INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

ULTRAVIOLET-B INDUCED CHANGES IN THE LEAF EPIDERMAL AND ANATOMICAL CHARACTERISTICS OF *VIGNA MUNGO* L. var. KM-2

Kokilavani, V. & Rajendiran, K.
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry – 605 008
Corresponding Authors email- rajeworks@yahoo.com

ABSTRACT
Foliar epidermis has a critical duty as a structural element, holding the cellular tissues compact and firm. Epidermis forms the boundary layer between the internal parts of the leaf and its environment. Its functions include conservation of water in the plant, prevention of loss of plant components by leaching and protection of plants against injuries due to wind, frost, and radiation and physical abrasion. Depletion in the ozone layer allowing increased flux of ultraviolet-B radiation has a direct effect on the epidermis of the leaves.The present study is an attempt to assess the effects of ultraviolet-B (UV-B) radiation in the epidermis and the anatomy of *Vigna mungo* L. var. KM-2 leaf. The epidermal and anatomical characters of the fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Vigna mungo* L. var. KM-2 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 all treatments. The cuticles on the adaxial epidermis and the mesophylls were thinner in controls. But they were thicker under UV-B (46.7%). The trichomes were longer (8.7% to 20.2%) but brittle in UV-B treated leaves which were short and healthy in control. The trichome frequency was also higher (10.3% to 36.5%) in UV-B stressed plants. The small, shiny and thick leaves of UV-B exposed plants compared poorly to broader, longer and thinner leaves of control plants. The stomatal frequency (16.3% to 56.6%) and the stomatal indices (12.5%) declined in UV-B stressed plants. Abnormal stomata like, non-functional, reduced size, malformations were more under UV-B stress (9% to 28%). Such aberrations were absent in leaves under control conditions. Suffering heavily by the radiation, the UV-B stressed plants modified the foliar characteristics to attenuate the impact to some extent.

KEY WORDS: Ultraviolet-B, black gram, 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) in sensitive plants. The epidermis constitutes a dynamic barrier between the plant's internal and external environment. Externally, it is impregnated with waxes and cutins but punctuated by stomata which regulate the flow of gases. Also dotting the dermal surface are the 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). The present study is carried out to record the mechanism of defense against UV-B radiation in the leave anatomy of an important legume, *Vigna mungo* L. var. KM-2.

MATERIALS & METHODS

The seeds of *Vigna mungo* L. var. KM-2 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} 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 mungo* L. var. KM-2 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).

RESULT & DISCUSSION

The leaves under UV-B exposure were small, wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface (Plate 1. Fig. 1 to 4). On the adaxial surface of unstressed leaves the costal cells are uniformly similar in being axially elongated, thin and straight walled and have unicellular thin walled trichomes.

FIGURE 1: Control and UV-B stressed leaves

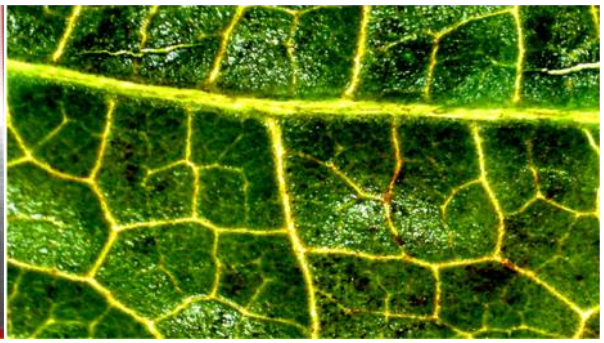

FIGURE 2: Shiny adaxial surface under UV-B

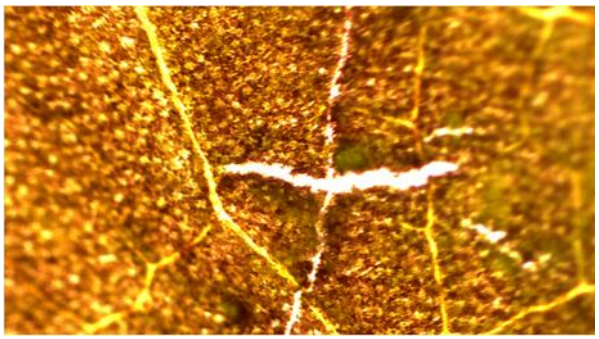

FIGURE 3: UV-B adaxial - Brittle and dead

FIGURE 4: UV-B - Chlorosis and necrosis

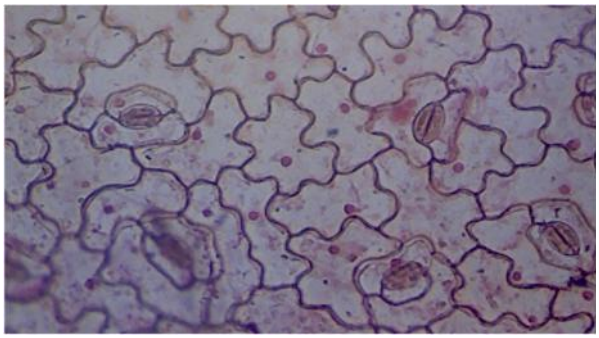

FIGURE 5: Control adaxial - Normal stomata

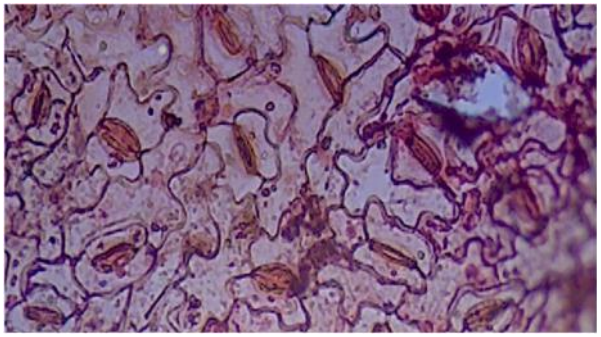

FIGURE 6: UV-B adaxial - Abnormal stomata

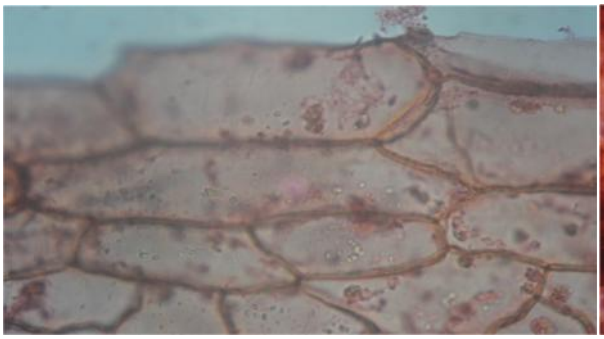

FIGURE 7: UV-B adaxial - Multiseriate epidermis

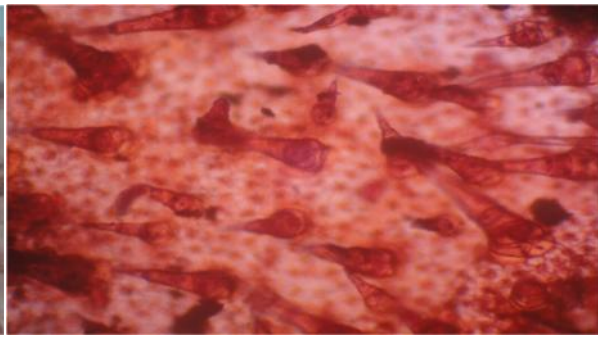

FIGURE 8: UV-B Abaxial - More trichomes

PLATE 1. Epidermal and anatomical characteristics of leaves of 30 DAS black gram under control condition and supplementary UV-B radiation exposure. (Fig. 5 to 8: 400 x)

The costal cells and trichomes on adaxial surface differ from abaxial surface in being shorter in length (Table 1). The intercostal epidermal cells both on abaxial and adaxial surfaces are sinuous and thin walled with unicellular trichomes occurring sporadically. The epidermal cells with dense, deeply stained nuclei were observed in control and in all the treated leaf samples (Plate 1. Fig. 5, 6). Epidermal cell frequency was higher (55.55%) over control in UV-B exposed plants 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 multiseriate epidermis thickness on adaxial surface were of the deleterious effects.

increased markedly by 28.28% and 36.25% respectively over control (Table 2; Plate 1. Fig. 7). The overall 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). Plants retard 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 (Wellmann 1976, Caldwell *et al.* 1983). At the structural level, increased leaf and cuticle thickness reduces UV-B penetration to internal tissues (Bornman and Vogelmann 1991, Rajendiran 2001) alleviating some

TABLE 1. Changes in the epidermal characteristics of leaves of 30 DAS black gram exposed to supplementary UV-B radiation

Parameter		Control		UV-B	
		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency mm^{-2}		186.67 ± 0.24	122.16 ± 0.42	157.58 ± 0.34	207.25 ± 0.42
Epidermal cell frequency mm^{-2}		157.13 ± 0.34	147.56 ± 0.26	244.42 ± 0.13	201.92 ± 0.32
Stomatal index		54.23 ± 0.82	45.16 ± 0.91	40.34 ± 1.26	50.53 ± 1.12
S/E ratio		1.62	0.78	0.57	1.05
Frequency of abnormal stomata mm^{-2}		-	-	24.22 ± 0.23	7.56 ± 0.22
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	20.67 ± 0.14	-
Frequency of trichome mm^{-2}		16.13 ± 0.12	10.56 ± 0.24	17.64 ± 0.08	14.42 ± 0.12
Stomatal size	Length (µm)	22.64 ± 0.15	23.85 ± 0.17	17.24 ± 0.17	20.45 ± 0.12
	Breadth(µm)	18.33 ± 0.17	12.33 ± 0.18	11.33 ± 0.24	12.55 ± 0.15
Epidermal cell size	Length(µm)	41.87 ± 0.13	71.17 ± 0.26	31.85 ± 0.28	57.15 ±1.24
	Breadth(µm)	23.85 ± 0.15	21.55 ± 0.14	21.23 ± 0.24	17.85 ± 0.13
Trichome length (µm)		255.32 ± 8.65	245.23 ± 11.25	276.65 ±5 .89	257.86 ± 8.43

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). 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 (Bornman and Vogelmann 1991 Kokilavani *et al.* 2013). 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. UV-B exposure increased the trichome frequency (9.36%) compared to control, especially on the adaxial surface (Table 1). Longest trichomes (8.35%) were recorded on the abaxial side of UV-B stressed plants (Plate 1. Fig.8).

TABLE 2. Changes in the anatomical characteristics of leaves of 30 DAS black gram exposed to supplementary UV-B radiation.

Parameter		Control	UV-B
Cuticle	Upper (µm)	7.58 ± 0.47	10.57 ± 0.45
thickness	Lower (µm)	8.56 ± 0.48	9.27 ± 0.34
Epidermis thickness	Upper (µm)	15.23 ± 0.45	23.89 ± 0.67
	Lower (µm)	16.53 ± 1.26	18.29 ± 0.65
Leaf thickness (µm)		216.67 ± 2.53	248.67 ± 2.56
Mesophyll thickness (µm)		155.15 ± 2.25	177.24 ± 2.23
Mesophyll volume (cm^3 dm^{-2})		1.61 ± 0.12	1.86 ± 0.12

The same pattern was observed on the distribution of trichome on the adaxial surface too (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. 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 and collapsed epidermal cells (26.67%) were found in large numbers only on the adaxial leaf surface of UV-B exposed plants (Table 1; Plate 1. 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). Analysis of epidermal cell size showed that the cells were smaller (19.69% to 23.93%) after UV-B irradiation (Table 1; Plate 1. Fig. 5, 6). The leaves are amphistomatic and the stomata are spherical 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, stomatal index and S/E ratio ware reduced significantly (12.62% to 82.57%) below control by UV-B on the adaxial side while they recorded an increase over control on the abaxial surface (Table 1). 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 1. Fig. 5, 6). Similar results were reported by Wright and Murphy (1982) and Kokilavani *et al.* (2013) on the adaxial side of the leaves after exposure to UV-B radiation. Pea plants, responding to UV-B treatment also had higher stomatal frequency (Nogues *et el.* 1998). UV-B irradiated leaves developed abnormalities like persistent stomatal initials**,** stomata with single guard cell and thickened pore and stomata with unequal guard cells (Table 1; Plate 1. Fig. 6). Such aberrations did not occur in the leaves of the plants grown under control conditions. (Table 1; Plate 1. Fig. 5). The data obtained from the present work proves the defence mechanism performed by the leaves of black gram suffering heavily under ultraviolet-B impact, as the leaves responded quickly by increasing the thickness of the cuticle, epicuticular wax deposition, frequency of trichomes and the volume of the internal organ system to offer additional mechanical barrier to UV-B penetration.

ACKNOWLEDGEMENT

The authors thank Prof. Dr. V. Ramassamy, Director, KMCPGS, Puducherry and Prof. Dr. S. Nadanakunjitham, Head, Department of Botany, KMCPGS, Puducherry for providing research facilities.

REFERENCES

Bornman, J. F., Vogelmann, T. C. (1991) Effect of UV-B radiation on leaf optical properties measured with fibre optics. J. Exp. Botany **42:** 647 - 554.

Caldwell, M. M. (1971) Solar UV irradiance end the growth and development on higher plants. In: Photophysiology. Ed. Giese, A. C. Academic Press, New York. **6:** 131 - 177.

Caldwell, M. M., Gold, W. G., Harris, G. and Ashurst, C. W. (1983) A modulated lamp systam for solar UV-B (280-320 nm supplementation studies in the field. Photochem. Photobiol. **37:** 479 - 485.

Cline, M. G. and Salisbury, F. B. (1966) Effects of ultraviolat fadlation on the leaves of higher piants. Radiat. Bot. **6:** 151 - 163.

Ehleringer, J. R. (1984) Ecology and ecophysiology of leaf pubescence in North American desert plants In: Biology and Chemistry of Plant Trichomes. Eds. Rodriguez, E., Healy, P. L. and Mahta, I. Plenum Publishing Corp. New York. pp. 113 - 132.

Johnson, H, B. (1975) Plant pubescence: An ecological perspective Bot. Rev. **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. Can. J. Bot. **73:** 376 - 383.

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. pp. 42.

Nobel, P. S. (1983) Biophysical Plant Physiology and Ecology. W.H. Freeman and Co. San Francisco.

Nogues, S., Allen, D. J., Morison, J. I. L. and Baker, N. R. (1998) UV-B radiation effects on water relations, leaf development and photosynthesis in droughted pea plants. Plant physiol. **117:** 173 - 181.

Patterson, D. T., Duke, S. O. and Hoagland, R. E. (1978) Plant Physiol. **61:** 402 - 405.

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

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

Rajendiran, K., Ramanujam, M.P. (2003) Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. - Biol. Plant. **46**: 621-624.

Rajendiran, K., Ramanujam, M.P. (2004) Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. - Biol. Plant. **48**: 145-148.

Rajendiran, K., Ramanujam, M.P. (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. Phil. Trans. R. Soc. **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) 2013: 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.

Wellmann, E. (1976) Specific ultraviolet effects in plant morphogenesis. Photochem. Photobiol., **50:** 479 - 487.

Woodman, R. L. and Fernandez, O. W. (1991)Differential mechanical defence: Herbivory, evapotranspiration and leaf hairs. Oikos. **80:** 11 - 19.

Wright, L. A. and Murphy, T. M. (1982) Short-wave ultraviolet light closes leaf stomata. Am. J. Bot. 89: 1196 - 1199.