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Research Article

INFLUENCE OF ELEVATED ULTRAVIOLET-B RADIATION ON THE COTYLEDONARY EPIDERMIS OF F_1 SEEDLINGS OF *LAGENARIA SICERARIA* (MOLINA) STANDL. VAR. WARAD

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ABSTRACT
Ozone depletion is facilitated by increased stratospheric particles provided by stratospheric clouds in the polar regions and globally by volcanic eruptions as explained by halogen-related chemistry. The volcanic aerosol-ozone interaction, suggests that with present halogen levels, a major eruption could decrease total column ozone by as much as 10% at mid-latitudes. Decrease in the level of ozone allows ultraviolet-B (UV-B) radiation into the earth surface, affecting the plants and animals including human. Even though several works are concentrated on the morphology, anatomy and metabolism of the UV-B exposed plants, very little research was carried out with the F_1 progenies of the UV-B affected plants. This study deals with the cotyledonary epidermis of F_1 seedlings germinated from seeds of UV-B survived *Lagenaria siceraria* (Molina.) Standl. var. Warad (bottle gourd) plants. The well filled seeds were harvested from *Lagenaria siceraria* (Molina.) Standl. var. Warad after exposure to supplementary UV-B radiation (2 hours daily @ 12.2 kJ m^{-2} d^{-1}) and from the control plants, grown under natural solar radiation (10 kJ m^{-2} d^{-1}). In UV-B stressed cotyledons number of stomata were less by 15.30 and 63.94 % than control on adaxial and abaxial surfaces respectively. Stomatal indices of stressed plants showed decreases by 22.14 to 6.46 % on both the surfaces. Cotyledons of UV-B stressed parents had smaller stomata while the abnormalities increased. No stomatal aberrations were recorded in normal cotyledons. The trichomes were shorter by 8.85 % and 5.15 % on adaxial and abaxial surfaces respectively and were also brittle in UV-B treated cotyledons compared to healthier ones in control. Frequency of trichome was increased by 8.63 % on adaxial side and by 33.44 % on abaxial surfaces in UV-B exposed plants. UV-B induced structural changes in the architecture were to protect the vital organs inside the cotyledons.

***Keywords:** Ultraviolet-B, Bottle Gourd, Variety Warad. Cotyledonary Epidermis, Abnormal Stomata*

INTRODUCTION
UV-B radiation an integral component of sunlight has dangerous effects on the plants. Maximum radiation is absorbed by the stratospheric ozone layer, but any depletion in it will enhance UV-B penetration into earth's surface. UV-B has the potential to damage macromolecules such as DNA and proteins, generate reactive oxygen species (ROS) and impair cellular processes. At the plant metabolism level, it severely inhibits photosynthesis (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and suppresses nodulation and nitrogen fixation (Rajendiran and Ramanujam, 2006; Rajendiran and Ramanujam, 2003; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014) in sensitive plants. Cotyledons are built with dynamic barrier in the form of epidermis demarking the plant's internal from external environment. Epidermis is impregnated with waxes and cutins on the exterior and possesses stomata to regulate the exchange of gases along with appendages like trichomes and receive major proportion of the ultraviolet radiation, reacting quickly to prevent its entry into the internal organs (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013). This work reports the changes in the epidermal architecture of cotyledons of F_1 progenies of *Lagenaria siceraria* (Molina.) Standl. var. Warad (bottle gourd) suffering under supplementary UV-B irradiation.

MATERIALS AND METHODS
The seeds of bottle gourd (*Lagenaria siceraria* (Molina.) Standl. var. Warad) obtained from Saravan Farms, Villupuram, Tamil Nadu, were grown in pot culture in the naturally lit greenhouse (day

temperature maximum 38 ± 2 ºC, night temperature minimum 18 ± 2 ºC, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 µmol m^{-2} s^{-1}, photoperiod 12 to 14 h). Supplementary UV-B radiation was provided in UV garden by three UV-B lamps (*Philips TL20W/12 Sunlamps*, The Netherlands), which were suspended horizontally and wrapped with cellulose diacetate filters (0.076 mm) to filter UV-C radiation (< 280 nm). UV-B exposure was given for 2 h daily from 10:00 to 11:00 and 15:00 to 16:00 starting from the 5th day after sowing. Plants received a biologically effective UV-B dose (UV-B_{BE}) of 12.2 kJ m^{-2} d^{-1} equivalents to simulated 20 % ozone depletion at Pondicherry (12º2'N, India). The control plants, grown under natural solar radiation, received UV-B_{BE} 10 kJ m^{-2} d^{-1}.
Seeds (F_1 generation) were harvested from both unstressed and supplementary UV-B stressed parent crops grown in the *in situ* condition. The seeds of test plant were thoroughly washed with water containing 0.1% Bavistin (a systemic fungicide BASF, India Ltd., Bombay) for 4-5 minutes. They were surface sterilized with 0.1% $HgCl_2$ for 4-5 minutes and washed 6 to 8 times with autoclaved water under Laminar Air Flow Cabinet (Technico Systems, Chennai) and inoculated aseptically onto sterilized petriplates lined with moist filter paper for germination. The cotyledons were fixed from 7 day old seedlings. Their epidermal peels were stained in 1 % aqueous safranin and mounted in 50 glycerine and sealed with DPX (Distrene, Plasticiser, Xylene). The final wash was given with aqueous sterilized solution of (0.1%) ascorbic acid. The surface sterilized explants were dipped in 90% ethanol for a short period (40 seconds). The seeds were inoculated horizontally and the stem explants were inoculated vertically on MS medium for culture initiation.
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 cotyledons 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. Stomatal 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.

RESULTS AND DISCUSSION

The epidermal cells with dense, deeply stained nuclei occurred in control and in all the UV-B irradiated cotyledons (Plate 1. Figure 1 to 8). Epidermal cell frequency was higher (34.28 to 45.96 %) over control in UV-B exposed cotyledons on both the surfaces, the highest being on adaxial surface (Table 1). Frequency of unicellular trichomes present in the costal as well as intercostal regions of both the surfaces was comparatively less on the abaxial side than the adaxial side (Table 1). Under UV-B exposure trichomes were more on adaxial surface (8.65%) and on abaxial (33.44 %) surface over control (Table 1). Trichomes were longer by 8.35 % on adaxial side and by 5.15 % on abaxial side in UV-B irradiated cotyledons (Table 1; Plate 1. Figure 3). The trichomes form a mechanical barrier against biotic attack (Johnson, 1975; Woodman and Fernandez, 1991), provide additional resistance to the diffusion of water vapour from the leaf interior to the atmosphere (Nobel, 1983) and as a reflector reducing the radiant energy absorbed by the leaf (Ehleringer, 1984; Rajendiran, 2001). Trichomes form additional mechanical shield to UV-B penetration by reflecting the radiant energy (Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b). The increased trichome frequency which could have been an adaptive feature to UV-B treatment as reported by Kokilavani and Rajendiran (2014c) differs from the reductions observed by Karabourniotis *et al*., (1995). Dead and collapsed epidermal cells were very deeply stained on both the surfaces of UV-B irradiated cotyledons (Table 1; Plate 1. Figure 4). Injury in the form of collapsed cells with signs of bronzing of tissue surfaces have been attributed to oxidised phenolic compounds (Cline and Salisbury, 1966) followed by tissue degradation (Caldwell, 1971). Size of epidermal cell (10.65 to 23.30 %) and stomatal (11.56 to 36.02 %) were decreased below normal due to UV-B irradiation (Table 1; Plate 1. Figure 5). The cotyledons are amphistomatic and the stomata are diacytic and paracytic and distributed all over the surface except over costal regions without any definite pattern or orientation.

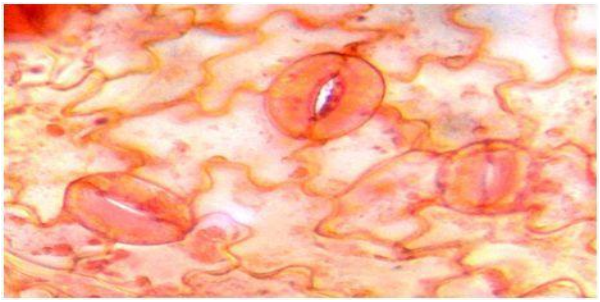

Figure 1: Control ada - Normal stomata

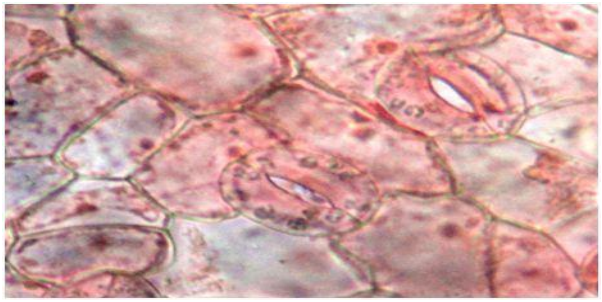

Figure 2: Control aba - Normal stomata

Fig. 3: UV-B ada - trichome

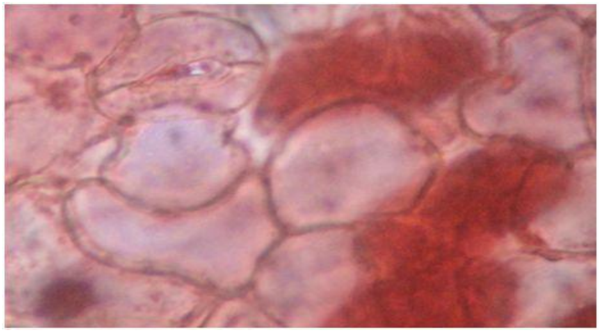

Fig. 4: UV-B ada - Dead epidermal cells

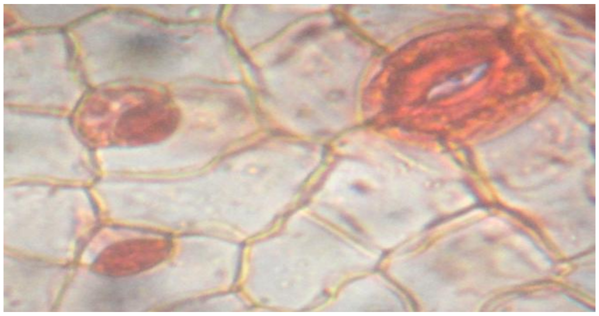

Figure 5: UV-B aba - Dwarf stomata

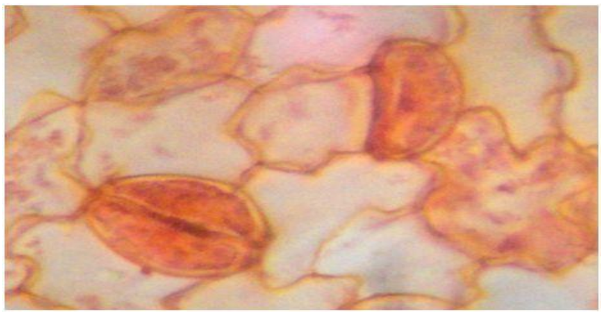

Figure 6: UV-B ada - Single guard cell

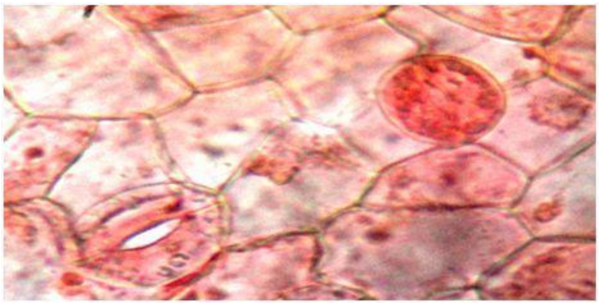

Figure 7: UV-B ada - Persistant stomatal initial

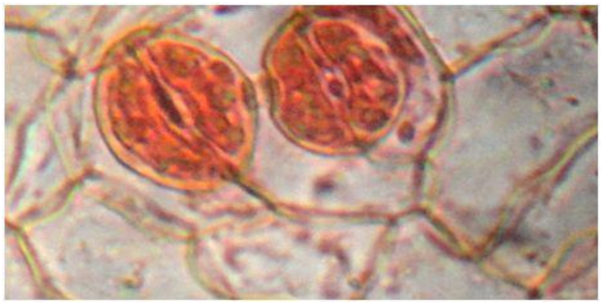

Figure 8: UV-B ada - Contiguous stomata

Plate 1: Epidermal characteristics of cotyledon of *Lagenaria siceraria* (Molina.) Standl. var. Warad under control condition and UV-B radiation exposure (400 x)

Table 1: Changes in the epidermal characteristics of cotyledons of *Lagenaria siceraria* (Molina.) Standl. var. Warad exposed to UV-B radiation

Parameter		Control		UV-B	
		Adaxial	**Abaxial**	**Adaxial**	**Abaxial**
Stomatal frequency mm^{-2}		197.83 ±0.26	133.12 ±0.44	167.56 ±0.36	218.25 ±0.24
Epidermal cell frequency mm^{-2}		168.14 ±0.32	158.57 ±0.24	245.41 ±0.16	212.93 ±0.30
Stomatal index		55.24 ±0.84	56.17 ±0.92	41.35 ±1.24	52.54 ±1.14
S/E ratio		1.74	0.82	0.66	1.14
Frequency of abnormal stomata mm^{-2}		-	-	25.24 ±0.24	8.58 ±0.24
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	22.65 ±0.15	-
Frequency of trichome mm^{-2}		17.15 ±0.15	11.57 ±0.25	18.63 ±0.09	15.44 ±0.12
Stomatal size	Length (µm)	23.63 ±0.12	24.22 ±0.17	18.22 ±0.16	21.42 ±0.12
	Breadth(µm)	19.32 ±0.16	13.32 ±0.17	12.36 ±0.23	13.57 ±0.15
Epidermal cell size	Length(µm)	42.83 ±0.12	72.14 ±0.27	32.85 ±0.29	58.16 ±1.23
	Breadth(µm)	24.87 ±0.14	22.54 ±0.15	22.22 ±0.23	18.87 ±0.12
Trichome length (µm)		255.32 ±8.65	245.23 ±11.25	276.65 ±5.89	257.86 ±8.43

Frequency of stomata decreased (15.30 %) on adaxial and increased (63.94 %) on abaxial surfaces compared to control and stomatal indices were decreased significantly (6.46 to 25.14 %) compared to control with S/E ratio on adaxial surface showing low value (62.06 %) while a higher value (39.02 %) was recorded on abaxial surface under UV-B exposure (Table 1). Pea plants responding to UV-B treatment had higher stomatal frequency on the adaxial surface (Nogues *et al.*, 1998). Stomata were smaller (11.56 to 36.02 %) than control on both surfaces of the foliage under UV-B and the abnormal stomata were more frequent with the maximum on the adaxial surface (Table 1; Plate 1. Figure 6 to 8). Abnormalities under UV-B include stomata with single guard cell, persistent stomatal initials, and contiguous stomata (Plate 1. Figure 6 to 8).

Several stomatal abnormalities were earlier reported in cotyledonary epidermis of *Helianthus annuus* after irradiation with gamma rays (Rajendiran and Selvaraj, 1989). However, abnormalities were not recorded in the cotyledons from unstressed parents (Table 1; Plate 1. Figure 1, 2). Surviving under elevated ultraviolet-B stress the F_1 progenies readily brought about a series of modifications in the epidermal architecture of cotyledons to tide over the environmental stress.

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