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ALTERATIONS IN LEAF ARCHITECTURE OF *OCIMUM SANCTUM* L. UNDER ELEVATED ULTRAVIOLET-B STRESS

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ABSTRACT
A leaf is composed of many layers that are sandwiched between two layers of tough skin cells which are further covered by waxy cuticle. These layers protect the leaf from loss of water, loss of plant components by leaching and protection of plants against injuries due to insects, bacteria, pests, wind, frost, and radiation and physical abrasion. Among the epidermal cells are pairs of sausage-shaped guard cells supporting gaseous exchange for photosynthesis and respiration and regulating transpiration. Any change in the environment has a direct impact on leaf architecture as these organs are designed by the habitat where the plants have adapted to live. 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 *Ocimum sanctum* L. leaf. The epidermal and anatomical characters of the fully developed third trifoliate leaf from the top on 30 DAS (days after seed germination) *Ocimum sanctum* L. 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 stress by 30.95 and 16.94 % respectively. The trichomes were longer by 7.73 % on adaxial and by 4.50 % on abaxial surfaces but brittle in UV-B treated leaves which were short and healthy in control. The trichome frequency was also higher by 38.37 % on adaxial and by 18.05 % on abaxial surfaces in UV-B exposed plants. The small, shiny and thick leaves of UV-B exposed plants compared poorly to broader, longer and thinner leaves of control plants. Suffering under UV-B, the stomatal frequency was reduced by 7.73 % on adaxial surface but was compensated by an addition of 77.92 % on the abaxial surface. However, the stomatal indices was declined on both adaxial (29.54 %) as well as on abaxial (27.08 %) surfaces of stressed plants compared to control. Abnormal stomata like, non-functional, 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 stressed *Ocimum sanctum* L. plants developed anatomical barriers to reduce UV-B penetration as a measure to alleviate the impact to some extent.

KEY WORDS: Ultraviolet-B, Holi basil, leaf morphology, leaf epidermis, leaf anatomy, abnormal stomata.

INTRODUCTION

The size and shape of leaves are the result of a compromise between leaf energy exchange, leaf temperature and photosynthesis. Leaves growing in sunny environments are smaller and more deeply lobed than leaves growing in shaded environments. Leafy plants growing in the hot, arid environment of deserts or cold arctic and alpine environments have small leaves. In part, this is related to the influence of leaf dimension on leaf boundary layer resistance and the efficiency with which heat and moisture are transported away from a leaf. 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 depletion of ozone layer which has become an insurmountable environmental problem in the recent past, 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, Arulmozhi and Rajendiran 2014, Vijayalakshmi and Rajendiran. 2014) in sensitive plants. The present study is carried out to record the mechanism of defense against UV-B radiation in the leaf anatomy of an important herbaceous sacred plant, *Ocimum sanctum* L. which is known widely for its medicinal values.

MATERIALS & METHODS

The *Ocimum sanctum* L. plants, 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) *Ocimum sanctum* L. 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 of *Ocimum sanctum* L. under UV-B exposed condition were small, wrinkled, highly shiny and brittle with chlorotic and necrotic lesions all over the adaxial surface (Plate 1, Plate 2 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. 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 2. Fig. 5, 6). Epidermal cell frequency was higher (72.91 %) over control in UV-B exposed plants but the effect was subdued on the abaxial side compared to adaxial surface (Table 1).

PLATE1. Changes in the morphological characteristics of leaves of 30 DAS *Ocimum sanctum* L. exposed to supplementary UV-B radiation.

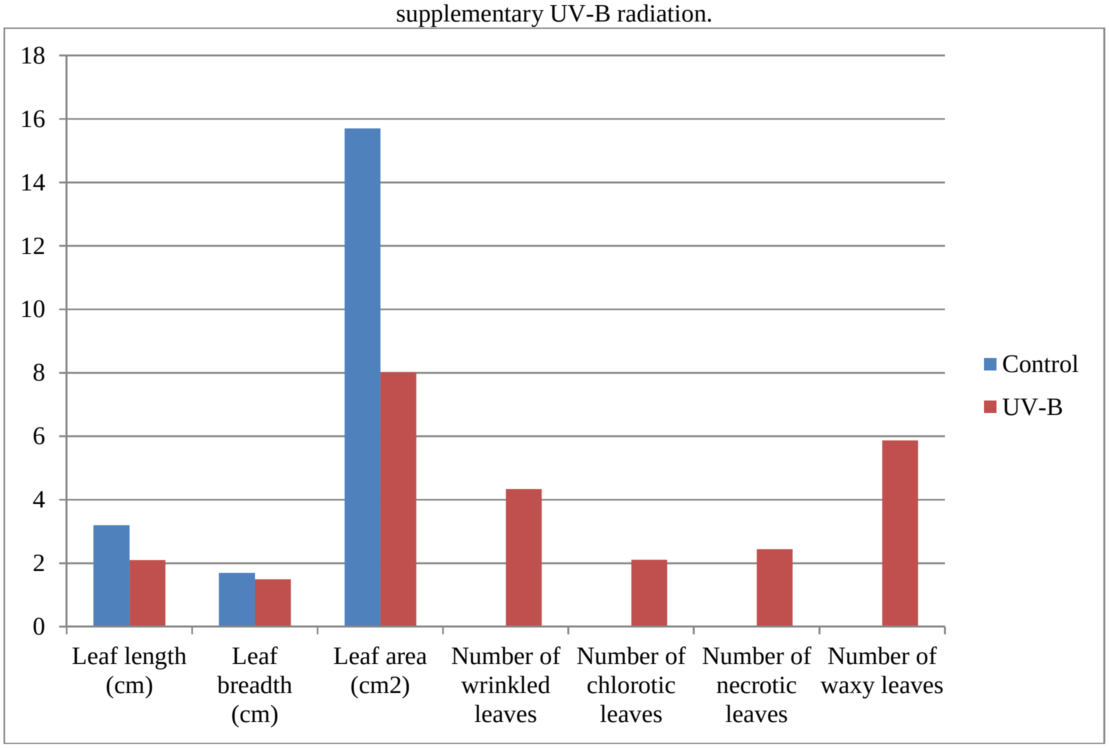

PLATE 2. Epidermal and anatomical characteristics of leaves of 30 DAS *Ocimum sanctum* L. under control condition and supplementary UV-B radiation exposure. Cont: Control, UV-B: UV-B stressed, Ada: Adaxial surface, Aba: Abaxial surface. (Fig. 4 to 8: 400 x)

FIGURE 1: Control and UV-B exposed leaves

FIGURE 2: UV-B Ada - Shiny waxy surface

Figure 3: UV-B Ada - Brittle with chlorosis

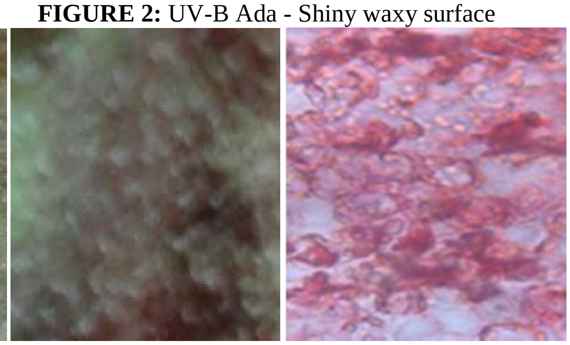

Figure 4: UV-B Ada - Necrosis and dead cells

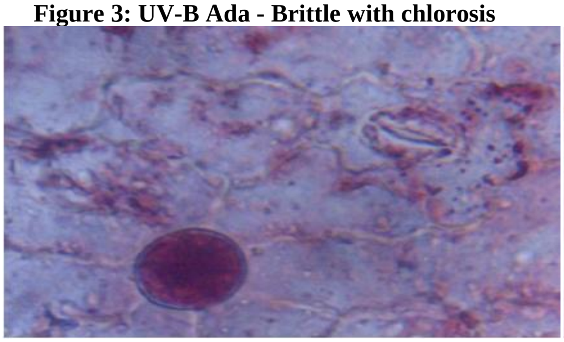

FIGURE 5: Cont Ada - Normal stomata, gland

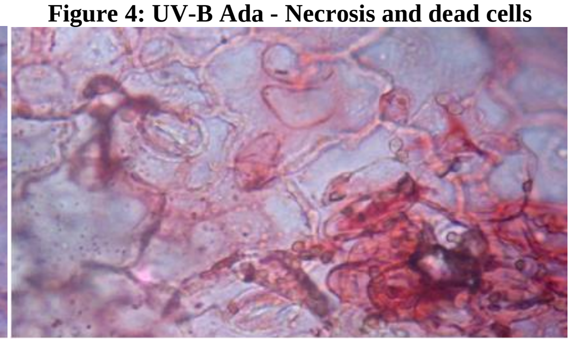

FIGURE 6: UV-B Ada - Collapsed stomata, gland

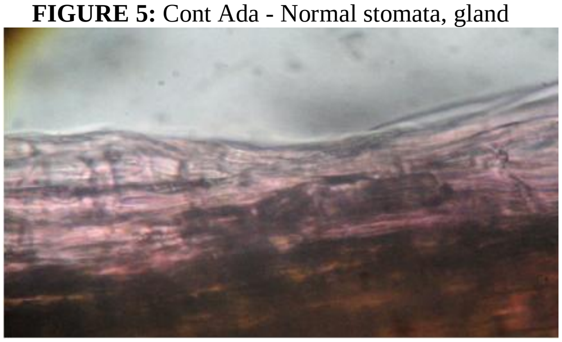

FIGURE 7: UV-B Ada - Multiseriate epidermis

FIGURE 8: UV-B Ada - Broken trichome

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 increased markedly by 30.95 % and 72.34 % respectively over control (Table 2; Plate 2. 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). According to Wellmann (1976) and Caldwell *et al.* (1983), 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. At the structural level, increased leaf and cuticle thickness reduces UV-B penetration to internal tissues (Bornman and Vogelmann 1991, Rajendiran 2001) 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 (Bornman and Vogelmann 1991). Bornman and Vogelmann (1991) and

Kokilavani and Rajendiran (2013) 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. 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 (38.37 %) compared to control, especially on the adaxial surface (Table 1). Longest trichomes (7.73 %) as well as broken trichomes were observed more on the adaxial side of UV-B irradiated plants (Plate 2. Fig.8). 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 (Kokilavani and Rajendiran 2013).

TABLE 1: Changes in the epidermal characteristics of leaves of 30 DAS *Ocimum sanctum* L. exposed to elevated UV-B radiation.

Parameter		Control		UV-B	
		Adaxial	Abaxial	Adaxial	Abaxial
Stomatal frequency mm^{-2}		175.18 ±0.35	119.24 ±0.46	161.63 ±0.23	212.16 ±0.13
Epidermal cell frequency mm^{-2}		148.16 ±0. 32	145.67 ±0. 34	256.19 ±0.41	199.29 ±0. 26
Stomatal index		29.04±1.12	31.34±0.97	20.46±1.36	22.85±1.52
S/E ratio		1.18	0.82	0.63	1.06
Frequency of abnormal stomata mm^{-2}		-	-	28.59 ±0.73	5.12 ±0.42
Frequency of dead/collapsed epidermal cells mm^{-2}		-	-	27.59 ±0.28	-
Frequency of trichome mm^{-2}		14.36 ±0.17	11.19 ±0.28	19.87 ±0.11	13.21 ±0.13
Stomatal size	Length (µm)	21.26±0.17	22.38±0.15	14.72±0.12	19.23±0.16
	Breadth(µm)	17.38±0.12	12.56±0.14	10.42±0.14	11.76±0.18
Epidermal cell size	Length(µm)	42.39±0.15	70.31±0.23	29.18±0.22	56.76±1.27
	Breadth(µm)	26.37±0.18	20.14±0.21	22.65±0.25	18.45±0.23
Trichome length (µm)		261.52±8.65	252.43±11.25	281.76±5.89	263.812±8.43

TABLE 2: Changes in the anatomical characteristics of leaves of 30 DAS *Ocimum sanctum* L. exposed to elevated UV-B radiation.

Parameter		Control	UV-B
Cuticle thickness	Adaxial (µm)	8.56 ± 0.24	11.21 ± 0.35
	Abaxial (µm)	7.86 ± 0.35	9.87 ± 0.24
Epidermis thickness	Adaxial (µm)	14.32 ± 0.33	24.68 ± 0.25
	Abaxial (µm)	17.12 ± 0.54	19.47 ± 0.57
Leaf thickness (µm)		227.23 ± 2.25	256.81 ± 2.43
Mesophyll thickness (µm)		156.75 ± 2.32	183.31 ± 2.17
Mesophyll volume (cm^3 dm^{-2})		1.72 ± 0.14	1.94 ± 0.16

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 (27.59 %) were found in large numbers only on the adaxial leaf surface of UV-B stressed plants (Table 1; Plate 2. 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 (19.27 % to 31.31 %) after UV-B irradiation (Table 1; Plate 2. Fig. 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 anomocytic. Stomatal frequency, stomatal index and S/E ratio ware reduced significantly (7.73 % to 46.61 %) below control by UV-B on the adaxial side while they recorded an increase over control on the abaxial surface (Table 1). On the contrary, pea plants responding to UV-B treatment had higher stomatal frequency on the adaxial surface (Nogues *et al.* 1998). 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 and Rajendiran (2013) on the adaxial side of the leaves after exposure to UV-B radiation. UV-B irradiated leaves developed abnormalities like persistent stomatal initials**,** stomata with single guard cell and thickened pore and collapsed stomata and glands

(Table 1; Plate 2. Fig. 6). Such aberrations did not occur in the leaves of the plants grown under control conditions (Table 1; Plate 2. Fig. 5). The data obtained from the present work provides enough evidences for the defence mechanism performed by the leaves of *Ocimum sanctum* L. as the plant under ultraviolet-B environment responded quickly by increasing the thickness of cuticle, multiseriate epidermis, epicuticular wax deposition, number of trichomes and the volume of the internal organ system to avoid penetration of UV-B rays. In response to UV-B radiation, *Ocimum sanctum* L. leaves reacted quickly to modify their architecture as evident by increase in thickness of cuticle, epicuticular wax deposition, number of trichomes and volume of internal organ system to create mechanical barriers against the penetration of UV-B rays.

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