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STUDY OF LEAF ARCHITECTURE IN *VIGNA UNGUICULATA* (L.) WALP. CV. PUDUVAI UNDER ELEVATED ULTRAVIOLET-B RADIATION

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ABSTRACT

The present study aims at assessing the effects of ultraviolet-B (UV-B) radiation in the morphology, epidermis and the anatomy of *Vigna unguiculata* (L.) Walp. cv. PUDUVAI leaf. Fully developed third trifoliate leaves from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. PUDUVAI under 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 stress induced changes in the leaf morphology and caused several injuries which were not recorded in normal plants. The epidermis both on the adaxial as well as abaxial surfaces exhibited many changes after UV-B exposure. The cuticle on both the surfaces was two to three times thicker under UV-B irradiation, followed by mesophyll which was thicker by 38.70 % compared to control leaves. UV-B stressed leaves had mesophyll volume increased by two and a half times more than normal plants, thus making the leaves thicker by 85.71 %. UV-B treated leaves possessed shorter and brittle trichomes on adaxial (26 %) and abaxial surfaces (30 %) compared with longer and healthier ones of control. However, the trichome frequency showed manifold increase on both surfaces in UV-B stressed leaves. UV-B irradiated plants had small, wrinkled, necrotic and shiny leaves compared to longer, broader and greener leaves of control plants. The stomatal frequency under UV-B was increased by 60 % on adaxial and by 96 % on the abaxial surface. Stomatal indices also showed increases on adaxial (28.11 %) and on abaxial (29.24 %) surfaces of irradiated crop compared to control. However, the stressed leaves had smaller stomata on both surfaces (20 to 45 %). Similar trend of smaller epidermal cells (15 to 32 %) and increased cell frequencies (28 to 29 %) 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. PUDUVAI variety of cowpea in response to ultraviolet-B irradiation modified the leaf architecture in order to alleviate the stress.

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

INTRODUCTION

The response of leaves to the forecasted increase in climate stress occurrence is considered to be the key issue in global climate change. Although leaf productivity increased in most ecosystems during the past century, several studies underlined an emerging trend of heat and drought induced decline and dieback at global scale. Generally combined physical and biological causes contribute to observed decline in foliage productivity. The green house gases accumulating around the earth due to human activity, increases in thickness day by day and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, there by cooling the stratosphere. Colder than normal temperatures act to deplete ozone layer, allowing enormous ultraviolet-B (UV-B) radiation into earth's surface, affecting the plants in several ecosystems. 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. An elevation in the flux of ultraviolet-B (UV-B) radiation (280-320 nm) due to ozone depletion is detrimental to plant growth and development and 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 crops. As leaves are the organs that receive major amount of UV-B radiation, they react quickly to prevent its entry into the internal organs (Bornman and Vogelmann 1991, Rajendiran and Ramanujam 2000, Kokilavani and Rajendiran 2013). The present study reports the variations brought about by ultraviolet-B rays in the leaves of *Vigna unguiculata* (L.) Walp. cv. PUDUVAI.

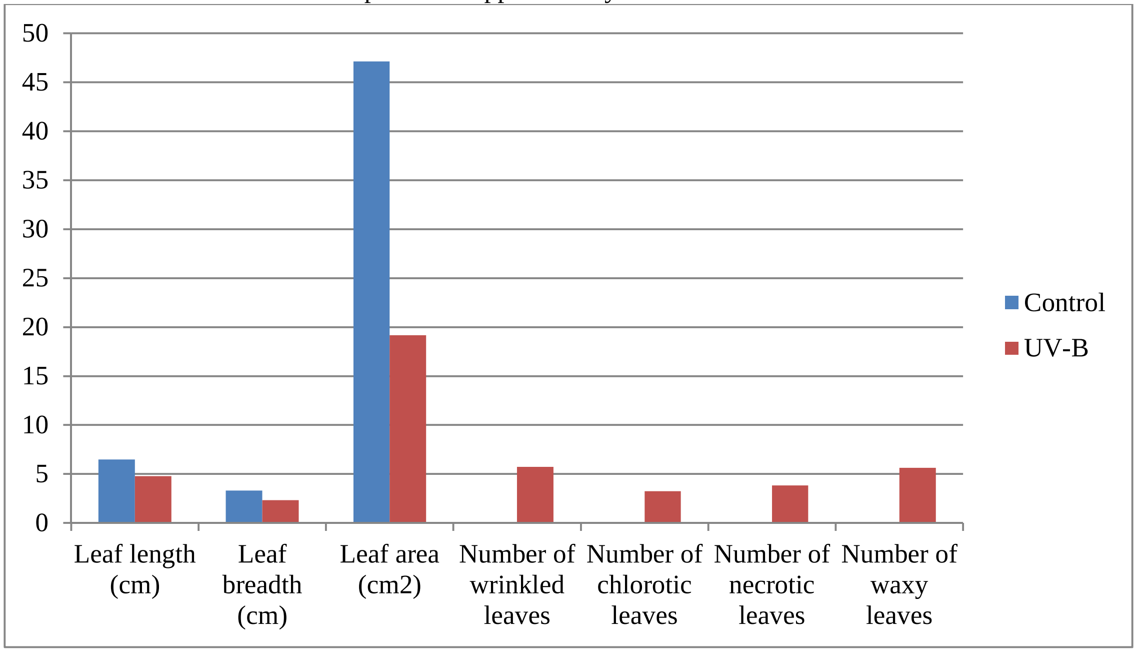
MATERIALS & METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. PUDUVAI obtained from local farmers, Puducherry, India, 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. PUDUVAI 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

The leaves of *Vigna unguiculata* (L.) Walp. cv. PUDUVAI were 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 2). The adaxial surface of normal leaves had costal cells which are uniformly similar in being axially elongated, thin and straight walled with unicellular thin walled trichomes.

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



The costal cells and trichomes on adaxial surface differ from abaxial surface in being shorter in length (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 with dense, deeply stained nuclei were observed in control and in all the UV-B irradiated leaves. 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 (Plate 3). However the cuticle and multilayered epidermis were two to three times more thicker on adaxial surface of stressed leaves than normal 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). With the mesophyll becoming voluminous, a thicker leaf would result (Rajendiran 2001).

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

Parameter		Control		UV-B	
		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency (mm^{-2})		176.0±0.32	150.8±1.31	281.5±1.97	296.1±0.64
Epidermal cell frequency (mm^{-2})		329.7±1.59	337.5±1.98	422.4±0.88	436.2±0.52
Stomatal index		33.07±0.21	33.85±2.98	42.34±1.25	43.72±1.52
S/E ratio		0.53	0.44	0.66	0.67
Frequency of abnormal stomata (mm^{-2})		-	-	39.6±0.19	35.2±0.86
Frequency of dead/collapsed epidermal cells (mm^{-2})		-	-	79.3±1.42	75.6±0.49
Frequency of trichome (mm^{-2})		13.2±1.86	14.9±1.04	34.0±0.49	30.5±0.32
Stomatal size	Length (µm)	23.3±1.47	27.7±0.32	12.6±0.18	14.2±1.17
	Breadth (µm)	14.2±1.34	15.6±0.22	11.3±1.40	12.3±0.77
Epidermal cell size	Length (µm)	64.3±0.41	67.7±0.18	37.7±0.63	45.4±1.57
	Breadth (µm)	47.2±0.29	43.6±2.75	39.9±1.41	41.0±0.67
Trichome length (µm)		83.5±0.64	89.1±1.13	61.7±0.92	62.0±2.37

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

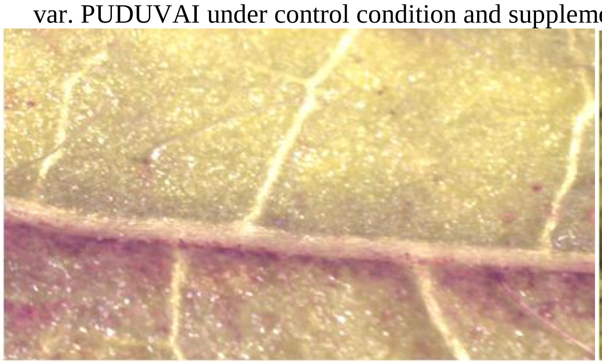


FIGURE 1: Shiny adaxial surface under UV-B

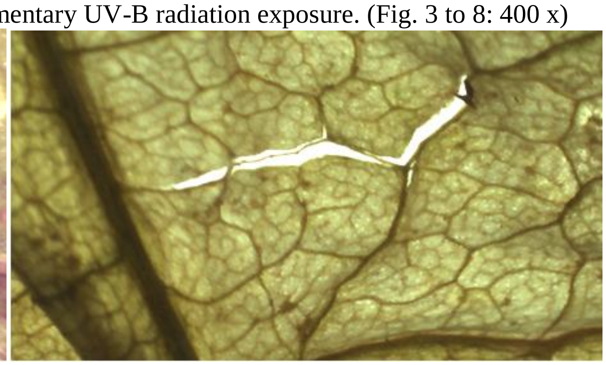


FIGURE 2: UV-B adaxial - Brittle and dead

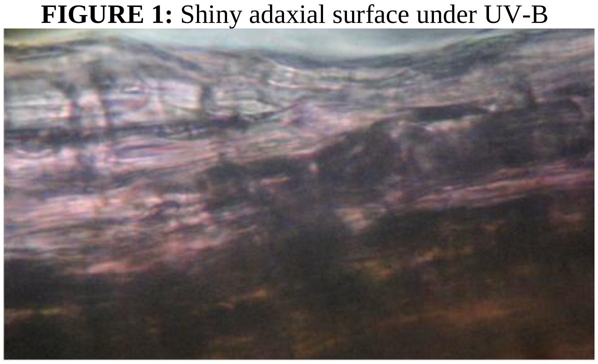


FIGURE 3: UV-B adaxial - Multiseriate epidermis

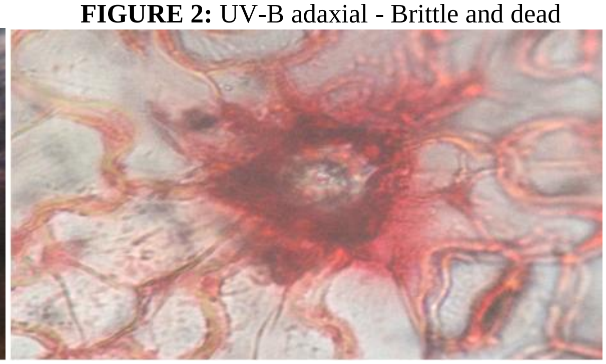


FIGURE 4: UV-B adaxial - Broken trichome

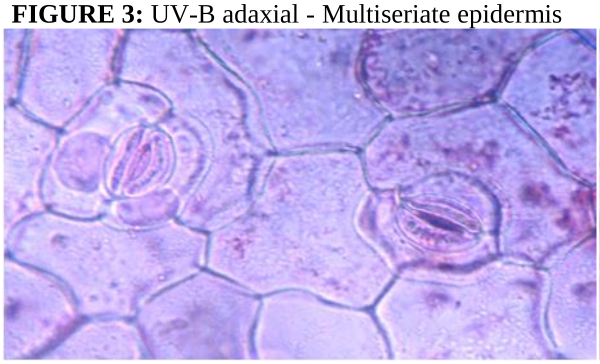


FIGURE 5: Control adaxial - Normal stomata

FIGURE 6: UV-B adaxial - Abnormal stomata

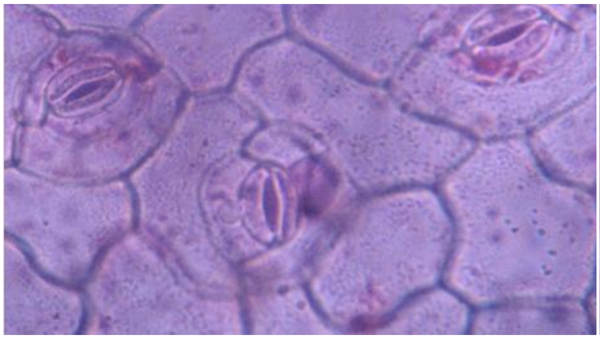


FIGURE 7: Control abaxial - Normal stomata

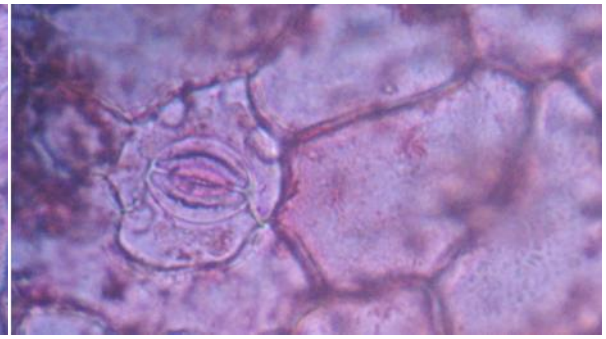
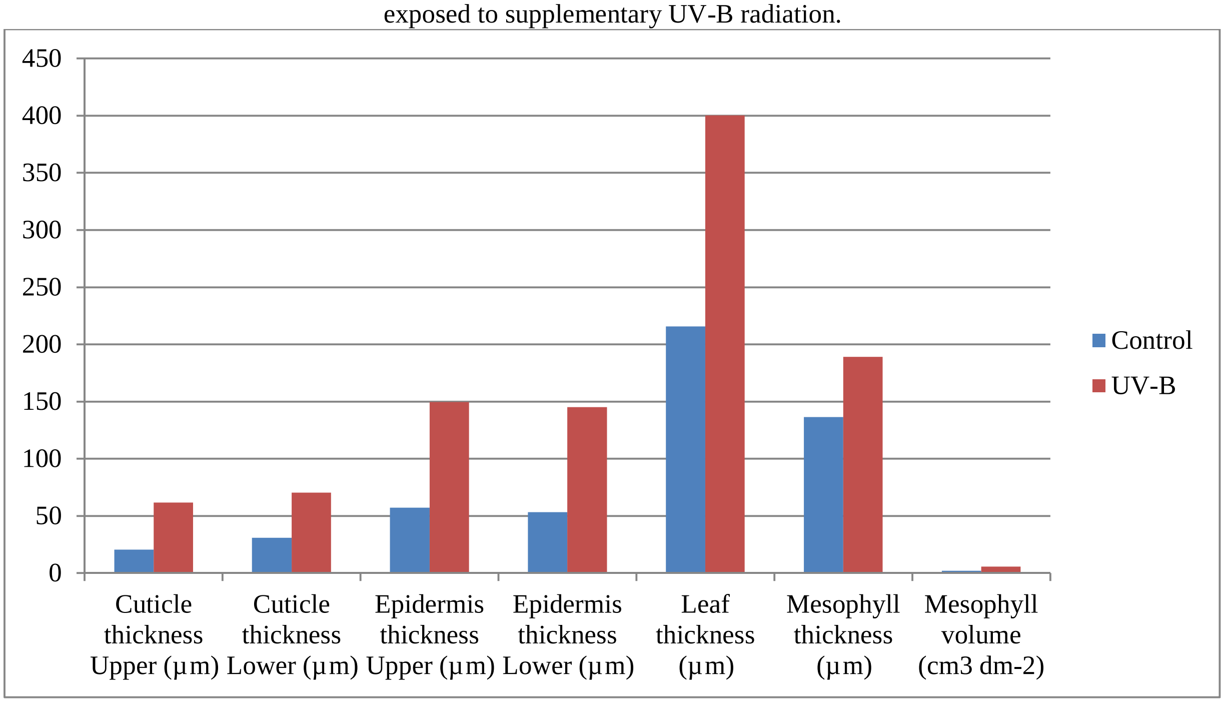


FIGURE 8: UV-B abaxial - Abnormal stomata

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 alleviating some of the deleterious effects (Wellmann 1976, Caldwell *et al.* 1983, Bornman and Vogelmann 1991, Rajendiran 2001). 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), Kokilavani *et al.* (2013) and Kokilavani and Rajendiran (2014a), 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. 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). Trichome frequencies under UV-B exposure increased on adaxial (157 %) as well as on abaxial (104 %) surfaces compared to control leaves (Table 1).

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



Shorter trichomes (26 %) along with broken ones were observed more on the adaxial side of UV-B irradiated leaves (Table 1; Plate 2. Fig. 4). However, the length of trichomes on the abaxial surface of stressed leaves was also decreased below control (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, 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) is at variance from the reductions observed by Karabourniotis *et al*. (1995). Very deeply stained dead and collapsed epidermal cells were found in large numbers on both the leaf surfaces of UV-B stressed plants (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). This may in some cases also be followed by tissue degradation (Caldwell 1971). The epidermal cell size (15 to 32 %) and stomatal size (20 to 45 %) were decreased below normal after UV-B irradiation (Table 1; Plate 2. Fig. 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. Stomatal frequency (60 to 96 %) and stomatal indices were increased significantly (28.11 to 29.24 %) above control with S/E ratio exhibiting high values (24 to 52 %) 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). 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), Kokilavani and Rajendiran (2014a) and Kokilavani and Rajendiran (2014b) on the adaxial side of UV-B stressed leaves. Leaves under UV-B exposure developed abnormalities like persistent stomatal initials**,** stomata with single guard cell and thickened pore and collapsed stomata (Plate 2. Fig. 6, 8). No such abnormalities were recorded in the leaves of the crops grown in control conditions (Table 1; Plate 2. Fig. 5, 7). From the results obtained from this study it is evident that *Vigna unguiculata* (L.) Walp. cv. PUDUVAI responded quickly to supplementary ultraviolet-B radiation and changed its foliar characteristics to withstand the stress.

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