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ANATOMICAL AND EPIDERMAL ALTERATIONS IN THE LEAVES OF THREE VARIETIES OF COWPEA GROWN UNDER *IN SITU* UV-B RADIATION

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ABSTRACT

The present study aims at assessing the effects of ultraviolet-B (UV-B) radiation on the foliar morphology, epidermis and anatomy of three varieties of cowpea *viz.*, GOWMATHI, FOLA and NS-634. Fully developed third trifoliate leaves from the top of 30 DAS (days after seed germination) cowpea varieties under *in situ* supplementary UV-B radiation (2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) were taken for assessment. UV-B induced changes were recorded in the leaf morphology, including injuries, which did not occur in normal plants. The epidermis both on the adaxial as well as abaxial surfaces exhibited many changes after UV-B exposure. Thickness of cuticle, epidermis, leaf and mesophyll and volume of mesophyll increased in all varieties of cowpea after UV-B irradiation. As a result, UV-B irradiated GOWMATHI, FOLA and NS-634 possessed very thick leaves. On prolonged exposure to UV-B, the leaves developed many stomatal abnormalities. Abnormal stomata *viz.*, stomata with single guard cell, reduced size and contiguous stomata were more along with dead epidermal cells in UV-B irradiated plants on the adaxial leaf surface. Stomata were healthier in leaves grown under control conditions. From the results obtained, it is evident that cowpea varieties responded quickly to supplementary ultraviolet-B radiation by modifying the foliar anatomy to withstand the stress.

KEY WORDS

Abnormal stomata, cowpea, leaf anatomy, leaf epidermis, leaf morphology, three varieties and ultraviolet-B.

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INTRODUCTION

The presence or absence of light in the environment has profound effects on the structure of plant leaves. Because of the role of light in plant metabolism, however, light and its availability clearly control the structures as well as the functioning of leaves. Human activities have depleted the ozone layer and as a result ultraviolet-B (UV-B) radiation (280-320 nm) in the sunlight increased to alarming level, causing a dangerous atmospheric stress to plants[1], as it affects leaf epidermis[2-12], causes abnormalities in cotyledonary epidermis[13-17], suppresses photosynthesis[18-20], retards growth[21-29], reduces harvest[23,25,30-34] and disturbs nodulation and symbiotic nitrogen fixation[35-47] in a variety of crop plants. As leaves are the organs that receive major amount of UV-B radiation, they react quickly to prevent its entry into the internal organs[48-50]. The present study reports the variations brought about by ultraviolet-B rays in the leaves of three varieties of cowpea.

MATERIAL AND METHODS

In situ UV-B irradiation

Cowpea (*Vigna unguiculata* (L.) Walp.) belonging to the family Fabaceae which is a nitrogen fixing grain legume was chosen for the study. Viable seeds of the three varieties of cowpea *viz.* GOWMATHI, FOLA and NS-634 (Namdhari Seeds) were procured from Saravana Farms, Villupuram, Tamil Nadu and from local farmers in Pondicherry, India. The seeds were selected for uniform colour, size and weight and used in the experiments. The crops 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 5 DAS (days after seed germination). 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}.

Epidermal and anatomical studies

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) 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[51] 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 and 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.

Dendrogram

At least ten replicates were maintained for all treatments and control to confirm the trends. The result of single linkage clustering[52] was displayed graphically in the form of a diagram called dendrogram[53]. The similarity indices between the three varieties of cowpea under study were calculated using the formula given by Bhat and Kudesia[54].

$$\textbf{Similarity index} = \frac{\textbf{Total number of similar characters}}{\textbf{Total number of characters studied}} \times 100$$

Based on the similarity indices between the three varieties of cowpea, dendrogram was draw to derive the interrelationship between them and presented in Table No.6 and Plate No.4.

RESULTS AND DISCUSSION

Leaf epidermis

The three varieties of cowpea had small, shiny and brittle leaves with chlorotic and necrotic lesions all over the adaxial surface after UV-B exposure (Plate No.1 to 3, Figure No.1, 2). The costal cells were uniformly similar in being axially elongated, thin and straight walled (Plate No.1 to 3, Figure No.3) on the adaxial surface of normal leaves. Intercostal epidermal cells on both abaxial and adaxial surfaces of leaves of three varieties of cowpea are sinuous and thin walled with unicellular trichomes occurring intermittently. The epidermal cells had dense and deeply stained nuclei in control and in all UV-B irradiated leaves of cowpea varieties (Plate 1 to 3; Figure No.3 to 6). Epidermal cell frequency was higher over control in UV-B exposed plants (Table No.1). The epidermal cell frequency was 22.57 % to 39.29 % more under UV-B stress but the effect was less on the abaxial side compared to adaxial surface. Epidermal cells were smaller in all varieties of cowpea by 9.20 % to 45.38 % on both the surfaces after UV-B irradiation. Stomata were smaller too by 8.48 % to 57.21 % after UV-B exposure in all the three varieties of cowpea (Table No.2; Plate No.1 to 3; Figure No.3 to 6). Stomatal frequency, stomatal index and S / E ratio were increased significantly above control after UV-B on the adaxial surface (5.88 % to 54.50 %) as well as on the abaxial surface (5.17 to 74 %), the maximum enhancement being in NS-634 (Table No.1). Dead and collapsed epidermal cells were more (67.78 % to 89 %) on the adaxial as well as on the abaxial leaf surfaces of UV-B stressed crops (Table No.3; Plate No.1 to 3, Figure No.4, 6). The adaxial epidermis recorded damages in the form of collapsed cells and the leaves became glazed with signs of bronzing of tissue surfaces due to formation of oxidised phenolic compounds[55]. According to Caldwell[56] this may in some cases also be followed by tissue degradation.

Trichomes

Unicellular and thin walled trichomes occurred on the costal and intercostal regions of both the surfaces of the leaves. The costal cells and trichomes on abaxial surface differ from adaxial surface in being longer in length after UV-B exposure (Table No.4). Trichome frequency was comparatively less on the abaxial side than on the adaxial side. UV-B treatment enhanced the trichome frequency by 66 % to 147 % in all varieties compared to their controls, especially on the adaxial surface (Table No.4). Longer trichomes (12.50 % to 28.89 %) along with broken ones were observed more on the adaxial side of UV-B stressed leaves (Table No.4; Plate No.1 to 3, Figure No.7). However, the length of trichomes on the abaxial surface of stressed leaves was more or less equal to that of control plants (Table No.4). The trichomes serve several functions *viz.*, mechanical barrier against biotic attack[57-59], providing additional resistance to the diffusion of water vapour from the interior of leaf to the atmosphere[60] and as a reflector reducing the radiant energy absorbed by the leaf[20,61]. The non-glandular hairs also form additional mechanical barrier to UV-B penetration by reflecting the radiant energy[2]. However, Karabourniotis *et al*.[62] observed reductions in trichome frequency after UV-B treatment.

Leaf anatomy

Cuticles and the epidermis in UV-B exposed leaves, on both surfaces, increased significantly in thickness over control by 96.15 % to 278 % (Table No.5). In UV-B irradiated plants, the epidermis was markedly thicker than the control on both the sides of the leaf by 93.25 to 295.67 %, the maximum thickness being on upper epidermis due to multiple layers (Table No.5; Plate No.1 to 3, Figure No.8). The overall trend expressed in cuticle and epidermis thickness continued in leaf thickness, mesophyll thickness and volume also (Table No.5). With the mesophyll becoming voluminous, a thicker leaf would result[20]. The highest values for leaf thickness were for UV-B irradiated NS-634 followed by GOWMATHI and FOLA (Table No.5). Plants obstruct UV-B penetration 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[1,57]. According to Rajendiran[20] and Bornman and Vogelmann[48], at the structural level, increased leaf and cuticle thickness reduces UV-B transmission to internal tissues alleviating the harmful effects to some extent. Addition of spongy mesophyll cells in *Medicago sativa* leaves and an enhancement in the number of palisade cells in *Brassica campestris* leaves increased the leaf thickness[48]. Greater thickness increased the amount of scattered light which could be due to less chlorophyll, increased number of intercellular spaces, cytoplasmic changes or altered cellular arrangements, like the palisade becoming broader and cell layers increasing in number as opined by

Kokilavani and Rajendiran[2], Rajendiran[20] and Bornman and Vogelmann[48].

Aberrations in epidermal cells and stomata

Aberrations observed in UV-B irradiated leaves were contiguous stomata, persistent stomatal initials, stomata with single guard cell and thickened pore and stomata with unequal guard cells (Table No.3; Plate No.1 to 3, Figure No.4, 6). Such abnormalities were not recorded in the leaves of the plants grown under normal photoperiodic conditions (Table No.3; Plate No.1 to 3, Figure No.3, 5). Similar results were reported in tobacco by Wright and Murphy[63], *Vigna unguiculata* (L.) Walp. cv. BCP-25 by Kokilavani and Rajendiran[3], *Cucumis sativus* L. var. CO-1 by Kokilavani and Rajendiran[4], *Vigna mungo* L. var. KM-2 by Kokilavani and Rajendiran[5], *Vigna unguiculata* (L.) Walp. cv. CW-122 by Kokilavani and Rajendiran[7], Vigna *unguiculata* (L.) Walp. cv. COVU-1 by Kokilavani and Rajendiran[8], *Vigna unguiculata* (L.) Walp. cv. COFC-8 by Kokilavani and Rajendiran[9], *Vigna unguiculata* (L.) Walp. cv. Vamban by Kokilavani and Rajendiran[10], *Vigna unguiculata* (L.) Walp. cv. CO-6 by Kokilavani and Rajendiran[11], *Vigna unguiculata* (L.) Walp. cv. CO-1 by Kokilavani and Rajendiran[12], *Vigna unguiculata* (L.) Walp. cv. CO-3 by Kokilavani and Rajendiran[39], *Vigna unguiculata* (L.) Walp. cv. Puduvai by Kokilavani and Rajendiran[50], *Vigna unguiculata* (L.) Walp. cv. KM-1 by Kokilavani and Rajendiran[64] and *Vigna unguiculata* (L.) Walp. cv. COVU-2 by Kokilavani and Rajendiran[65] on the adaxial side of the leaves after exposure to UV-B radiation. Severe stomatal abnormalities were also reported in the cotyledonary epidermis of F_1 seedlings grown after harvesting from *in situ* ultraviolet-B treated parents of *Momordica charantia* L.[13], *Benincasa hispida* (Thunb.) Cogn.[16] and *Macrotyloma uniflorum* (Lam.) Verdc.[17].

Dendrogram

The epidermal and anatomical parameters recorded in three varieties of cowpea showed differences in number and size of epidermal cell and stomata, including frequency of abnormal stomata and dead epidermal cells and increased leaf thickness after irradiation with *in situ* supplementary UV-B on 30 DAS. The similarity index value between FOLA and NS-634 was 40 %. These two varieties as one group joined with GOWMATHI which had similarity indices of 36.24 % and 33.33 % with FOLA and NS-634 respectively (Table No.6; Plate No.4).

Table No.1: Changes in the frequency of stomata and epidermal cells in the leaves of three varieties of 30 DAS *Vigna unguiculata* (L.) Walp. under control and supplementary UV-B exposed conditions

S.No	Varieties	Treatment	Stomatal frequency (mm^{-2})		Epidermal cell frequency (mm^{-2})		Stomatal index		S/E ratio	
			Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
1	GOWMATHI	Control	223.42±4.35	218.83±0.51	351.04±3.46	367.50±0.55	34.31±0.32	35.71±1.56	0.67	0.58
		UV-B	291.42±0.68	273.62±1.61	465.42±1.38	450.48±0.60	45.73±1.88	44.28±1.06	0.63	0.61
2	FOLA	Control	161.83±2.44	174.83±0.73	361.43±0.47	371.43±1.07	37.15±0.90	36.33±2.07	0.44	0.46
		UV-B	250.03±1.01	237.23±0.60	480.23±1.80	472.35±0.18	47.23±0.64	46.15±1.18	0.53	0.51
3	NS-634	Control	166.23±0.26	147.34±1.58	333.32±0.56	318.21±2.02	34.54±0.16	31.07±3.06	0.51	0.47
		UV-B	244.24±2.45	256.52±0.56	427.05±0.45	443.23±0.68	45.91±0.27	45.52±1.89	0.54	0.56

Table No.2: Changes in the size of stomata and epidermal cells in the leaves of three varieties of 30 DAS *Vigna unguiculata* (L.) Walp. under control and supplementary UV-B exposed conditions

S.No	Varieties	Treatment	Stomatal size (µm)				Epidermal cell size (µm)			
			Adaxial		Abaxial		Adaxial		Abaxial	
			Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
1	GOWMATHI	Control	41.22±1.31	26.51±0.93	37.86±2.64	16.81±1.47	73.65±1.27	46.26±5.08	79.85±0.47	44.44±0.67
		UV-B	32.12±0.45	12.32±1.45	28.97±0.26	12.35±0.37	64.48±1.55	46.48±1.55	54.47±1.73	40.35±0.37
2	FOLA	Control	36.71±1.36	21.12±0.44	33.46±0.56	16.86±1.38	68.97±0.65	47.63±2.35	57.63±1.28	48.26±0.26
		UV-B	16.86±0.68	11.18±0.56	17.95±1.62	15.43±0.24	37.67±0.24	42.26±0.87	35.43±0.35	37.86±1.36
3	NS-634	Control	41.25±1.76	35.47±3.06	34.53±1.56	16.88±0.43	57.26±1.67	46.15±0.8	57.54±0.67	52.17±0.25
		UV-B	17.65±0.85	13.46±0.67	19.96±0.77	14.76±0.95	35.62±2.56	34.57±1.65	38.65±0.46	38.24±1.16

Table No.3: Frequency of abnormal stomata and dead cells in the leaves of three varieties of 30 DAS *Vigna mungo* (L.) Hepper under control and supplementary UV-B exposed conditions

S.No	Varieties	Treatment	Frequency of abnormal stomata (mm^{-2})		Frequency of dead epidermal cells (mm^{-2})	
			Adaxial	Abaxial	Adaxial	Abaxial
1	GOWMATHI	Control	-	-	-	-
		UV-B	53.47±1.46	47.16±0.17	88.97±0.67	82.23±2.35
2	FOLA	Control	-	-	-	-
		UV-B	56.27±0.35	63.45±1.34	72.65±0.18	67.78±0.26
3	NS-634	Control	-	-	-	-
		UV-B	46.85±2.46	41.63±0.28	83.86±0.37	82.55±1.14

Table No.4: Changes in the frequency and length of trichomes in the leaves of three varieties of 30 DAS *Vigna unguiculata* (L.) Walp. under control and supplementary UV-B exposed conditions

S.No	Varieties	Treatment	Trichome frequency (mm^{-2})		Trichome length (μm)	
			Adaxial	Abaxial	Adaxial	Abaxial
1	GOWMATHI	Control	15.72±0.78	14.26±1.45	75.76±1.28	77.25±0.53
		UV-B	31.82±0.55	26.85±0.28	92.84±1.43	76.63±1.17
2	FOLA	Control	15.76±1.84	14.63±1.67	84.36±0.86	84.84±0.87
		UV-B	38.93±0.67	37.85±0.67	108.74±0.76	85.34±0.47
3	NS-634	Control	18.94±0.83	18.87±0.16	84.63±1.67	86.73±1.53
		UV-B	31.43±1.12	27.23±1.46	10.83±1.56	84.35±0.45

Table No.5: Changes in anatomical characteristics of leaves of three varieties of 30 DAS *Vigna unguiculata* (L.) Walp. under control and supplementary UV-B exposed conditions

S.No	Varieties	Treatment	Cuticle thickness (μm)		Epidermis thickness (μm)		Mesophyll thickness (μm)	Leaf thickness (μm)
			Adaxial	Abaxial	Adaxial	Abaxial		
1	GOWMATHI	Control	36.24 ± 0.57	44.68 ± 0.66	71.52 ± 1.67	87.38 ± 0.85	148.54 ± 0.27	274.56 ± 2.53
		UV-B	75.69 ± 0.54	87.64 ± 1.24	174.32 ± 1.45	168.86 ± 2.47	205.73 ± 1.32	487.45 ± 0.34
2	FOLA	Control	38.53 ± 1.42	34.24 ± 1.53	73.84 ± 0.56	78.75 ± 0.53	155.46 ± 0.56	265.76 ± 1.54
		UV-B	78.25 ± 1.44	96.47 ± 0.56	164.25 ± 1.79	173.44 ± 0.56	227.83 ± 1.55	456.24 ± 0.84
3	NS-634	Control	16.61 ± 0.94	20.33 ± 1.93	36.23 ± 1.93	54.24 ± 0.55	146.55 ± 0.64	205.73 ± 1.37
		UV-B	54.63 ± 0.47	76.83 ± 0.85	143.35 ± 0.78	148.65 ± 1.52	214.45 ± 0.62	405.23 ± 0.73

Table No.6: The similarity indices in epidermal and anatomical characteristics of three varieties of *Vigna unguiculata* (L.) Walp. under supplementary UV-B exposed conditions

S.No	Varieties	VAMBAN-3	NIRMAL-7	T-9
1	VAMBAN-3	100%	56.2%	52.8%
2	NIRMAL-7	56.2%	100%	60%
3	T-9	52.8%	60%	100%

Plate No.1: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. GOWMATHI under control condition and supplementary UV-B radiation exposure. (Figure No.3 to 8: 400 x)

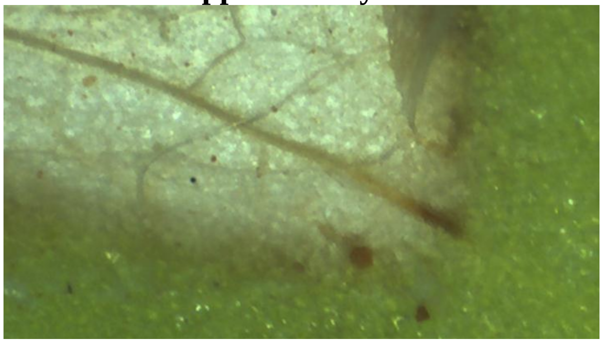


Figure No.1: Shiny adaxial surface under UV-B

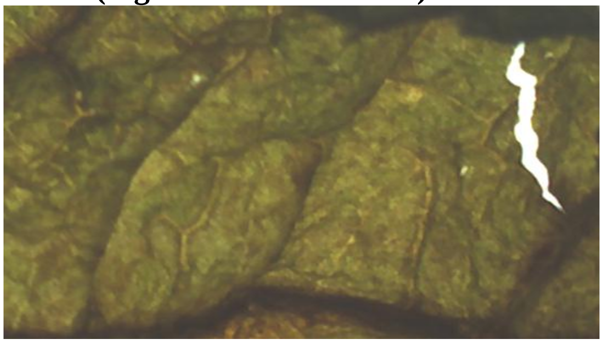


Figure No.2: UV-B adaxial - Brittle and dead

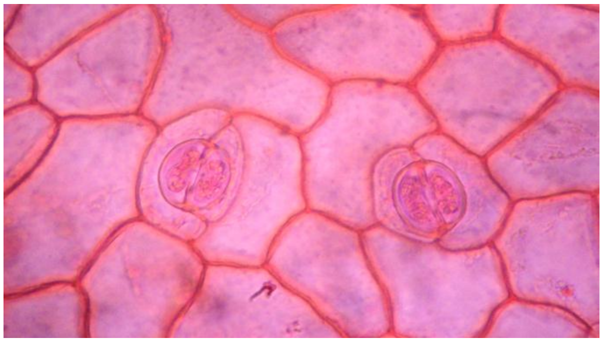


Figure No.3: Control adaxial - Normal stomata

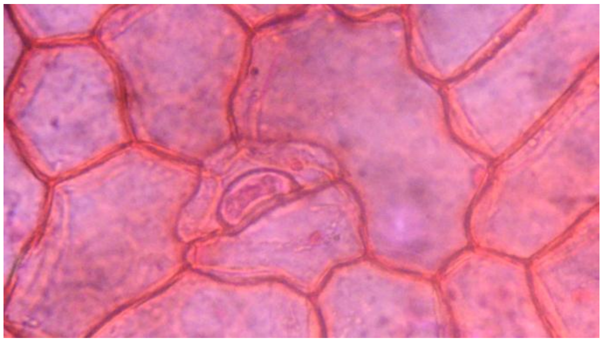


Figure No.4: UV-B adaxial - Single guard cell

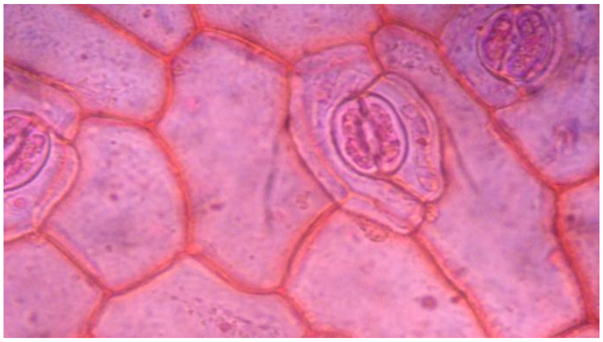


Figure No.5: Control abaxial - Normal stomata

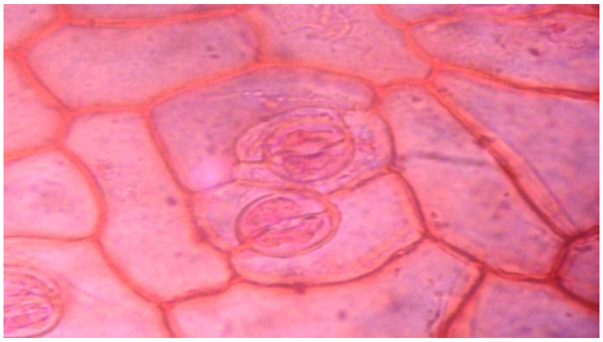


Figure No.6: UV-B abaxial - Contiguous stomata

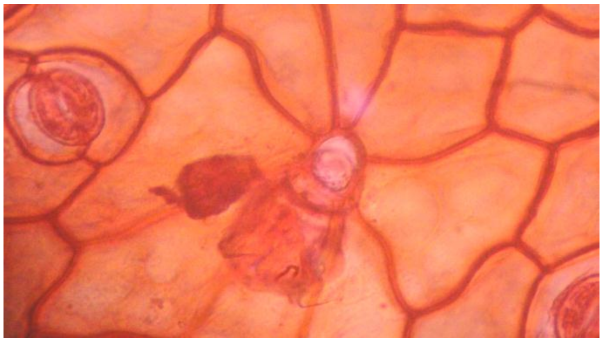


Figure No.7: UV-B adaxial - Broken trichome

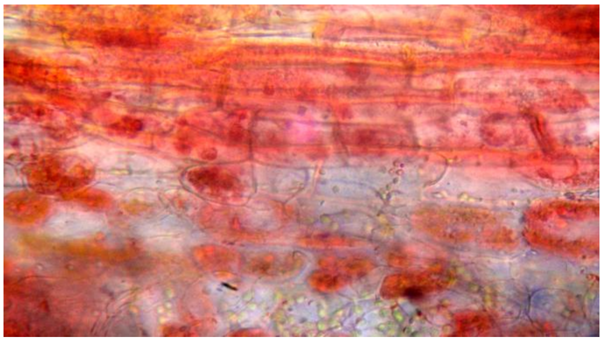


Figure No.8: UV-B adaxial - Multiseriate epidermis

Plate No.2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. FOLA under control condition and supplementary UV-B radiation exposure. (Figure No.3 to 8: 400 x)

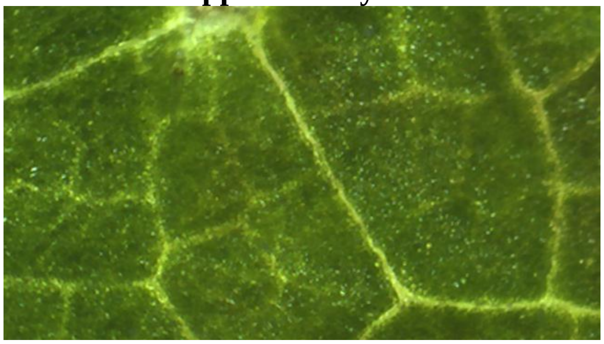


Figure No.1: Shiny adaxial surface under UV-B

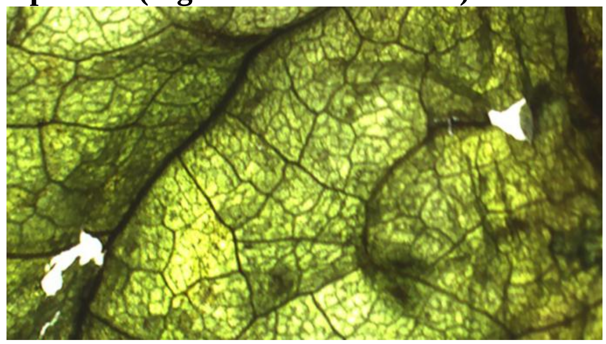


Figure No.2: UV-B adaxial - Brittle and dead

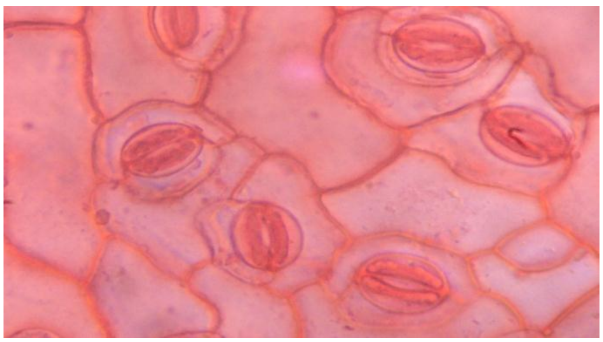


Figure No.3: Control adaxial - Normal stomata

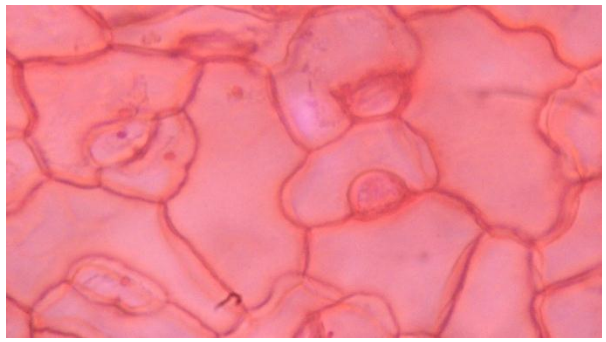


Figure No.4: UV-B adaxial - Persistant initial

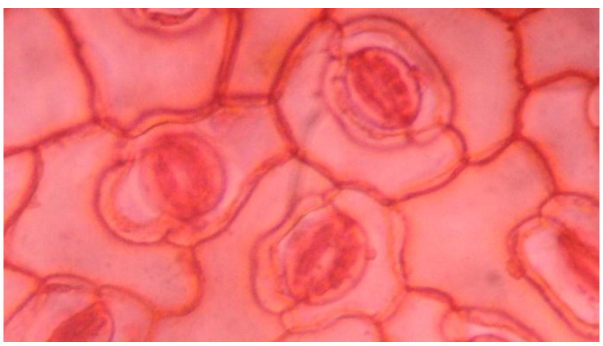


Figure No.5: Control abaxial - Normal stomata

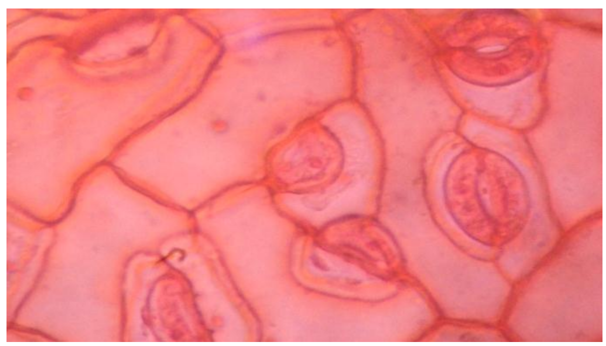


Figure No.6: UV-B abaxial - Single guard cell

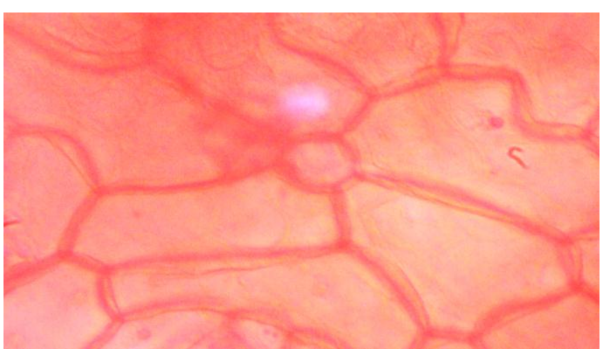


Figure No.7: UV-B adaxial - Broken trichome

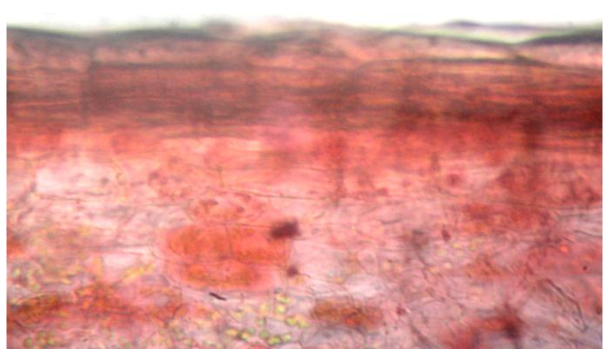


Figure No.8: UV-B adaxial - Multiseriate epidermis

Plate No.3: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna unguiculata* (L.) Walp. var. NS-634 under control condition and supplementary UV-B radiation exposure. (Fig. 3 to 8: 400 x)

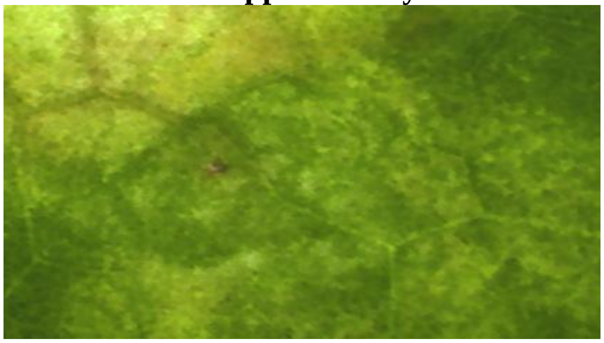


Figure No.1: Shiny adaxial surface under UV-B

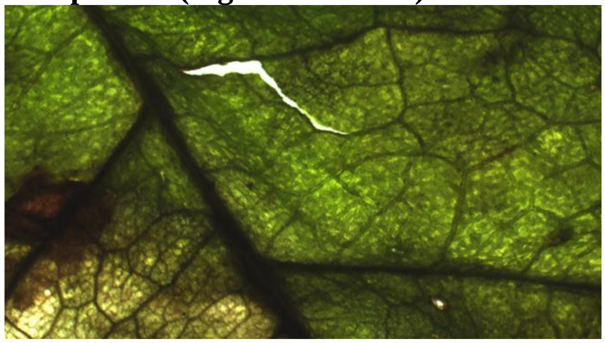


Figure No.2: UV-B adaxial - Brittle and dead

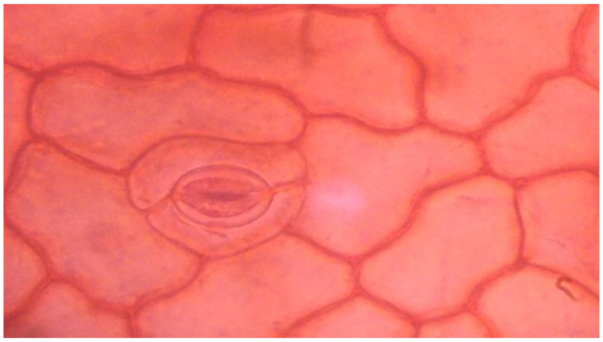


Figure No.3: Control adaxial - Normal stomata

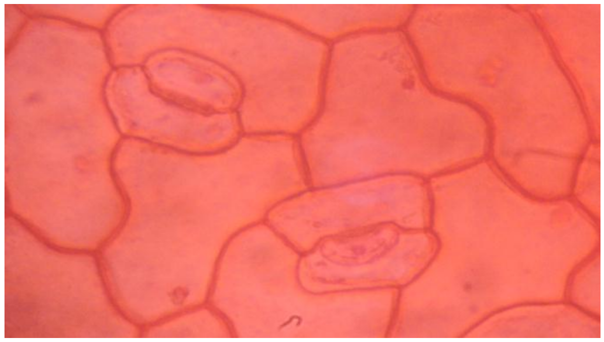


Figure No.4: UV-B adaxial - Single guard cell

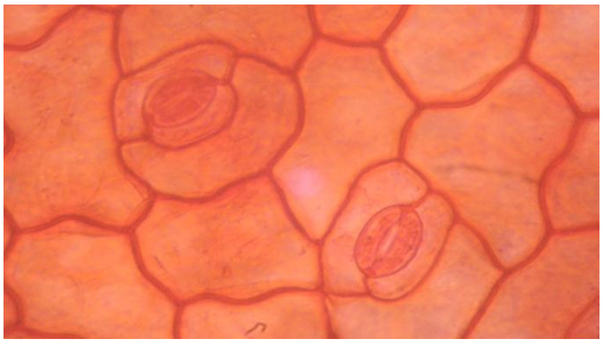


Figure No.5: Control abaxial - Normal stomata

Figure No.6: UV-B abaxial - Single guard cell

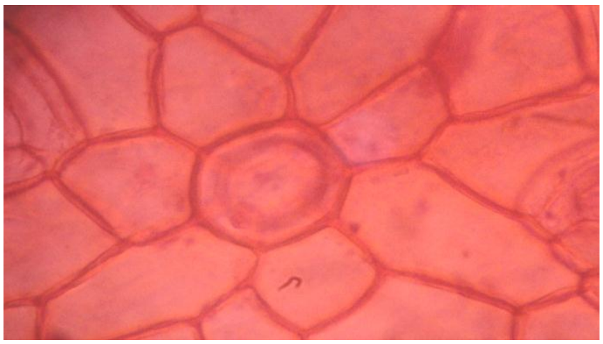


Figure No.7: UV-B adaxial - Broken trichome

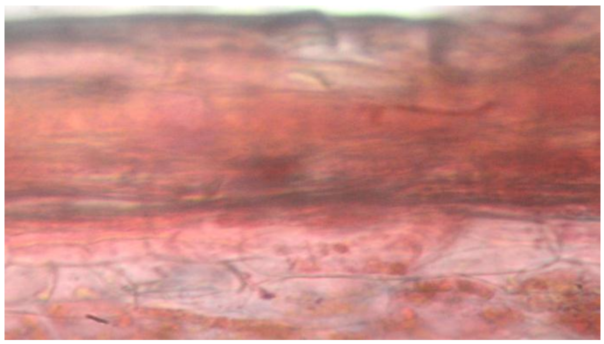
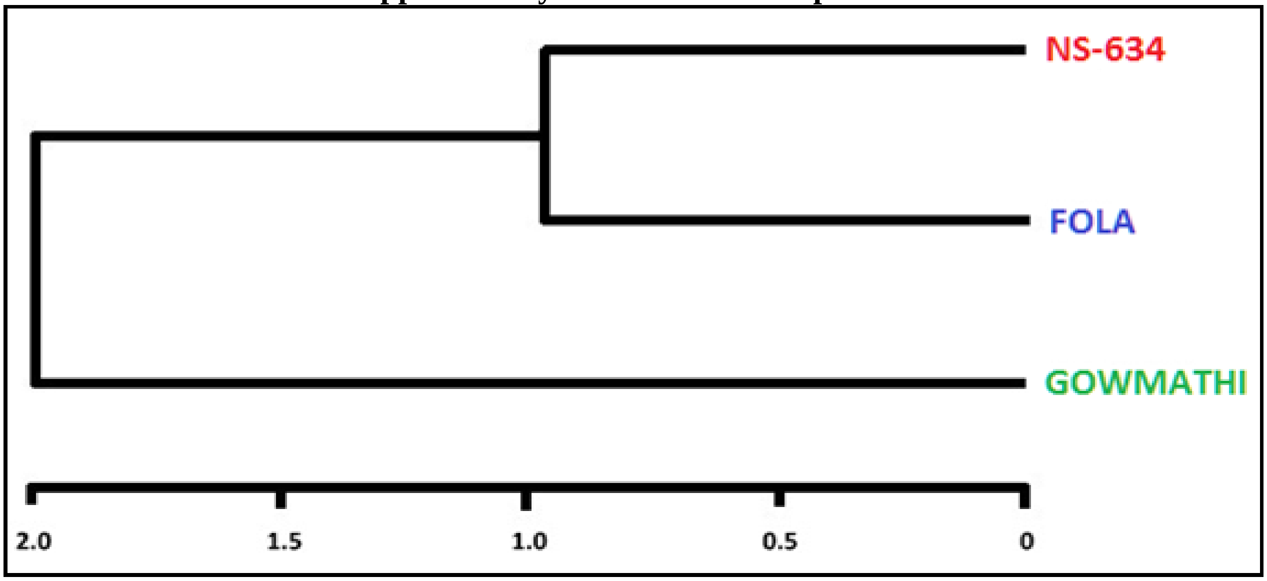


Figure No.8: UV-B adaxial - Multiseriate epidermis

Plate No.4: Dendrogram showing the interrelationship between three varieties of *Vigna unguiculata* (L.) Walp. in epidermal characteristics under control condition and supplementary UV-B radiation exposure



CONCLUSION

The changes in leaf architecture recorded in three varieties of cowpea provided evidence for the defence mechanism performed by the leaves suffering under ultraviolet-B impact, as they responded quickly by increasing the thickness of the cuticle, epidermis, frequency of trichomes and the volume of internal organs to create additional mechanical barrier to prevent UV-B penetration.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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