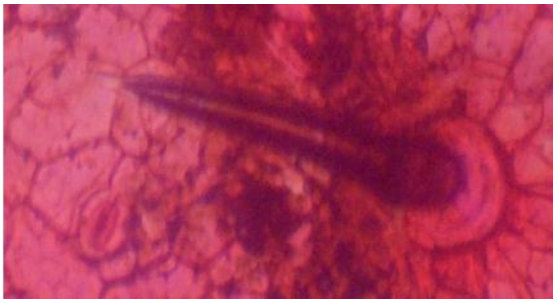

FIGURE 8: UV-B Ada - Longer trichome

Similar trend expressed in cuticle and epidermis thickness continued in leaf thickness, mesophyll thickness and volume also (Table 2). With the mesophyll becoming voluminous, a thicker leaf would result (Rajendiran 2001). 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). Bornman and Vogelmann (1991), Kokilavani and Rajendiran (2013) and Kokilavani *et al.* (2013) 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. Unicellular trichomes were present in the costal as well as intercostal regions of both the surfaces, and their frequency was comparatively less on the abaxial side than the adaxial side. A trichome frequency under UV-B exposure increased on adaxial (30.81 %) as well as on abaxial (32.54 %) surfaces compared to control leaves (Table 1). Longer trichomes (7.26 %) along with broken ones were observed more on the adaxial side of UV-B irradiated leaves (Table 1; Plate 2. Fig. 8). However, the length of trichomes (2.55 %) on the abaxial surface of stressed leaves was only slightly increased over contol (Table 1). The trichomes serve several functions as a mechanical barrier against biotic attack (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). The increased trichome frequency which could have been an adaptive feature to UV-B treatment is at variance from the reductions observed by Karabourniotis *et al*. (1995). Very deeply stained dead, collapsed epidermal cells (23.43 %) and glands were found in large numbers only on the adaxial leaf surface of UV-B stressed plants (Table 1; Plate 2. Fig. 6). 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). This may in some cases also be followed by tissue degradation (Caldwell 1971). The epidermal cell (9.14 % to 25.47 %) and stomata (18.91 % to 49.80 %) were smaller after UV-B irradiation (Table 1; Plate 2. Fig. 5, 6). 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 (19.72 %) and stomatal indices were reduced significantly (25.13 %) below control with S/E ratio exhibiting very low value (54.09 %) under UV-B exposure on the adaxial side, while they recorded an increase over control on the abaxial surface (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. 5, 6). Similar results were reported by Wright and Murphy (1982), Kokilavani and Rajendiran (2013), Kokilavani *et al.* (2013) and Kokilavani *et al.* (2014). on the adaxial side of the leaves after exposure to UV-B radiation. UV-B irradiated leaves developed abnormalities like persistent stomatal initials**,** stomata with single guard cell and thickened pore and collapsed stomata (Table 1; Plate 2. Fig. 6). No such abnormalities were recorded in the leaves of the crops grown in control conditions (Table 1; Plate 2. Fig. 5). The changes in architecture in the leaves of *Vigna unguiculata* (L.) Walp. cv. BCP-25 were to offer additional mechanical barrier to UV-B penetration, as the leaves responded quickly to UV-B stress by reducing its size and area, increasing the thickness of cuticle, number of upper epidermal layers, epicuticular wax deposition, frequency of trichomes and the volume of the internal organ system.

ACKNOWLEDGEMENT

The authors thank Prof. Dr. V. Jayachandiran, Head, Department of Botany, KMCPGS, Puducherry for providing research facilities.

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