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### INFLUENCE OF ELEVATED ULTRAVIOLET-B RADIATION ON FOLIAR ORGANISATION IN GREEN GRAM VARIETIES

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#### ABSTRACT

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. Cooler temperature favours depletion reaction in ozone layer, allowing enormous ultraviolet-B (UV-B) radiation into Earth's surface, affecting the plants in the biosphere. The present study was carried out to compare the effects of ultraviolet-B (UV-B) radiation on the foliar morphology, epidermis and the anatomy of three varieties of green gram viz., CO-8, NVL-585 and VAMBAN-2. Fully developed third trifoliate leaves from the top of 30 DAS (days after seed germination) green gram varieties under *in situ* supplementary UV-B radiation (2 hours daily @  $12.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ ; ambient =  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) were excised for assessment. UV-B stress induced changes in the leaf morphology and caused serious injuries which were not recorded in normal plants. The adaxial and abaxial surfaces of epidermis exhibited several changes after UV-B exposure. On prolonged irradiation with UV-B, leaves exhibited various kinds of abnormalities. Due to UV-B irradiation CO-8, NVL-585 and VAMBAN-2 developed very thick leaves. Thickness of cuticle, epidermis, leaf and mesophyll and volume of mesophyll enhanced in all varieties under UV-B exposure. UV-B irradiated leaves developed many stomatal abnormalities. Abnormal stomata like, stomata with single guard cell, reduced size and persistent stomatal initial were more along with dead epidermal cells on the adaxial surface of UV-B irradiated green gram crops. Such aberrations were absent in leaves of control crops. The leaves of green gram varieties developed structural barriers to screen UV-B radiation, by increasing cuticle thickness, epidermal layers, trichome frequency and volume of the internal organs, but reducing leaf size and area.

#### KEY WORDS

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

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#### INTRODUCTION

The amount of UV-B in present-day sunlight is enough to affect the physiological and developmental processes of plants. As the leaves develop mechanisms to reduce these effects and a limited ability to adapt to increased levels of UV-B, leaf architecture can be directly affected by UV-B radiation. Ozone depletion pumps enormous ultraviolet-B (UV-B) radiation (280-320 nm) into the

Earth's surface which is a dangerous atmospheric stress to plants<sup>1</sup> as it affects leaf epidermis<sup>2-12</sup>, causes abnormalities in cotyledonary epidermis<sup>13-17</sup>, suppresses photosynthesis<sup>18-20</sup>, stunts plant growth<sup>21-29</sup>, reduces yield<sup>23,25,30-34</sup>, and reduces nodulation and nitrogen metabolism<sup>35-47</sup>, in sensitive 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<sup>48-50</sup>. In this study the changes in the foliage of green gram varieties under *in situ* UV-B stress was discussed.

## MATERIAL AND METHODS

### *In situ* UV-B irradiation

Green gram (*Vigna radiata* (L.) Wilczek.), the nitrogen fixing grain legume was chosen for the study. Viable seeds of the three varieties of green gram viz. CO-8, NVL-585 and VAMBAN-2 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  $38 \pm 2$  °C, night temperature minimum  $18 \pm 2$  °C, relative humidity  $60 \pm 5$  %, maximum irradiance (PAR)  $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 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<sub>BE</sub>) of  $12.2 \text{ kJ m}^{-2} \text{d}^{-1}$  equivalents to simulated 20 % ozone depletion at Pondicherry ( $12^{\circ}2' \text{N}$ , India). The control plants, grown under natural solar radiation, received UV-B<sub>BE</sub>  $10 \text{ kJ m}^{-2} \text{d}^{-1}$ .

### 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<sup>51</sup> 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) =  $S / S + E \times 100$  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  $\mu\text{m}$ .

### Dendrogram

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

Total number of similar characters

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

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

## RESULTS AND DISCUSSION

### Leaf epidermis

In all the varieties of green gram the leaves were small, wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface after UV-B irradiation (Plate No.1 to 3, Figure No.1, 2). On the adaxial surface of normal leaves the costal cells are uniformly similar in being axially elongated, thin and straight walled (Plate No.1 to 3, Figure No.3). The costal cells and trichomes on adaxial surface differ from abaxial

surface in being shorter in length. In general, the intercostal epidermal cells both on abaxial and adaxial surfaces of three varieties are sinuous and thin walled with unicellular trichomes occurring intermittently. The epidermal cells with dense, deeply stained nuclei were recorded in control and in all the UV-B irradiated leaves of green gram varieties (Plate No.1 to 3, Figure No.3 to 6). Frequency of epidermal cells was higher over control in UV-B exposed plants (Table No.1). The epidermal cell frequency was 29.75 % to 82 % more under UV-B irradiation but the effect was subdued on the abaxial side compared to adaxial surface. Epidermal cells in UV-B irradiated leaves were smaller in all green gram varieties by 10.93 % to 36.82 % on the adaxial surface followed by abaxial surface (2 % to 19.29 %). Under UV-B irradiation, stomata were smaller by 4.20 % to 63.76 % than control on both surfaces of the foliage (Table No.2; Plate No.1 to 3; Figure No.3 to 6). However, after UV-B exposure stomatal frequency, stomatal index and S / E ratio increased significantly above control by 33.94 % to 84.31 % on the adaxial surface and by 15.43 % to 74.04 % on the abaxial surface, the maximum enhancement being in VAMBAN-2 (Table No.1). Very deeply stained dead and collapsed epidermal cells occurred more (73.35 % to 87.55 %) on the adaxial as well as on the abaxial leaf surfaces of UV-B exposed plants (Table No.3; Plate No.1 to 3, Figure No.4, 6). Epidermis on the adaxial surface recorded damages in the form of collapsed cells, the leaves becoming glazed showing signs of bronzing of tissue surfaces due to formation of oxidised phenolic compounds<sup>55</sup> and in some cases also be followed by tissue degradation<sup>56</sup>.

### Trichomes

Unicellular thin walled trichomes were present in the costal as well as intercostal regions of both the surfaces. The costal cells and trichomes on adaxial surface differ from abaxial surface in being shorter in length (Table No.4). UV-B exposure increased the trichome frequency by 48.56 % to 162 % in all varieties compared to their controls, especially on the adaxial surface (Table No.4). Longer trichomes (11 % to 24 %) along with broken ones were observed more on the adaxial side of UV-B

irradiated leaves. However, NVL-585 had trichomes shorter (8.24 %) than the control on the adaxial surface (Table No.4; Plate No.1 to 3, Figure No.7). The length of trichomes on the abaxial surface of stressed leaves was more or less equal to that of control plants (Table No.4). Leaves use trichomes for several functions viz., mechanical barrier against biotic attack<sup>57-59</sup>, additional resistance to the diffusion of water vapour from the leaf interior to the atmosphere<sup>60</sup> and reflector reducing the radiant energy absorbed by the leaf<sup>20,61</sup>. Non-glandular hairs also offer additional shield to UV-B penetration by reflecting the radiant energy<sup>2</sup>. However, in contrary to increased trichome frequency which could have been an adaptive feature to UV-B irradiation, Karabourniotis *et al.*<sup>62</sup> observed reductions in trichome number due to UV-B treatment.

### Leaf anatomy

The thickness of cuticles in UV-B exposed leaves both on upper and lower sides increased significantly over control by 142 % to 302 % (Table No.5). In UV-B irradiated plants, the epidermis was markedly thicker than the control on both the sides of the leaf by 87.67 % to 175.18 %, the maximum thickness being on upper epidermis (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 (16.48 % to 85 %) also (Table No.5). With the mesophyll becoming voluminous, a thicker leaf would result<sup>20</sup>. The highest values for leaf thickness were for UV-B irradiated VAMBAN-2 followed by CO-8 and NVL-585 (Table No.5). According to Caldwell *et al.*<sup>1</sup> and Wellmann<sup>57</sup>, 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. Increased leaf and cuticle thickness reduces UV-B penetration to internal tissues<sup>20,48</sup> alleviating the deleterious effects. Leaves of *Medicago sativa* became thick due to addition of spongy mesophyll cells, while in *Brassica campestris* there was an increase in the number of palisade cells<sup>20,48</sup>. According to Kokilavani and Rajendiran<sup>2</sup>, Rajendiran<sup>20</sup> and Bornman and Vogelmann<sup>48</sup> and 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.

### Stomatal abnormalities

Abnormal stomata were more frequent on UV-B treated leaves, the maximum being on the adaxial surface. Stomatal abnormalities in UV-B irradiated leaves include 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). No such abnormalities were recorded in the leaves of the crops grown in control conditions (Table No.3; Plate 1 to 3, Figure No.3, 5). Similar results were reported in tobacco<sup>63</sup>, *Vigna unguiculata* (L.) Walp. cv. BCP-25<sup>3</sup>, *Cucumis sativus* L. var. CO-1<sup>4</sup>, *Vigna mungo* L. var. KM-2<sup>5</sup>, *Vigna unguiculata* (L.) Walp. cv. CW-122<sup>7</sup>, *Vigna unguiculata* (L.) Walp. cv. COVU-1<sup>8</sup>, *Vigna unguiculata* (L.) Walp. cv. COFC-8<sup>9</sup>, *Vigna unguiculata* (L.) Walp. cv. Vamban<sup>10</sup>, *Vigna unguiculata* (L.) Walp. cv. CO-6<sup>11</sup>, *Vigna unguiculata* (L.) Walp. cv. CO-1<sup>12</sup>, *Vigna*

*unguiculata* (L.) Walp. cv. CO-3<sup>39</sup>, *Vigna unguiculata* (L.) Walp. cv. Pudukai<sup>50</sup>, *Vigna unguiculata* (L.) Walp. cv. KM-1<sup>64</sup> and in *Vigna unguiculata* (L.) Walp. cv. COVU-2<sup>65</sup> on the adaxial side of the leaves after exposure to UV-B. Many types of stomatal abnormalities and epidermal cell injuries were also reported in the cotyledonary epidermis of F<sub>1</sub> seedlings grown after harvesting from ultraviolet-B irradiated parents of *Momordica charantia* L.<sup>13</sup>, *Benincasa hispida* (Thunb.) Cogn.<sup>16</sup> and *Macrotyloma uniflorum* (Lam.) Verdc.<sup>17</sup>.

### Dendrogram

The epidermal parameters recorded on 30 DAS in three varieties of green gram showed differences in epidermal and stomatal number, epidermal cell and stomatal size, including frequency of abnormal stomata and dead epidermal cells after irradiation with supplementary UV-B. NVL-585 and VAMBAN-2 with 60.83 % similarity between them remained together as one group. On the other hand, CO-8 remained alone in the cluster showing more or less identical similarity values with NVL-585 (58.33 %) and VAMBAN-2 (56.66 %) (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 radiata* (L.) Wilczek under control and supplementary UV-B exposed conditions**

S.No	Varieties	Treatment	Stomatal frequency (mm <sup>-2</sup> )		Epidermal cell frequency (mm <sup>-2</sup> )		Stomatal index		S/E ratio	
			Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
1	CO-8	Control	163.82±2.23	158.45±0.56	302.67±3.45	303.56±0.97	31.67±0.78	31.94±1.13	0.51	0.50
		UV-B	219.43±0.42	217.65±0.47	471.82±0.52	393.87±0.66	48.68±1.89	38.37±0.67	0.43	0.57
2	NVL-585	Control	130.83±0.86	142.74±0.76	322.55±0.39	316.34±0.64	33.14±0.62	30.27±0.77	0.40	0.46
		UV-B	241.14±1.17	225.89±0.56	441.45±2.34	425.81±0.59	45.91±0.38	41.98±0.35	0.54	0.51
3	VAMBAN-2	Control	102.26±1.45	118.45±0.34	238.69±0.27	227.6±1.21	23.86±1.27	22.27±0.19	0.46	0.59
		UV-B	146.27±0.66	136.73±0.55	434.23±1.67	389.20±0.75	42.72±0.78	38.76±1.26	0.35	0.36

**Table No.2: Changes in the size of stomata and epidermal cells in the leaves of three varieties of 30 DAS *Vigna radiata* (L.) Wilczek 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	CO-8	Control	57.23±0.38	42.75±1.83	52.46±0.46	44.27±0.14	87.53±0.27	54.48±1.97	85.42±0.36	47.84±2.54
		UV-B	33.26±0.84	12.27±0.28	24.48±1.67	19.76±2.45	61.23±0.83	45.56±1.15	63.19±2.35	38.65±0.48
2	NVL-585	Control	42.93±1.34	21.42±0.26	42.45±0.47	18.56±1.53	79.43±1.34	42.45±0.66	82.5±1.27	42.96±1.13
		UV-B	23.47±0.56	14.59±0.27	23.65±2.89	17.78±1.78	57.65±0.67	34.54±0.65	52.12±0.74	42.07±2.45
3	VAMBAN-2	Control	42.37±3.62	22.6±0.49	41.66±0.73	17.85±1.27	78.88±1.07	42.86±0.27	58.91±3.77	48.87±0.78
		UV-B	24.83±0.37	16.62±2.55	26.83±1.77	14.64±0.47	56.45±1.34	39.32±2.49	52.47±0.59	47.88±0.15

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

S.No	Varieties	Treatment	Frequency of abnormal stomata (mm <sup>-2</sup> )		Frequency of dead epidermal cells (mm <sup>-2</sup> )	
			Adaxial	Abaxial	Adaxial	Abaxial
1	CO-8	Control	-	-	-	-
		UV-B	63.39±0.55	7.66±1.65	87.04±1.27	76.28±0.54
2	NVL-585	Control	-	-	-	-
		UV-B	47.78±2.73	45.52±0.76	74.35±0.73	73.35±0.43
3	VAMBAN-2	Control	-	-	-	-
		UV-B	25.76±0.78	33.78±0.86	87.55±1.44	83.33±0.56

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

S.No	Varieties	Treatment	Trichome frequency (mm <sup>-2</sup> )		Trichome length (µm)	
			Adaxial	Abaxial	Adaxial	Abaxial
1	CO-8	Control	16.76±1.56	16.05±0.17	76.68±1.18	74.87±0.38
		UV-B	38.93±0.36	31.09±1.87	85.16±0.38	75.62±0.42
2	NVL-585	Control	14.34±1.66	13.26±0.23	94.68±1.37	70.72±1.57
		UV-B	37.55±0.26	31.92±0.67	86.87±0.98	74.91±1.19
3	VAMBAN-2	Control	18.76±0.75	15.98±0.55	77.73±0.48	73.36±0.28
		UV-B	27.87±1.38	27.56±0.65	96.38±0.38	74.28±0.35

**Table No.5: Changes in anatomical characteristics of leaves of three varieties of 30 DAS *Vigna radiata* (L.) Wilczek 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	CO-8	Control	31.22 ± 0.95	24.46 ± 0.2	55.66 ± 0.28	53.68± 1.29	184.55 ± 1.65	266.69 ± 0.37
		UV-B	75.82 ± 0.46	70.48 ± 0.81	111.64 ± 0.93	119.73 ± 1.45	233.62 ± 2.22	493.63 ± 1.17
2	NVL-585	Control	25.42± 1.45	22.14 ± 3.27	48.42 ± 3.43	63.64 ± 0.45	212.41 ± 0.34	297.36 ± 0.75
		UV-B	102.26 ± 0.28	79.26± 0.63	133.24± 2.92	130.17 ± 1.26	247.41 ± 0.66	475.66 ± 0.42
3	VAMBAN-2	Control	32.85± 0.59	24.56 ± 1.64	75.84 ± 0.87	71.73 ± 2.47	161.83 ± 0.97	265.86 ± 1.65
		UV-B	112.18 ± 0.83	97.81 ± 0.95	142.33 ± 0.74	157.12± 0.67	274.52 ± 0.27	490.88 ± 0.97

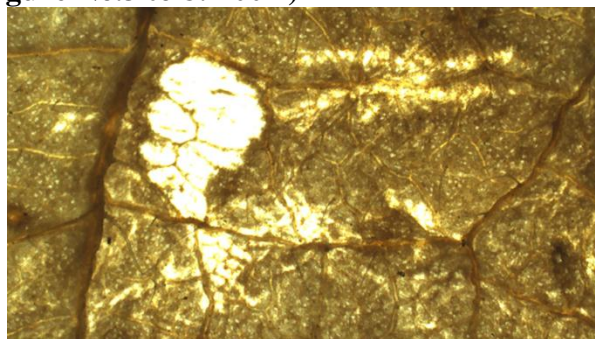
**Table No.6: The similarity indices in epidermal and anatomical characteristics of three varieties of *Vigna radiata* (L.) Wilczek under supplementary UV-B exposed conditions**

S.No	Varieties	CO-8	NVL-585	VAMBAN-2
1	CO-8	100%	58.33%	56.66%
2	NVL-585	58.33%	100%	60.83%
3	VAMBAN-2	56.66%	60.83%	100%

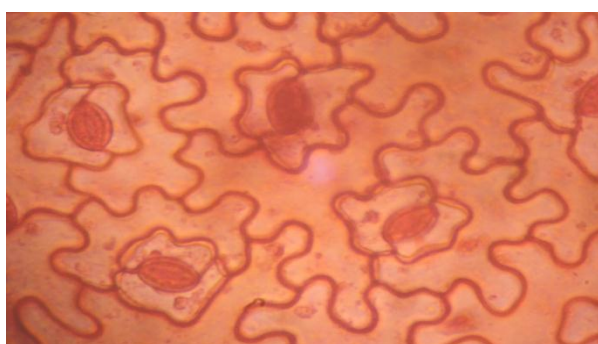
**Plate No.1: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna radiata* (L.) Wilczek var. CO-8 under control condition and supplementary UV-B radiation. (Figure No.3 to 8: 400 x)**



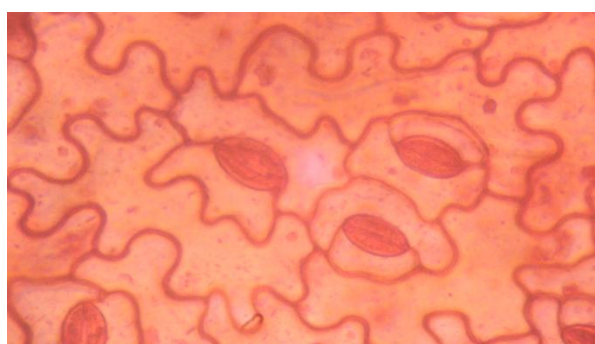
**Figure No.1: Shiny adaxial surface under UV-B**



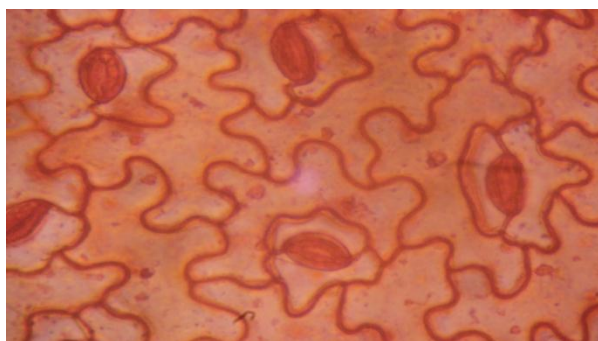
**Figure No.2: UV-B adaxial - Brittle and dead**



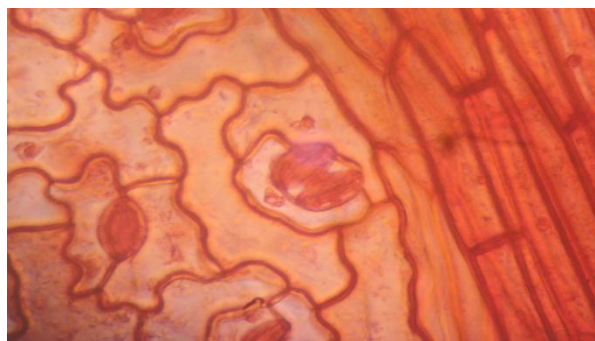
**Figure No.3: Control adaxial - Normal stomata**



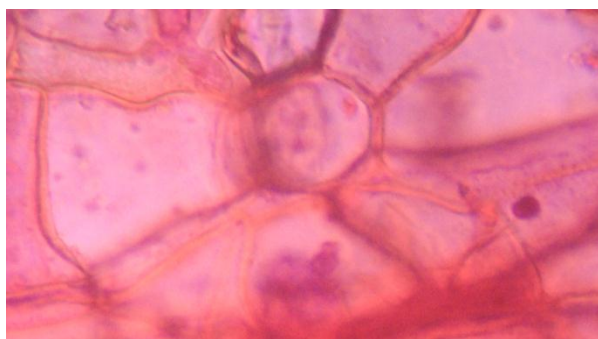
**Figure No.4: UV-B adaxial - Contiguous stomata**



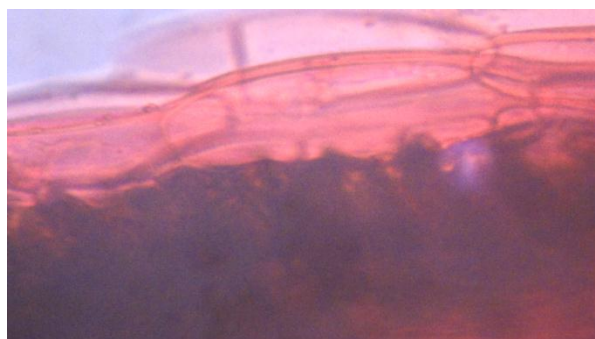
**Figure No.5: Control abaxial - Normal stomata**



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

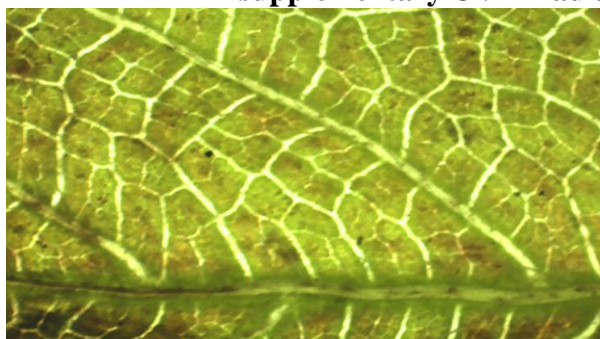


**Figure No.7: UV-B adaxial - Broken trichome**

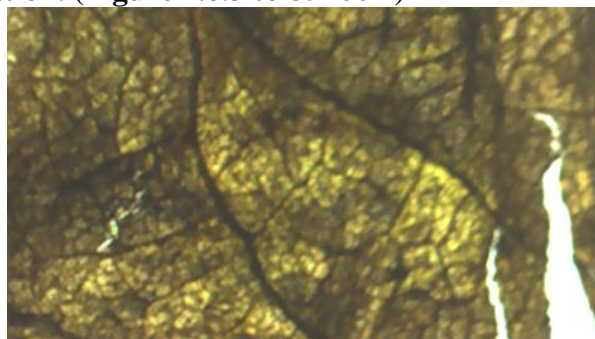


**Figure No.8: UV-B adaxial - Multiseriate epidermis**

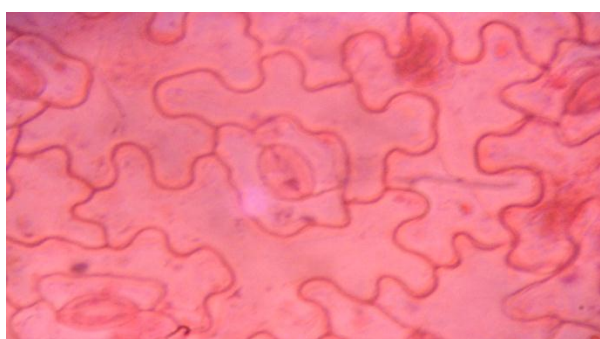
**Plate No.2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna radiata* (L.) Wilczek var. NVL-585 under control condition and supplementary UV-B radiation. (Figure No.3 to 8: 400 x)**



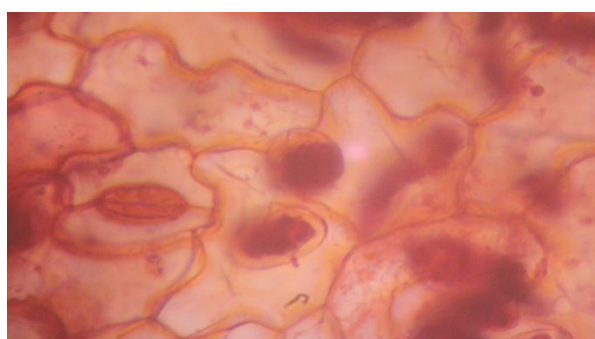
**Figure No.1: Shiny adaxial surface under UV-B**



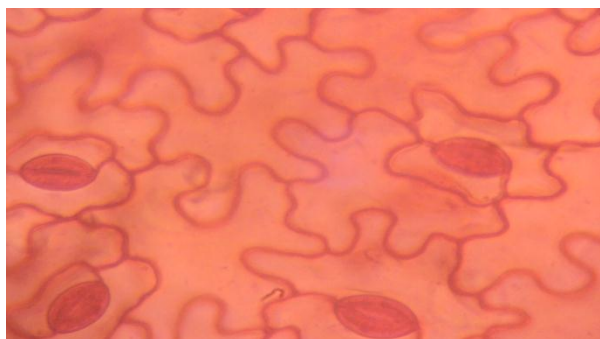
**Figure No.2: UV-B adaxial - Brittle and dead**



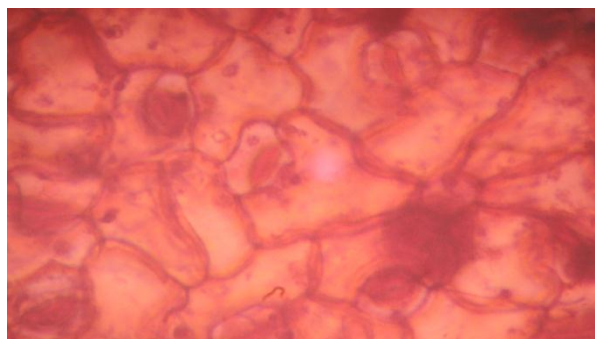
**Figure No.3: Control adaxial - Normal stomata**



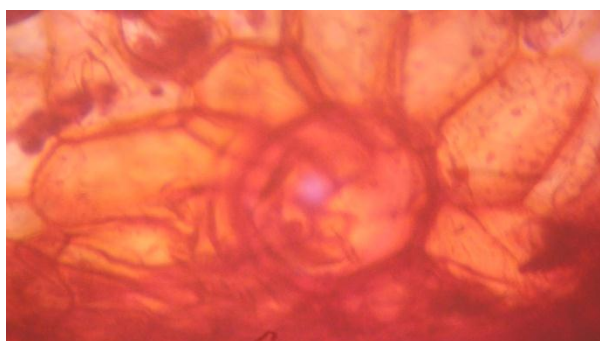
**Figure No.4: UV-B adaxial - Persistent initials**



**Figure No.5: Control abaxial - Normal stomata**



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

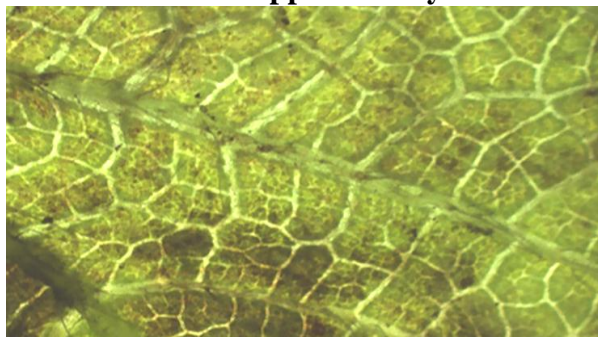


**Figure No.7: UV-B adaxial - Broken trichome**

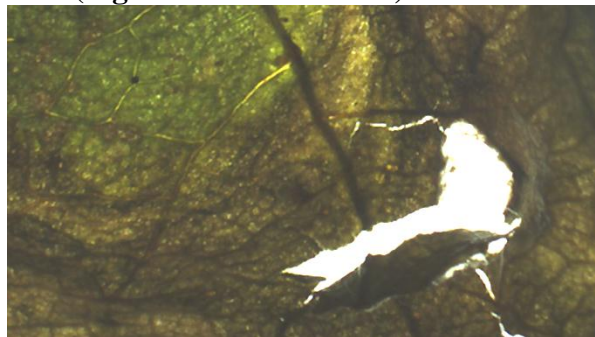


**Figure No.8: UV-B adaxial - Multiple epidermis**

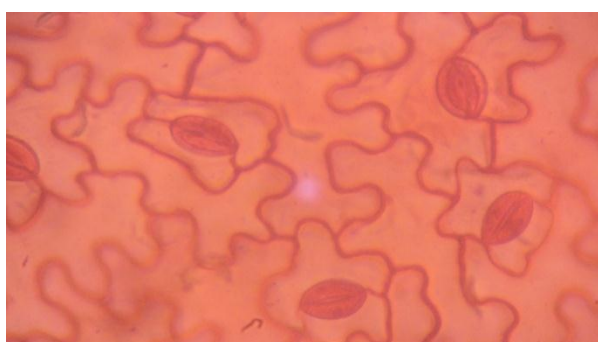
**Plate No.3: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna radiata* (L.) Wilczek var. VAMBAN-2 under control condition and supplementary UV-B radiation. (Figure No.3 to 8: 400 x)**



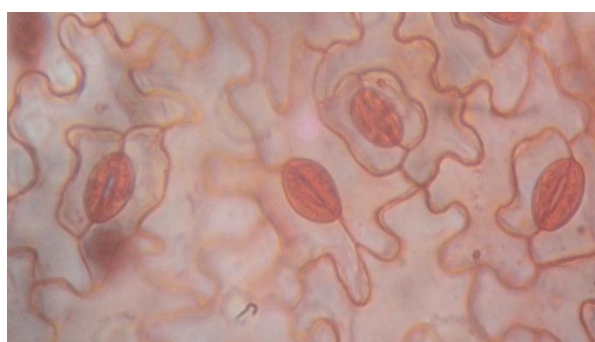
**Figure No.1: Shiny adaxial surface under UV-B**



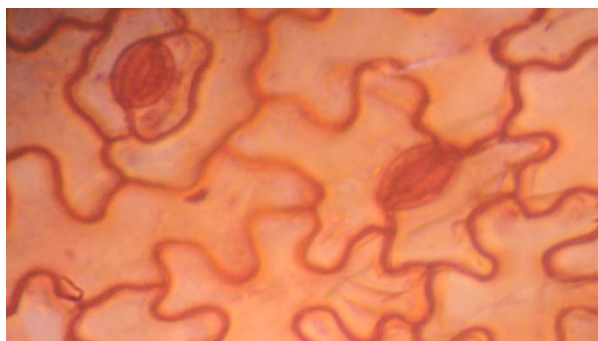
**Figure No.2: UV-B adaxial - Brittle and dead**



**Figure No.3: Control adaxial - Normal stomata**



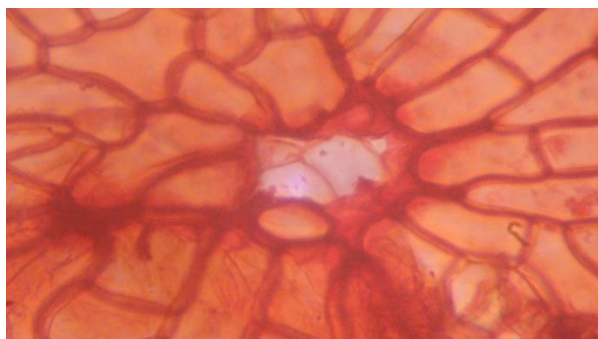
**Figure No.4: UV-B adaxial - Contiguous stomata**



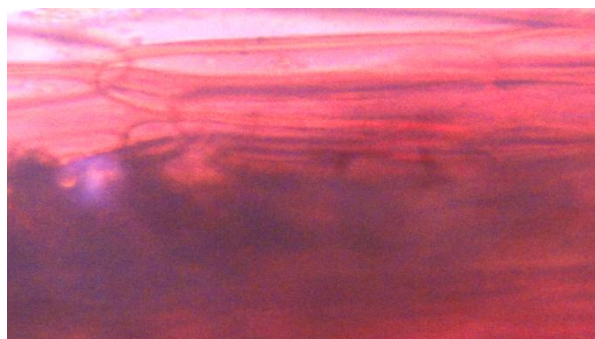
**Figure No.5: Control abaxial - Normal stomata**



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

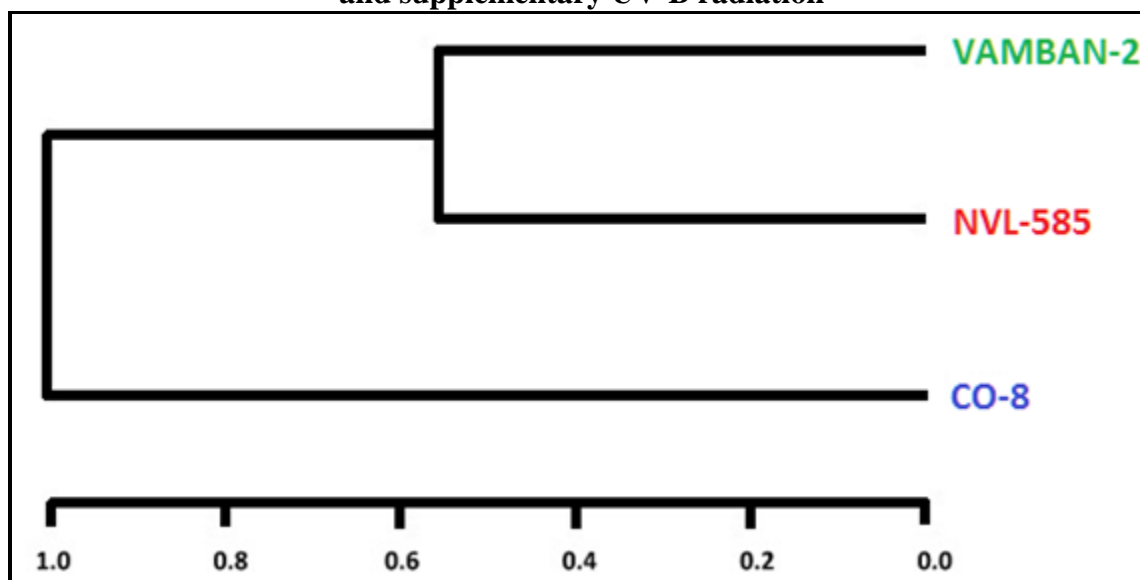


**Figure No.7: UV-B adaxial - Broken trichome**



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

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



## CONCLUSION

The three varieties of green gram in response to *in situ* UV-B stress reduced leaf size and area, and developed foliage with thicker cuticle, multiple epidermal layers, numerous trichomes and increased mesophyll volume to avoid UV-B penetration.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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