International Journal of Innovative Research and Review ISSN: 2347 – 4424 (Online)
An Online International Journal Available at http://www.cibtech.org/jirr.htm
2014 Vol. 2 (4) October-December, pp.61-68/Kokilavani and Rajendiran
Research Article

EVALUATION OF THE IMPACT OF ULTRAVIOLET-B RADIATION ON THE FOLIAR EPIDERMAL AND ANATOMICAL CHARACTERISTICS OF *VIGNA UNGUICULATA* (L.) WALP. CV. COVU-1

Kokilavani V. and *Rajendiran K.
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry – 605 008
**Author for Corresponde4nce*

ABSTRACT
In nature, plants exhibit remarkable diversity of leaf morphology, which is the result of adaptation to local environment for optimization of photosynthesis. Leaf shape, leaf size and leaf angle are prime determinants of plant architecture, which significantly affect the photosynthetic potential. In addition to manufacture of food, leaf performs vital functions of interchange of gases between the atmosphere and the plant body and evaporation of water. 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 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. COVU-1 leaf. The fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna unguiculata* (L.) Walp. cv. COVU-1 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. Many types of malformations in the leaf structure with injuries were observed under UV-B exposure. On adaxial surface cuticle and epidermis were several times thicker after UV-B irradiation. The volume and thickness of mesophyll increased by 30.46 % and 16.66 % making the leaves thicker by 60.24 % under UV-B exposure. The trichomes were shorter by 38.92 % and 39.43 % on adaxial and abaxial surfaces respectively and were also brittle in UV-B treated leaves compared to healthier ones in control. Frequency of trichome was increased by 174.24 % on adaxial and by 131.06 % on abaxial surfaces in UV-B exposed plants. The leaves of crops in elevated UV-B were small, shiny and thick compared to broader, longer and thinner leaves of normal plants. Stomatal frequency in UV-B was increased by 83.33 and 59.83 % over control on adaxial and abaxial surfaces respectively. Same trend was noticed in stomatal indices of stressed plants which showed increases by 36 to 37 % on both the surfaces. Abnormalities in stomata like, stomata with only one guard cell and persistant stomatal initial were more in number together with dead and collapsed epidermal cells on the adaxial surface of UV-B irradiated plants. No aberrations were recorded in leaves grown under control conditions. The UV-B induced structural changes in the leaves were to obstruct the radiation from penetrating into the inner region.

Keywords: *Ultraviolet-B, Cowpea, Variety COVU-1, 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 and Rajendiran, 2013a; Sudaroli 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 work reports the changes in the leaf development in *Vigna unguiculata* (L.) Walp. cv. COVU-1 to withstand UV-B stress.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. cv. COVU-1 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. COVU-1 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

Leaves of *Vigna unguiculata* (L.) Walp. cv. COVU-1 was small, wrinkled, shiny and brittle along with chlorotic and necrotic lesions developing all over the adaxial surface when exposed with UV-B irradiation (Plate 1; Plate 2. Figure 1 to 2). Adaxial side of control leaves had uniformly similar costal cells that are axially elongated, thin and straight walled with unicellular thin walled trichomes. The costal and intercostal cells and trichomes on adaxial surface were different from abaxial surface in being slightly shorter in length (Table 1). The epidermal cells had dense and deeply stained nuclei in control and in all UV-B irradiated samples. Epidermal cell frequency was higher by 37 % over control in UV-B exposed leaves on both the sides (Table 1). Cuticle deposition was heavier and the epidermis was thicker under UV-B irradiation compared to control (Plate 3). As the epidermis was multilayered on adaxial surface, it appeared three times thicker in ultraviolet-B exposed leaves (Plate 2. Figure 3; Plate 3). UV-B stress also increased the thickness of leaf and mesophyll and the volume of mesophyll (Plate 3). According to Wellmann (1976), Caldwell *et al.,* (1983), Bornman and Vogelmann (1991) and (Rajendiran, 2001) 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. The thickness of leaf 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). Kokilavani and Rajendiran (2013), Kokilavani *et al.*, (2013) and Kokilavani and Rajendiran (2014a) opined 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. UV-B exposure increased trichome frequency on adaxial (131.06 %) and on abaxial (174.24 %) sides over normal leaves (Table 1). Trichomes were shorter by 38.92 % on adaxial side and by 39.43 % on abaxial side in UV-B irradiated leaves with numerous broken trichomes on the adaxial side (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).

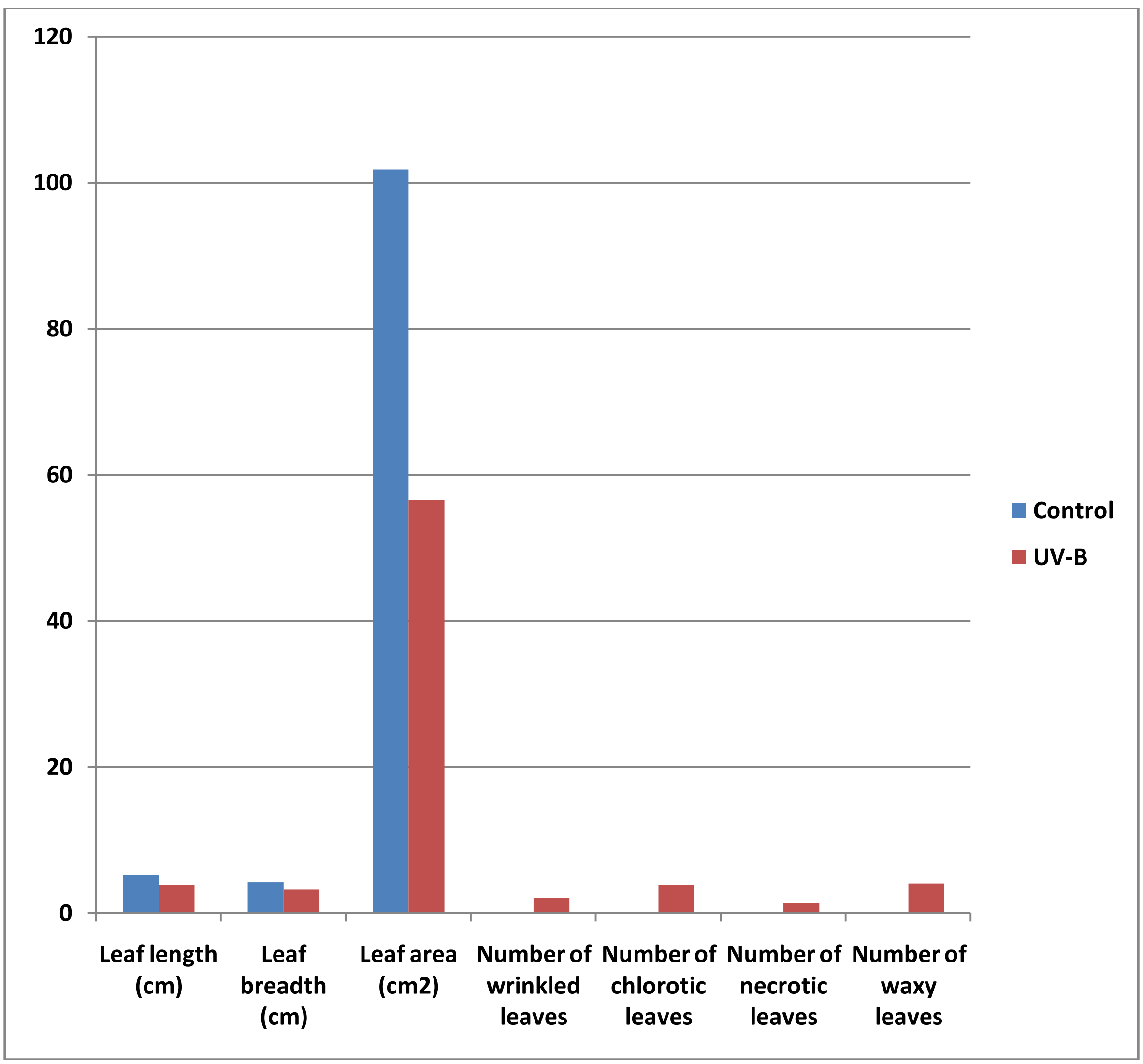

Plate 1: Changes in the morphological characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. COVU-1 exposed to elevated UV-B radiation

International Journal of Innovative Research and Review ISSN: 2347 – 4424 (Online)
An Online International Journal Available at http://www.cibtech.org/jirr.htm
2014 Vol. 2 (4) October-December, pp.61-68/Kokilavani and Rajendiran
Research Article

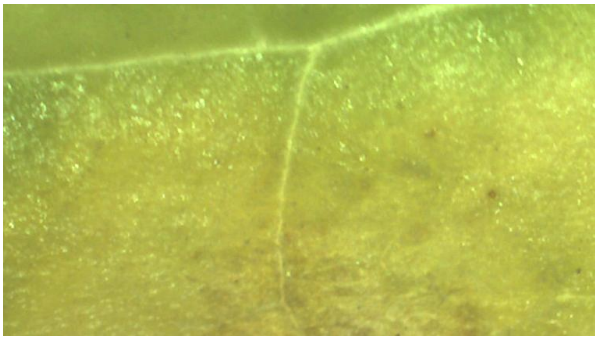

Figure 1: Shiny adaxial surface under UV-B

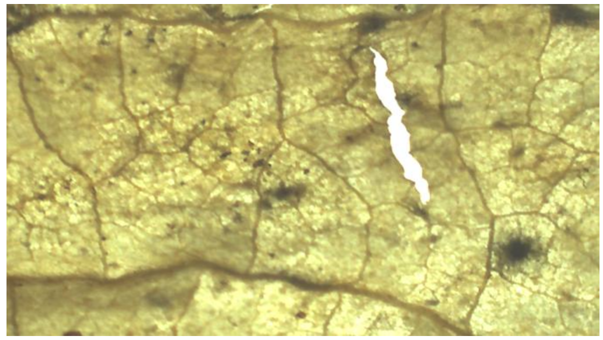

Figure 2: UV-B adaxial - Brittle and dead

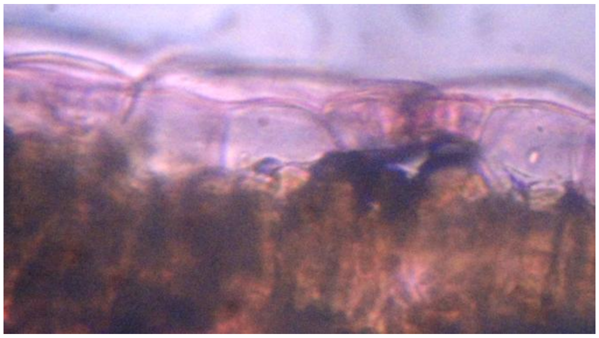

Figure 3: UV-B adaxial - Multiseriate epidermis

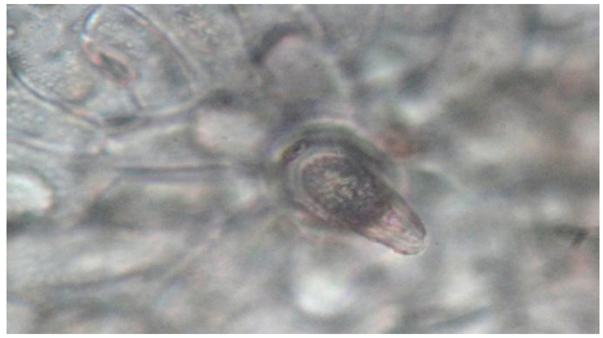

Figure 4: UV-B adaxial - Broken trichome

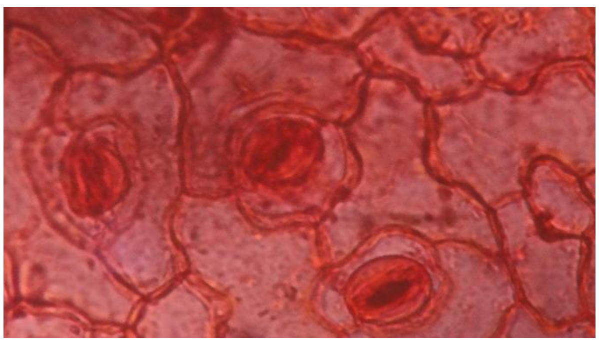

Figure 5: Control adaxial - Normal stomata

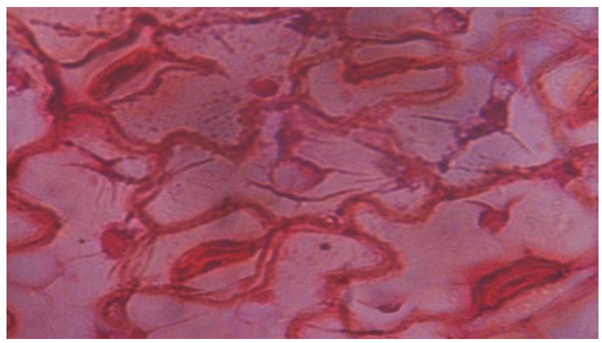

Figure 6: UV-B adaxial - Abnormal stomata

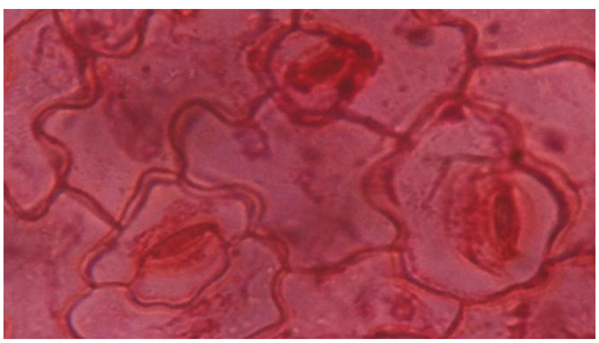

Figure 5: Control adaxial - Normal stomata

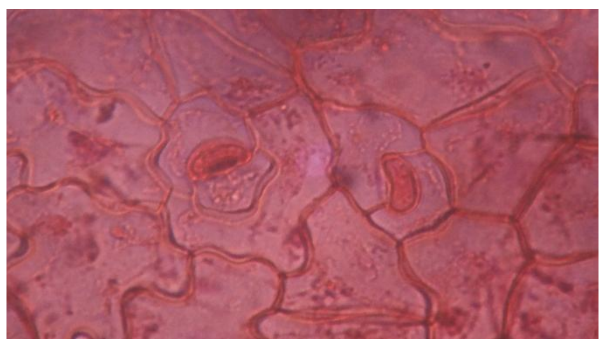

Figure 8: UV-B abaxial - Abnormal stomata

Plate 2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. COVU-1 under control condition and supplementary UV-B radiation exposure (Figure 3 to 8: 400 x)

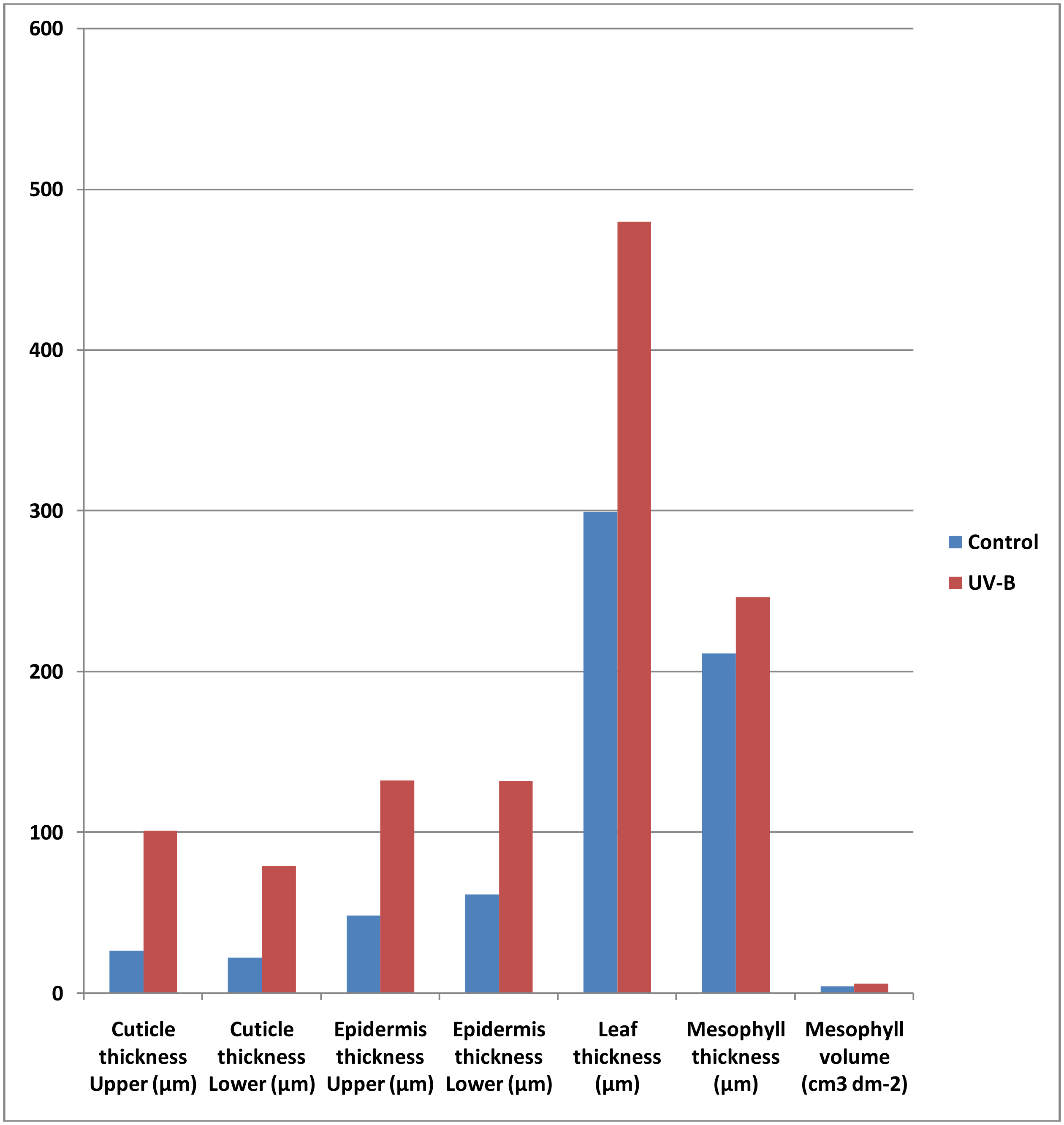

Plate 3: Changes in the anatomical characteristics of leaves of 30 DAS *Vigna unguiculata* (L.) Walp. cv. COVU-1 exposed to elevated UV-B radiation

The trichomes also contribute an 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). Epidermal cells were dead and collapsed taking on very deep stain on both the leaf surfaces of UV-B exposed plants (Table 1; Plate 2. Figure 6, 8). 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).

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

Parameter		**Control**		**UV-B**	
		Adaxial	**Abaxial**	**Adaxial**	**Abaxial**
Stomatal frequency mm^{-2}		132.0±0.82	141.9±0.71	242.0±1.12	226.8±0.93
Epidermal cell frequency mm^{-2}		320.9±0.39	311.8±0.68	440.0±2.75	428.1±0.52
Stomatal index		32.19±0.64	31.28±0.77	44.10±0.58	42.91±0.34
S/E ratio		0.41	0.45	0.55	0.52
Frequency of abnormal stomata mm^{-2}		-	-	48.4±2.03	44.2±0.66
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	74.8±0.72	72.0±0.29
Frequency of trichome mm^{-2}		13.2±1.86	13.2±0.11	30.5±0.29	36.2±0.48
Stomatal size	Length (µm)	43.3±1.30	42.1±0.41	24.4±0.58	23.3±2.75
	Breadth(µm)	21.1±0.27	18.7±1.83	14.1±0.29	17.8±1.49
Epidermal cell size	Length(µm)	79.9±1.52	81.0±1.22	57.3±0.97	34.4±0.62
	Breadth(µm)	42.2±0.83	43.0±1.13	52.1±0.84	41.2±2.01
Trichome length (µm)		79.9±1.34	71.0±1.51	48.8±0.48	43.0±1.10

Size of epidermal cells (28 to 35 %) and stomata (33.17 to 43.64 %) 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 costal regions without any definite pattern or orientation. Frequency of stomata (83.33 %) and stomatal indices were increased significantly (36 to 37 %) above control with S/E ratio on both sides showing more value (37 %) under UV-B exposure on the adaxial as well as abaxial surfaces (Table 1). In contrast, pea plants responding to UV-B treatment had higher stomatal frequency on the adaxial surface (Nogues *et al.*, 1998). Smaller stomata along with many abnormalities were more frequent on both surfaces of UV-B stressed crops with the maximum being on the adaxial surface (Table 1; Plate 2. Figure 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 irradiated leaves. Abnormalities like persistent stomatal initials**,** stomata with single guard cell and thickened pore and collapsed stomata were seen after UV-B exposure (Plate 2. Figure 6, 8), but were not recorded in control leaves (Table 1; Plate 2. Figure 5, 7). To conclude, *Vigna unguiculata* (L.) Walp. cv. COVU-1 receiving supplementary ultraviolet-B radiation created several barriers to combat the stress.

ACKNOWLEDGEMENT
The authors thank Prof. Dr. Thamizharasi Thamilmani, Director, KMCPGS, Puducherry for providing research facilities.

REFERENCES

Arulmozhi D and Rajendiran K (2014). Effect of supplementary ultraviolet-B radiation on nodulation and nitrogen metabolism in *Lablab purpureus* L. var. Goldy. *International Journal of Advanced Biological Research* **4**(3) 343-346.

Bornman JF and Vogelmann TC (1991). Effect of UV-B radiation on leaf optical properties measured with fibre optics. *Journal of Experimental Botany* **42** 647 - 554.

Caldwell MM (1971). Solar UV irradiance ends the growth and development on higher plants. In: *Photophysiology,* edited by Giese AC (Academic Press) New York **6** 131 - 177.

Caldwell MM, Gold WG, Harris G and Ashurst CW (1983). A modulated lamp systam for solar UV-B (280-320 nm supplementation studies in the field. *Photochemistry and Photobiology* **37** 479 - 485.

Cline MG and Salisbury FB (1966). Effects of ultraviolat fadlation on the leaves of higher piants. *Radiation Botany* **6** 151 - 163.

Ehleringer JR (1984). Ecology and ecophysiology of leaf pubescence in North American desert plants In: *Biology and Chemistry of Plant Trichomes,* edited by Rodriguez E, Healy PL and Mahta I (Plenum Publishing Corp.) New York 113 - 132.

Johnson HB (1975). Plant pubescence: An ecological perspective. *Botanical Review* **41** 233-258.

Karabourniotis G, Kotsabassidis D and Manatas Y (1995). Trichome density and its protective potential against ultraviolet-B radiation damage during leaf development. *Canadian Journal of Botany* **73** 376 - 383.

Kokilavani V and Rajendiran K (2013). Ultraviolet-B induced changes in the leaf epidermal and anatomical characteristics of *Vigna mungo* L. var. KM-2. *International Journal of Science and Nature* **5**(1) 126-130.

Kokilavani V, Rajendiran K, Vijayarengan P and Ranganathan R (2013). Epidermal and anatomical characteristics in the leaves of UV-B irradiated cucumber. National Conference on Environmental Issues and Challenges - Vision 2020, Department of Zoology, Annamalai University. Tamil Nadu, India 42.

Kokilavani V and Rajendiran K (2014a). Changes in leaf architecture of *Vigna unguiculata* (L.) Walp. cv. BCP-25 after exposure to elevated ultraviolet-B radiation. *International Journal of Science and Nature* **5**(3) 542-546.

Kokilavani V and Rajendiran K (2014b). Ultraviolet-B induced changes in the leaf architecture of *Cucumis sativus* L. var. CO 1. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 208-215.

Kokilavani V and Rajendiran K (2014c). Alterations in leaf architecture of *Ocimum sanctum* L. under elevated ultraviolet-B stress. *Global Journal of Bio-Science and Biotechnology* **3**(4) 374-378.

Kokilavani V, Sudaroli Sudha J, Vijayalakshmi R, Arulmozhi D and Rajendiran K (2014). Changes in the Foliar Epidermal and Anatomical Characteristics of *Ocimum basilicum* L. under elevated Ultraviolet-B stress. National Conference on Conservation Characterization and Cultivation of Medicinal Plants for Sustainable Utilization and Community Welfare, Department of Botany, Kanchi Mamunivar Centre for Post-graduate Studies (Autonomous), Pondicherry, India 83.

Nobel PS (1983). *Biophysical Plant Physiology and Ecology* (W.H. Freeman and Co.) San Francisco.

Nogues S, Allen DJ, Morison JIL and Baker NR (1998). UV-B radiation effects on water relations, leaf development and photosynthesis in droughted pea plants. *Plant Physiology* **117** 173 - 181.

Patterson DT, Duke SO and Hoagland RE (1978). *Plant Physiology* **61** 402 - 405.

Rajendiran K (2001). Amelioration of Ultraviolet-B radiation impacts in green gram by Triadimefon. PhD. Thesis, Pondicherry University.

Rajendiran K and Ramanujam MP (2000). Efficacy of triadimefon treatment in ameliorating the UV-B stress in green gram. In: *National Symposium on Environmental Crisis and Security in the New Millennium*, edited by Khan M, National Environmental Science Academy, New Delhi 41-42.

Rajendiran K and Ramanujam MP (2003). Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum* **46** 621-624.

Rajendiran K and Ramanujam MP (2004). Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. *Biologia Plantarum* **48** 145-148.

Rajendiran K and Ramanujam MP (2006). Interactive effects of UV-B irradiation and triadimefon on nodulation and nitrogen metabolism in *Vigna radiata* plants. *Biologia Plantarum* **50**(4) 709-712.

Salisbury W (1928). On the causes and ecological significance of stomatal frequency with special reference to woodland flora. *Philosophical Transactions of the Royal Society* **216** 1 - 85.

Sudaroli Sudha J and Rajendiran K (2013a). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Sesbania grandiflora* (L.) Pers. *International Journal of Science and Nature* **4**(4) 664 - 667.

Sudaroli Sudha J and Rajendiran K (2013b). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. c.v. BCP-25. *International Journal of Food, Agriculture and Veterinary Sciences* **3**(3) 77 - 81.

Vijayalakshmi R and Rajendiran K (2014). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Phaseolus vulgaris* L. cv. Prevail. *International Journal of Advanced Biological Research* **4**(3) 339 - 342.

Wellmann E (1976). Specific ultraviolet effects in plant morphogenesis. *Photochemistry and Photobiology* **50** 479 - 487.

Woodman RL and Fernandez OW (1991). Differential mechanical defence: Herbivory, evapotranspiration and leaf hairs. *Oikos* **80** 11 - 19.

Wright LA and Murphy TM (1982). Short-wave ultraviolet light closes leaf stomata. *American Journal of Botany* **89** 1196 - 1199.