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# COMPARISON OF LEAF ARCHITECTURE OF THREE VARIETIES OF BLACK GRAM AFTER *IN SITU* ULTRAVIOLET-B EXPOSURE

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## ABSTRACT

The architecture of leaves depend upon the light intensity available in the habitat in which they grow. The nature of cuticle, epidermis and the mesophyll including the amount of chloroplasts in them are designed based on the surrounding environment. Any imposed stress would have a direct effect on the leaf structure as they form the vital organs performing photosynthesis. The present study aims at comparing the effects of ultraviolet-B (UV-B) radiation on the foliar morphology, epidermis and the anatomy of three varieties of black gram (*Vigna mungo* (L.) Hepper) *viz.* VAMBAN-3, NIRMAL-7 and T-9. Fully developed third trifoliate leaves from the top of 30 DAS (days after seed germination) black gram 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 excised for assessment. UV-B stress induced changes in the leaf morphology and caused several injuries which were not recorded in normal plants. UV-B irradiated VAMBAN-3, NIRMAL-7 and T-9 produced very thick leaves. Thickness of cuticle, epidermis, leaf and mesophyll and volume of mesophyll increased in all varieties under UV-B exposure. The epidermis both on the adaxial as well as abaxial surfaces exhibited many changes after UV-B exposure. UV-B irradiated leaves developed many stomatal abnormalities. Abnormal stomata like, stomata with single guard cell, reduced size, malformations were more along with dead epidermal cells on the adaxial surface of UV-B irradiated plants. Such aberrations were absent in leaves under control conditions. The three varieties of black gram in response to ultraviolet-B irradiation modified the leaf architecture creating several barriers to combat the stress.

## KEY WORDS

Abnormal stomata, Black gram, Leaf anatomy, Leaf epidermis, Leaf morphology, Three varieties and Ultraviolet-B.

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## INTRODUCTION

Ultraviolet-B (UV-B) radiation (280-320 nm) is the most energetic part of the daylight spectrum that has the potential to damage macromolecules such as DNA and proteins, generate reactive oxygen species (ROS) and impair cellular processes[1]. At the structural level it affects leaf epidermis[2-12] and causes abnormalities in cotyledonary epidermis[13-17] and at metabolic level suppresses photosynthesis[18-20], retarding growth[21-29], reducing harvest[23-34] and disturbs nodulation and nitrogen metabolism[35-47] in sensitive crops. As leaves are the organs that receive major amount of UV-B radiation, they react quickly to prevent its entry into the internal organs[48-50]. In this context, an experiment was conducted to study the defense mechanism brought about by black gram varieties in the foliage against UV-B radiation.

## MATERIAL AND METHODS

### *In situ* UV-B irradiation

Black gram (*Vigna mungo* (L.) Hepper) the nitrogen fixing grain legume was chosen for the study. Viable seeds of the three varieties of black gram *viz.* VAMBAN-3, NIRMAL-7 and T-9 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 black gram 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 black gram, 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 leaves of three varieties of black gram were small, wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface due to UV-B irradiation (Plate No.1,3, Figure No.1,2). On the adaxial surface of normal leaves of three varieties of black gram, the costal cells are uniformly similar in being axially elongated, thin and straight walled (Plate No.1,3, Figure No.3). 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 observed in control and in all the UV-B irradiated leaves of black gram varieties (Plate No.1,3, Figure No.3,6). Epidermal cell frequency was higher over control in UV-B exposed plants (Table No.1). Among the treatments the epidermal cell frequency was 28.64 % to 77.31 % more under UV-B stress but the effect was subdued on the abaxial side compared to adaxial surface. Analysis of epidermal cell size showed that the cells were smaller in all varieties of black gram by 9.30 % to 34 % on both the surfaces after UV-B irradiation. In UV-B irradiated plants stomata were smaller by 12.11 % to 59 % than control on both surfaces of the foliage (Table No.2; Plate No.1,3; Figure No.3,6). However, stomatal frequency, stomatal index and S / E ratio were increased significantly above control by UV-B (4.76 % to 71.92 %) on the adaxial surface as well as on the abaxial surface (6.15 % to 69.49 %), the maximum enhancement being in VAMBAN-3 (Table No.1). Very deeply stained dead and collapsed epidermal cells were found in large numbers (62.48 % to 90.78 %) on the adaxial as well as on the abaxial leaf surfaces of UV-B exposed plants (Table No.3; Plate No.1,3, Figure No.4,6). 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[55]. This may in some cases also be followed by tissue degradation[56].

**Trichomes**

Trichomes were unicellular, thin walled and found scattered 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 4). In control leaves, trichome frequency was comparatively less on the abaxial side than the adaxial side. UV-B exposure increased the trichome frequency by 22.45 % to 200 % in all varieties compared to their controls, especially on the adaxial surface (Table 4). Longer trichomes (11.86 % to 22.37 %) along with broken ones were observed more on the adaxial side of UV-B irradiated leaves (Table No.4, Plate No.1,3, Figure No.7). However, the length of trichomes on the abaxial surface of stressed leaves was less by 5.87 % to 16.29 % to that of control plants (Table No.4). The trichomes serve as a mechanical barrier against biotic attack[57,59], as an additional resistance to the diffusion of water vapour from the leaf[60] and as a reflector reducing thermal energy absorbed by the leaf[20,61]. These non-glandular hairs also provide additional mechanical barrier to UV-B transmission by reflecting the radiant energy[2]. 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*.[62]

**Leaf anatomy**

The cuticles of UV-B irradiated leaves both on upper and lower sides increased significantly in thickness over control by 52.46 % to 212.03 % (Table No.5). In UV-B stressed plants, the epidermis was thicker than the control on both the sides of the leaf by 58.71 % to 225.37 %, the maximum thickness being on upper epidermis (Table No.5, Plate No.1,3, Figure No.8). The trend seen in cuticle and epidermis thickness continued in leaf thickness, mesophyll thickness and mesophyll volume also (Table No.5). With increased volume of cells, a thicker leaf would result[20]. The highest values for leaf thickness were for UV-B irradiated VAMBAN-3 followed by NIRMAL-7 and T-9 (Table No.5). According to Caldwell *et al.*[1] and Wellmann[57], plants obstruct the 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. At the structural level, increased leaf and cuticle thickness reduces UV-B penetration to internal tissues[20,48] alleviating some of the deleterious effects. 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[48]. Kokilavani and Rajendiran[2], Rajendiran[20] and Bornman and Vogelmann[48] opined that greater thickness increased the amount of scattered light which could be due to low chlorophyll content, increased number of intercellular air spaces, cytoplasmic changes or altered cellular arrangements like the palisade becoming wider and cell layers increasing in number.

**Abnormalities**

Abnormal stomata were more frequent on UV-B exposed leaves, the maximum being on the adaxial surface. 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.1,3, Figure No.4,6). No such abnormalities were recorded in the leaves of the crops grown in control conditions (Table No.3, Plate No.1,3, Figure No.3,5). Similar results were reported in tobacco[63], in *Vigna unguiculata* (L.) Walp. cv. BCP-25[3], in *Cucumis sativus* L. var. CO-1[4], *Vigna mungo* L. var. KM-2[5], *Vigna unguiculata* (L.) Walp. cv. CW-122[7], *Vigna unguiculata* (L.) Walp. cv. COVU-1[8], *Vigna unguiculata* (L.) Walp. cv. COFC-8[9], *Vigna unguiculata* (L.) Walp. cv. Vamban[10], *Vigna unguiculata* (L.) Walp. cv. CO-6[11], *Vigna unguiculata* (L.) Walp. cv. CO-1[12], *Vigna unguiculata* (L.) Walp. cv. CO-3[39], *Vigna unguiculata* (L.) Walp. cv. Puduvai[50], *Vigna unguiculata* (L.) Walp. cv. KM-1[64] and in *Vigna unguiculata* (L.) Walp. cv. COVU-2[65] on the adaxial side of the leaves after exposure to UV-B radiation. Gowsalya *et al.*[13] in *Momordica charantia* L., Thiruvarasan *et al.*[16] in *Benincasa hispida* (Thunb.) Cogn. and Vidya *et al.*[17] in *Macrotyloma uniflorum* (Lam.) Verdc. observed several abnormalities in the cotyledonary epidermis of $F_1$ seedlings grown after harvesting from ultraviolet-B irradiated parent crops.

**Dendrogram**

The three varieties of black gram showed differences in parameters *viz.*, epidermal and stomatal number, epidermal cell and stomatal size, including frequency of abnormal stomata and dead epidermal cells after irradiation with supplementary UV-B on 30 DAS. The similarity index between NIRMAL-7 and T-9 was the highest with 60 %. NIRMAL-7 and T-9 as one group showed similarity value of 56.2 and 52.8 % respectively with VAMBAN-3 which remained separately in the cluster (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 mungo* (L.) Hepper 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 | VAMBAN-3 | Control | 182.72±5.65 | 170.16±0.23 | 263.43±3.59 | 287.43±0.44 | 27.33±0.38 | 28.46±0.88 | 0.63 | 0.57 |
| | | UV-B | 276.83±0.76 | 288.38±0.67 | 452.87±0.51 | 423.57±1.79 | 46.47±1.45 | 42.66±0.65 | 0.66 | 0.76 |
| 2 | NIRMAL-7 | Control | 211.28±0.76 | 224.14±0.92 | 238.86±0.39 | 257.54±1.48 | 25.67±0.88 | 25.17±1.34 | 0.74 | 0.72 |
| | | UV-B | 281.69±0.73 | 279.75±0.15 | 340.47±2.75 | 456.64±0.78 | 41.43±0.34 | 40.46±0.68 | 0.65 | 0.67 |
| 3 | T-9 | Control | 222.67±0.47 | 231.74±1.81 | 312.67±0.29 | 346.43±1.27 | 30.61±0.17 | 34.57±1.34 | 0.76 | 0.65 |
| | | UV-B | 298.63±0.37 | 312.55±0.56 | 478.28±1.93 | 445.68±1.67 | 48.54±1.27 | 43.56±1.56 | 0.64 | 0.69 |

**Table No.2: Changes in the size of stomata and epidermal 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 | Stomatal size (µm) | | | | Epidermal cell size (µm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Adaxial | | Abaxial | | Adaxial | | Abaxial | |
| | | | Length | Breadth | Length | Breadth | Length | Breadth | Length | Breadth |
| 1 | VAMBAN-3 | Control | 41.56±3.16 | 24.3±0.87 | 37.85±1.84 | 20.73±1.65 | 80.77±0.87 | 44.52±0.84 | 74.45±1.67 | 43.20±0.87 |
| | | UV-B | 20.12±1.53 | 11.70±0.45 | 24.32±1.28 | 16.68±0.67 | 60.78±2.12 | 37.67±1.32 | 57.12±0.27 | 37.56±0.18 |
| 2 | NIRMAL-7 | Control | 41.24±1.38 | 27.29±0.86 | 37.44±1.56 | 15.27±0.28 | 64.67±0.37 | 44.22±2.65 | 64.65±3.67 | 43.28±1.56 |
| | | UV-B | 16.87±0.17 | 15.58±1.76 | 23.31±1.67 | 13.39±0.68 | 42.68±0.79 | 34.27±0.65 | 44.37±1.76 | 34.58±0.56 |
| 3 | T-9 | Control | 45.36±3.12 | 24.58±1.69 | 40.28±0.64 | 21.14±0.28 | 89.42±0.37 | 46.56±1.37 | 77.68±2.56 | 48.23±0.67 |
| | | UV-B | 30.49±0.72 | 18.28±1.12 | 27.68±1.29 | 15.65±3.01 | 68.24±0.35 | 42.23±0.65 | 61.23±0.67 | 42.58±1.68 |

**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 | VAMBAN-3 | Control | - | - | - | - |
| | | UV-B | 34.78±0.44 | 28.18±1.53 | 77.65±1.77 | 72.56±1.22 |
| 2 | NIRMAL-7 | Control | - | - | - | - |
| | | UV-B | 40.24±1.43 | 41.35±0.56 | 62.48±2.56 | 66.36±0.78 |
| 3 | T-9 | Control | - | - | - | - |
| | | UV-B | 32.11±0.56 | 36.08±0.46 | 90.78±1.32 | 87.67±1.67 |

**Table No.4: Changes in the frequency and length of trichomes 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 | Trichome frequency ($mm^{-2}$) | | Trichome length (μm) | |
|---|---|---|---|---|---|---|
| | | | Adaxial | Abaxial | Adaxial | Abaxial |
| 1 | VAMBAN-3 | Control | 21.18±0.56 | 14.44±0.36 | 81.22±1.24 | 82.45±0.24 |
| | | UV-B | 34.42±0.65 | 31.32±1.21 | 92.23±0.56 | 76.45±0.15 |
| 2 | NIRMAL-7 | Control | 13.36±1.56 | 12.78±0.56 | 70.13±0.58 | 86.56±1.67 |
| | | UV-B | 38.36±1.35 | 31.45±4.28 | 82.66±1.37 | 72.46±0.76 |
| 3 | T-9 | Control | 19.84±0.56 | 15.58±0.58 | 87.63±0.58 | 88.13±0.36 |
| | | UV-B | 28.03±1.59 | 21.79±1.52 | 107.23±0.67 | 87.76±0.59 |

**Table No.5: Changes in anatomical characteristics of leaves of ten varieties of 30 DAS *Vigna mungo* (L.) Hepper 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 | VAMBAN-3 | Control | 17.54 ± 2.98 | 23.81 ± 0.46 | 45.44 ± 0.33 | 51.76 ± 0.45 | 148.83 ± 1.84 | 214.75 ± 0.56 |
| | | UV-B | 54.73 ± 0.18 | 71.33 ± 1.87 | 147.85 ± 1.15 | 156.83 ± 0.66 | 197.52 ± 0.72 | 468.84 ± 0.49 |
| 2 | NIRMAL-7 | Control | 32.71 ± 0.68 | 34.22 ± 1.36 | 62.86 ± 0.57 | 67.94 ± 1.19 | 264.87 ± 3.31 | 276.97 ± 3.63 |
| | | UV-B | 65.82 ± 0.37 | 79.43 ± 0.25 | 146.97 ± 0.25 | 156.58 ± 0.87 | 227.61 ± 1.36 | 442.74 ± 0.23 |
| 3 | T-9 | Control | 46.53 ± 0.53 | 34.86 ± 1.28 | 84.63 ± 0.74 | 75.86 ± 1.46 | 175.71 ± 0.67 | 297.84 ± 1.28 |
| | | UV-B | 70.94 ± 1.86 | 78.55 ± 0.68 | 134.32 ± 0.54 | 137.98 ± 0.93 | 124.88 ± 2.08 | 449.67 ± 0.29 |

**Table No.6: The similarity indices in epidermal and anatomical characteristics of three varieties of *Vigna mungo* (L.) Hepper 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 mungo* (L.) Hepper var. VAMBAN-3 under control condition and *in situ* UV-B irradiation. (Figure No.3 to 8: 400 x)**

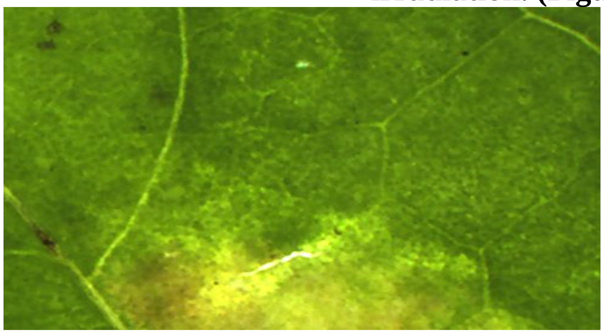


**Figure No.1: UV-B adaxial - Shiny surface**

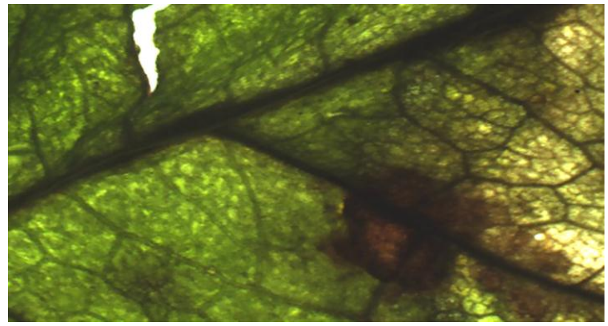


**Figure No.2: UV-B adaxial - Brittle and dead**

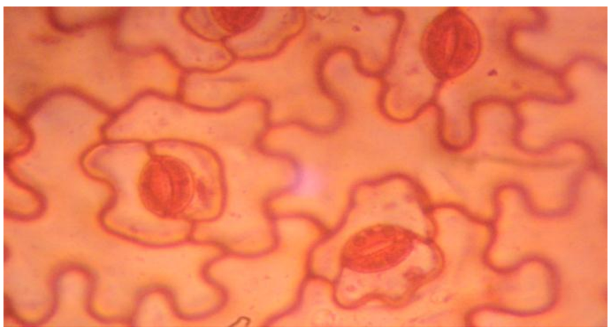


**Figure No.3: Control adaxial - Normal stomata**

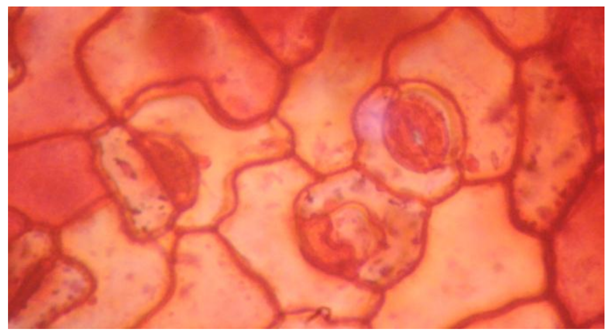


**Figure No.4: UV-B adaxial - Abnormal stomata**

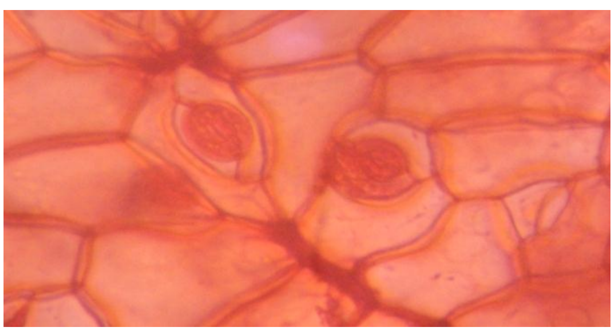


**Figure No.5: Control abaxial - Normal stomata**

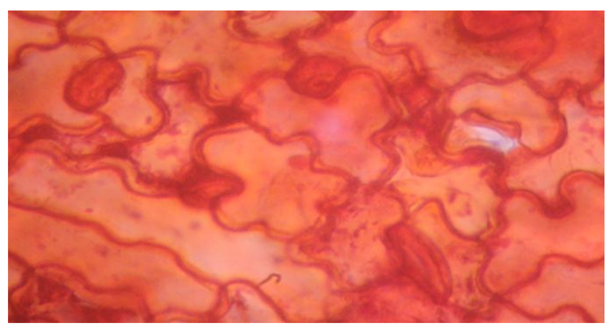


**Figure No.6: UV-B abaxial - Abnormal stomata**

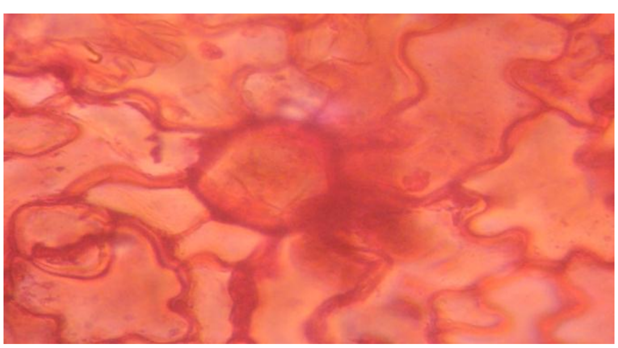


**Figure No.7: UV-B adaxial - Broken trichome**

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

**Plate No.2: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna mungo* (L.) Hepper var. NIRMAL-7 under control condition and *in situ* UV-B irradiation. (Figure No.3 to 8: 400 x)**

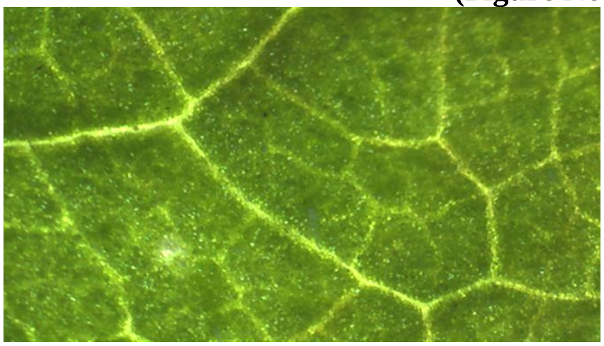


**Figure No.1: UV-B adaxial - Shiny surface**

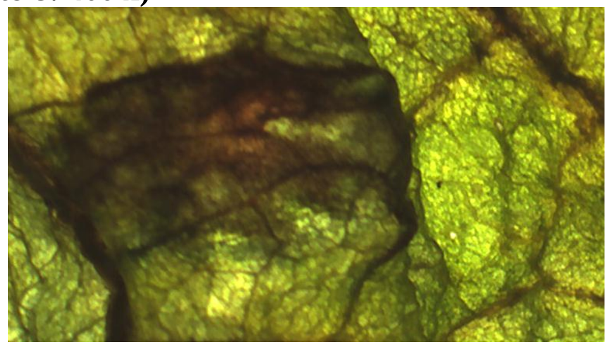


**Figure No.2: UV-B adaxial - Brittle and dead**

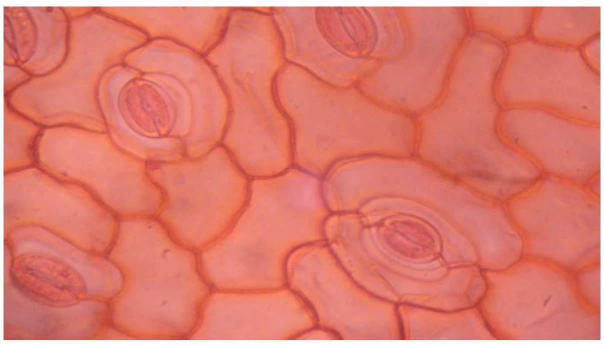


**Figure No.3: Control adaxial - Normal stomata**

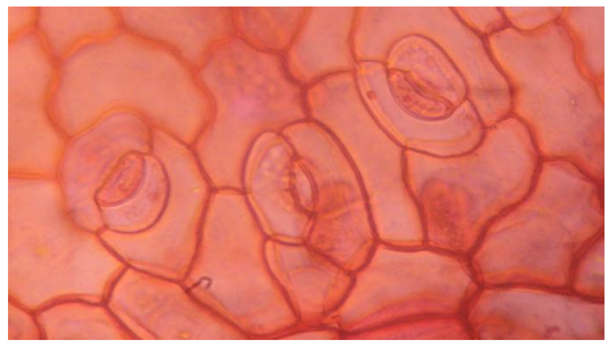


**Figure No.4: UV-B adaxial - Abnormal stomata**

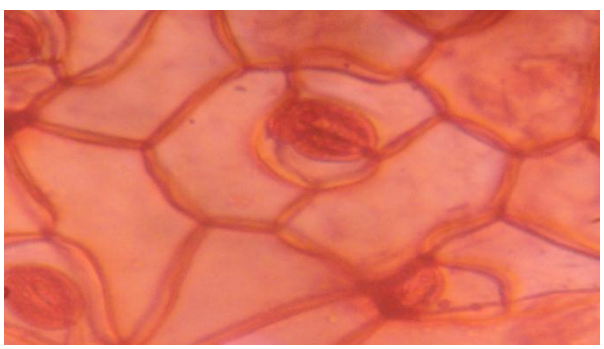


**Figure No.5: Control abaxial - Normal stomata**

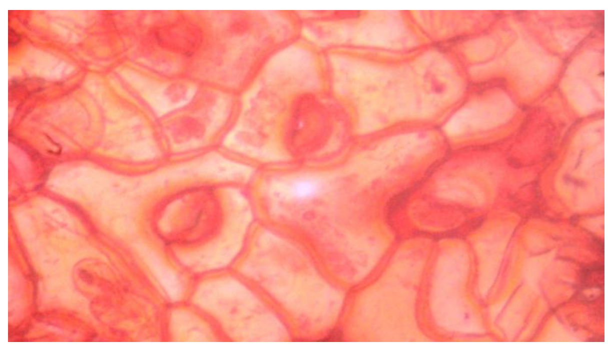


**Figure No.6: UV-B abaxial - Abnormal stomata**

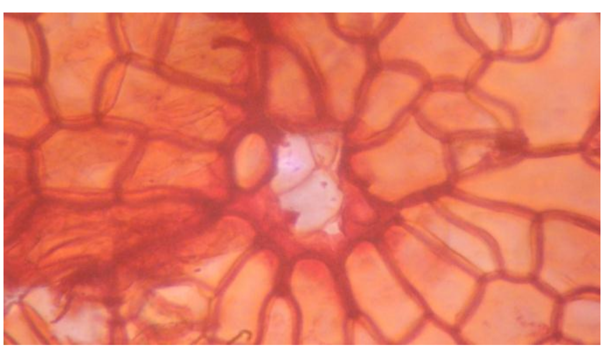


**Figure No.7: UV-B adaxial - Broken trichome**

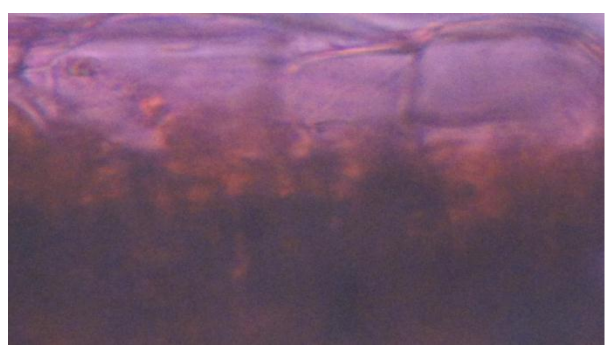


**Figure No.8: UV-B adaxial - Multiseriate epidermis**

**Plate No.3: Epidermal and anatomical characteristics of first fully expanded leaves of 30 DAS *Vigna mungo* (L.) Hepper var. T-9 under control condition and *in situ* UV-B irradiation. (Figure No.3 to 8: 400 x)**

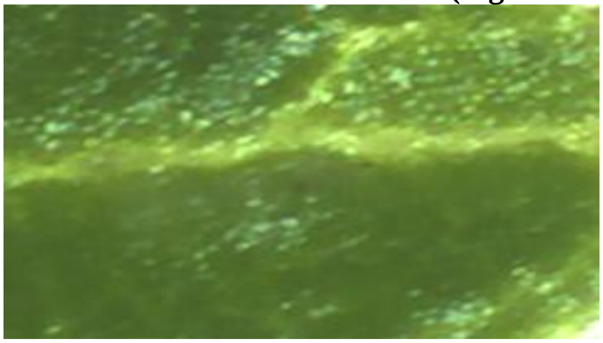


**Figure No.1: UV-B adaxial - Shiny surface**

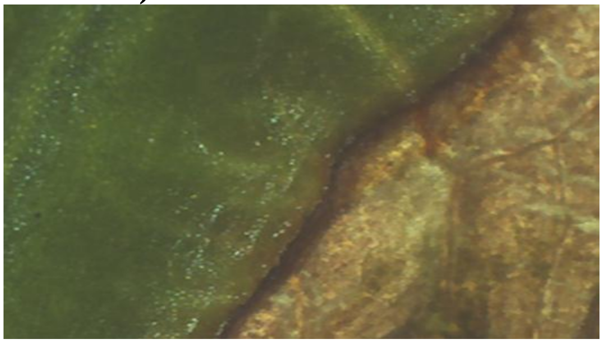


**Figure No.2: UV-B adaxial - Brittle and dead**

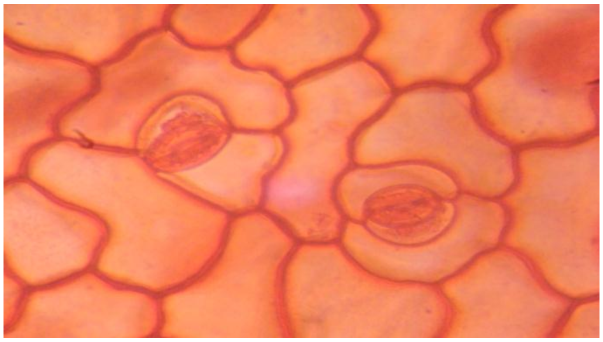


**Figure No.3: Control adaxial - Normal stomata**

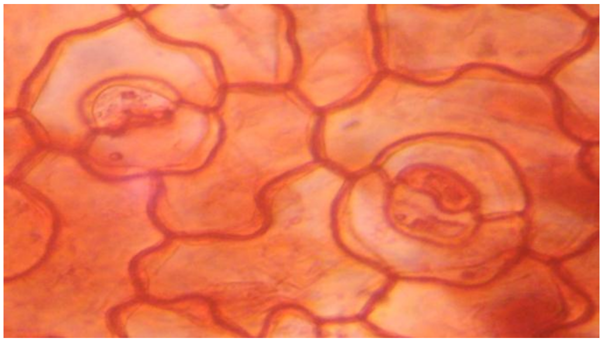


**Figure No.4: UV-B adaxial - Abnormal stomata**

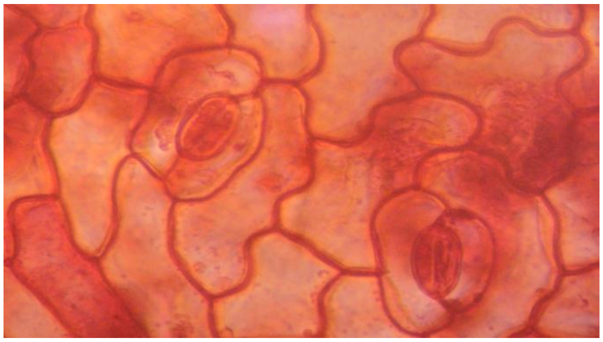


**Figure No.5: Control abaxial - Normal stomata**

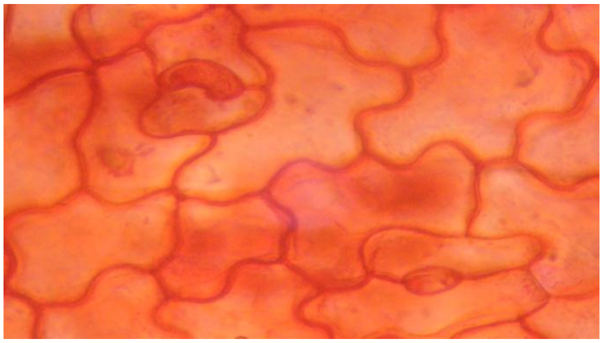


**Figure No.6: UV-B abaxial - Abnormal stomata**

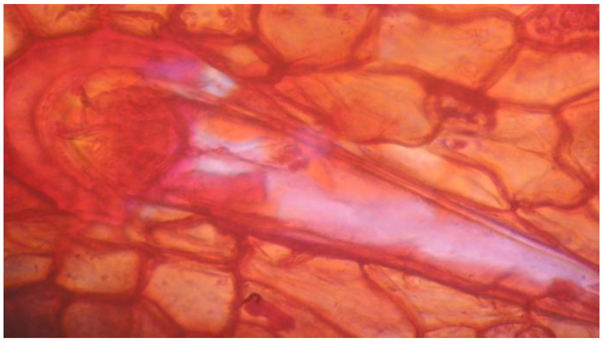


**Figure No.7: UV-B adaxial - Broken trichome**

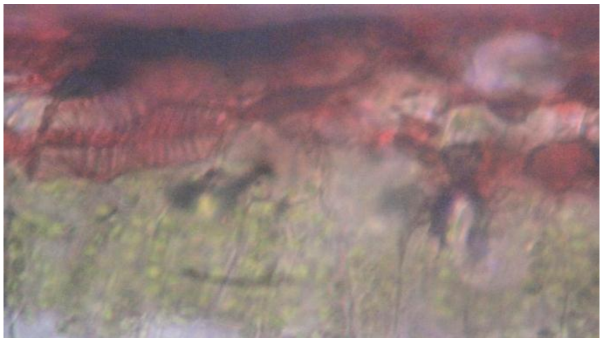
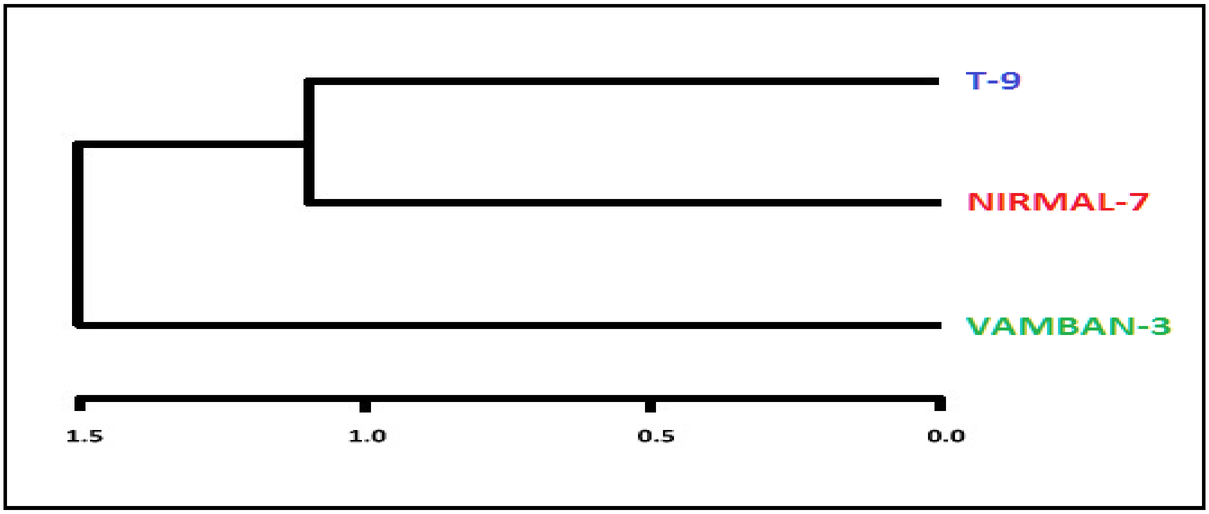


**Figure No.8: UV-B adaxial - Multiseriate epidermis**

**Plate No.4: Dendrogram showing the interrelationship between three varieties of *Vigna mungo* (L.) Hepper in epidermal and anatomical characteristics under control condition and *in situ* UV-B irradiation**




## CONCLUSION

All the three varieties of black gram in response to *in situ* UV-B impact developed maximum alterations in foliar morphology, epidermis and anatomy to survive in the abiotic stress.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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