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Research Article

ULTRAVIOLET-B INDUCED CHANGES IN THE COMPOSITION OF FOLIAR EPICUTICULAR WAX OF GREEN GRAM VARIETIES

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ABSTRACT
The differences in the composition of epicuticular wax in the leaves of three varieties of green gram (*Vigna radiata* (L.) Wilczek) *viz.* CO-8, NVL-585 and VAMBAN-2 grown under elevated ultraviolet-B (UV-B) radiation is reported. Fully developed third trifoliate leaves from the top of 30 DAS (days after seed germination) green gram crops under supplementary UV-B radiation (2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) were used for wax extraction. UV-B exposed CO-8, NVL-585 and VAMBAN-2 varieties of green gram accumulated 69.79, 64.43 and 55.68 % of foliar epicuticular waxes over the respective control plants. Wax samples from the leaves of control and UV-B exposed CO-8 resolved into five components. NVL-585 and VAMBAN-2 varieties under normal and UV-B exposed conditions resolved into three major components only. In all the varieties of green gram the three major spots corresponded to alkanes, monoesters and aldehydes, while the two minor spots in CO-8 were identified as primary and secondary alcohols. An intense spot corresponding to aldehydes appeared in control and UV-B stressed samples of VAMBAN-2 variety.

Keywords: *Epicuticular Wax, Green Gram, Three Varieties, Ultraviolet-B*

INTRODUCTION
Terrestrial plants have to cope with adverse conditions like elevated levels of ultraviolet rays, dry atmosphere, heavy rains, air pollutants and attack by microbes and insects. Plants counter these abiotic and biotic stresses effectively by the protective mechanisms located in the epidermal layer of the leaves in the form of a lipid coating called cuticle. Epicuticular waxes in the cuticle often form two- and three-dimensional structures, in dimensions between hundreds of nanometers and some micrometers, influencing the wettability, self-cleaning behaviour and light reflection at the cuticle interface (Koch and Ensikat, 2008). In contrast to the voluminous reports available on the effects of ultraviolet-B (UV-B) radiation (280-320 nm) on growth (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004; Kokilavani and Rajendiran, 2014a; Rajendiran *et al.,* 2015), yield (Kokilavani and Rajendiran, 2014b; Rajendiran *et al.,* 2015) and nodulation and nitrogen metabolism (Rajendiran and Ramanujam, 2006; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Kokilavani and Rajendiran, 2014c; Sudaroli and Rajendiran, 2014a; Sudaroli and Rajendiran, 2014b; Sudaroli and Rajendiran, 2014c; Arulmozhi and Rajendiran, 2014a; Arulmozhi and Rajendiran, 2014b; Arulmozhi and Rajendiran, 2014c; Vijayalakshmi and Rajendiran, 2014a; Vijayalakshmi and Rajendiran, 2014b; Vijayalakshmi and Rajendiran, 2014c) in many crops, the pattern of foliar epicuticular wax deposition in plants under UV-B exposure is yet to be assessed. This experiment presents the wax composition in the leaves of CO-8, NVL-585 and VAMBAN-2 varieties of green gram after supplementary UV-B radiation.

MATERIALS AND METHODS
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. 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 the 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} 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 epicuticular wax pattern the fully developed third trifoliate leaf from the top was taken from the 30 DAS green gram crops. Fresh leaf discs (0.1 g) were punched out with a cork-borer (1 cm diameter) and immediately dipped in 10 ml of redistilled chloroform for 10 seconds. The wax extract was evaporated to dryness, labelled and stored. The wax extracts were separated into single wax classes by thin layer chromatography (TLC). Aliquots (100µl**)** of wax extracts were spotted on activated silica gel (Merck Kieselgel-G) plates and developed in benzene as the solvent (Plate 1, Figure 1, 2). The plates were stained with iodine vapour, observed under ultraviolet light (Plate 1, Figure 3, 4) and the Rf values were calculated and compared with standard Rf values reported by Steinmuller and Tevini (1982). Rf is equal to the distance travelled by the substance divided by the distance travelled by the solvent. Its value is always between zero and one.

The experiments were repeated for three times to confirm the trends. The result of single linkage clustering (Maskay, 1998) was displayed graphically in the form of a diagram called dendrogram (Everstt 1985). The similarity indices between the three varieties of green gram under study were calculated using the formula given by Bhat and Kudesia (2011).

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

Based on the similarity indices between the three varieties of green gram, dendrogram was draw to derive the interrelationship between them and presented in Table 2 and Plate 2.

RESULTS AND DISCUSSION

Supplementary UV-B radiation enhanced epicuticular wax deposition in all the varieties (CO-8, NVL-585 and VAMBAN-2) of green gram over their respective controls. Maximum accumulation of wax occurred in CO-8 (69.79%), followed by NVL-585 (64.43%) and VAMBAN-2 (55.68%) (Table 1).

Table 1: Epicuticular wax content and its TLC analysis in the leaves of three varieties of 30 DAS *Vigna radiata* (L.) Wilczek under control and supplementary UV-B exposed conditions

Varieties	Treatment	Rf values					Wax content µg g^{-1} fw
		Spot 1	Spot 2	Spot 3	Spot 4	Spot 5	
CO-8	Control	0.86 (+)	0.60 (+)	0.40 (+)	0.24 (F)	0.14 (F)	3.74
	UV-B	0.86 (+)	0.60 (+)	0.40 (+)	0.24 (F)	0.14 (F)	6.35
NVL-585	Control	0.86 (+)	0.60 (+)	0.40 (+)	-	-	3.43
	UV-B	0.86 (+)	0.60 (+)	0.40 (+)	-	-	5.64
VAMBAN-2	Control	0.86 (+)	0.60 (+)	0.40 (++)	-	-	3.52
	UV-B	0.86 (+)	0.60 (+)	0.40 (++)	-	-	5.48

(+) : Major spot
(++) : Intensely present
(F) : Minor spot
- : No spot

Rf value	***Components***
0.86	*Alkanes*
0.60	*Monoesters*
0.40	*Aldehydes*
0.24	*Secondary alcohols*
0.14	*Primary alcohols*

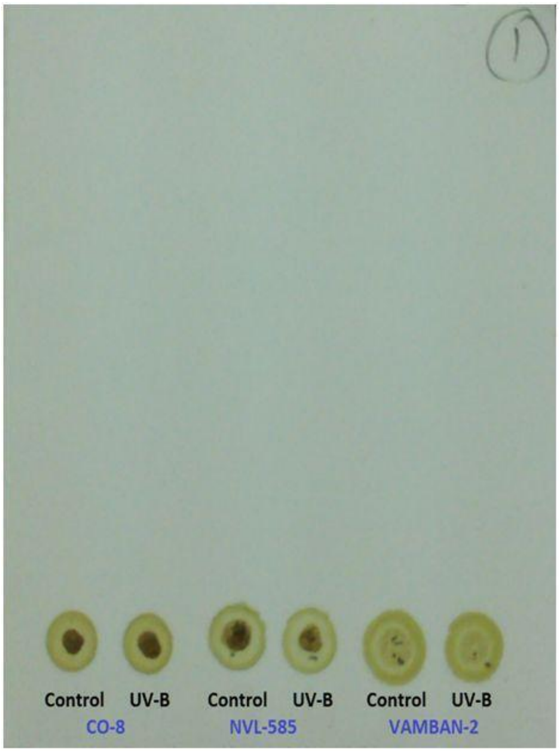

Figure 1: Spotted on activated silica gel

Figure 2: Developed in benzene as solvent

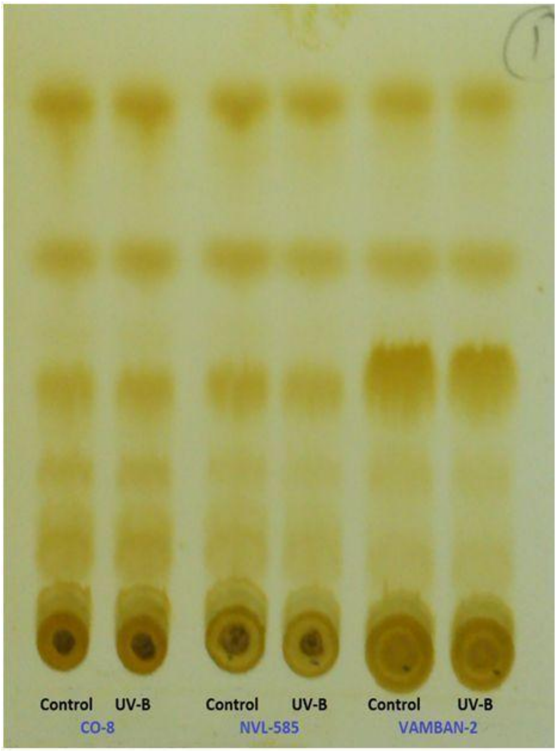

Figure 3: Stained with iodine vapour

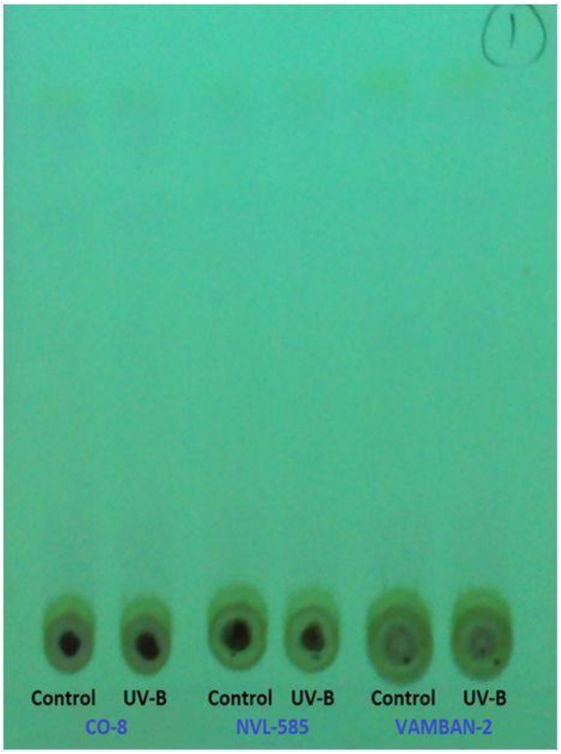

Figure 4: Under ultraviolet light

Plate 1: TLC analysis of epicuticular wax in the leaves of three varieties of 30 DAS *Vigna radiata* (L.) Wilczek under control and supplementary UV-B exposed conditions

Table 2: The similarity indices in epicuticular wax pattern in the leaves of three varieties of 30 DAS *Vigna radiata* (L.) Wilczek under control and supplementary UV-B exposed conditions

Varieties	CO-8	NVL-585	VAMBAN-2
CO-8	100%	60%	60%
NVL-585	60%	100%	100%
VAMBAN-2	60%	100%	100%

Plate 2: Dendrogram showing the interrelationship between three varieties of *Vigna radiata* (L.) Wilczek in leaf epicuticular wax pattern under control and supplementary UV-B exposed conditions

The wax samples of control and UV-B exposed CO-8 variety of green gram in TLC resolved into five components, of which three were major spots and two appeared as minor spots (Table 1; Plate 1). The three major ones corresponding to Rf 0.86, 0.60 and 0.40 resolved into alkanes, monoesters and aldehydes respectively (Table 1; Plate 1). Two minor spots which occurred only in CO-8 samples at Rf 0.24 and 0.14 were identified as secondary alcohols and primary alcohols respectively. The wax samples harvested from control and UV-B exposed NVL-585 and VAMBAN-2 varieties of green gram resolved into three major components only and did not contain any minor spots. The three major spots in NVL-585 and VAMBAN-2 wax samples corresponding to Rf 0.86, 0.60 and 0.40 resolved into alkanes, monoesters and aldehydes respectively. VAMBAN-2 differed from other two varieties in having an intense spot at Rf 0.40 (aldehydes) both in control as well as in UV-B stressed samples (Table 1; Plate 1). Such differences in the composition of epicuticular wax occurred in green gram plants when UV-B radiation and triadimefon treatment were given alone and in combination (Rajendiran 2001).
The epicuticular wax pattern in three varieties of green gram under control and UV-B exposed conditions showed differences between them when assessed through dendrogram (Table 2; Plate 2). NVL-585 and VAMBAN-2 had the highest similarity index of 100 % between them and formed a group, as their epicuticular wax samples resolved into three major components only and did not contain any minor spots. However, CO-8 which recorded five components both in control and UV-B exposed foliar epicuticular wax samples remained alone in the cluster showing 60 % affinity towards the group consisting of NVL-585 and VAMBAN-2 varieties of green gram.

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