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Research Article

COMPARISON OF EPICUTICULAR WAX PATTERNS IN THREE VARIETIES OF BLACK GRAM UNDER ELEVATED ULTRAVIOLET-B RADIATION

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ABSTRACT
Epicuticular wax composition in the leaves of three varieties of black gram (*Vigna mungo* (L.) Hepper) *viz.* VAMBAN-3, NIRMAL-7 and T-9 in response to elevated ultraviolet-B (UV-B) radiation is reported. Fully developed third trifoliate leaves from the top of 30 DAS (days after seed germination) black 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 excised and used for wax extraction. UV-B exposed VAMBAN-3, NIRMAL-7 and T-9 varieties of black gram accumulated 49.72, 52.99 and 75.08% more epicuticular waxes than the control plants. Wax samples from VAMBAN-3, NIRMAL-7 and T-9, both in control and UV-B exposed crops resolved into three major components *viz.,* monoketones, diketones and secondary alcohols. All the wax components appeared as major spots in T-9 variety. Diketones appeared as minor spot in VAMBAN-3, while secondary alcohols appeared as minor spot in NIRMAL-7.

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

INTRODUCTION
Epicuticular waxes often form two- and three-dimensional structures, in dimensions between hundreds of nanometers and some micrometers, which influence the wettability, self-cleaning behaviour and the light reflection at the cuticle interface (Koch and Ensikat, 2008). In addition, wax deposit plays a major part in maintaining the water balance of plants during unfavourable seasons. As the leaves are the organs that receive major amount of solar radiations including UV-B during climate change *viz.*, global warming and high UV-B flux due to ozone depletion, an assessment of the foliar epicuticular wax formation under such adverse conditions becomes necessary. Even though reports on ultraviolet-B (UV-B) radiation (280-320 nm) induced inhibitions of 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 sensitive crops are available in plenty, very little investigations have been carried out on the leaf epicuticular wax deposition in response to UV-B radiation. This study presents the variations in wax deposition brought about by ultraviolet-B rays in the leaves of *in situ* grown VAMBAN-3, NIRMAL-7 and T-9 varieties of black gram.

MATERIALS AND METHODS
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. 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 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 epicuticular wax pattern the fully developed third trifoliate leaf from the top was taken from the 30 DAS (days after seed germination) black 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 black 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 black gram, dendrogram was draw to derive the interrelationship between them and presented in table 2 and plate 2.

RESULTS AND DISCUSSION

UV-B exposed VAMBAN-3, NIRMAL-7 and T-9 varieties of black gram enhanced epicuticular wax content by 49.72, 52.99 and 75.08% over their respective controls (Table 1). All the wax samples from the leaves of three varieties of black gram grown under control and UV-B exposed conditions resolved into three components in thin layer chromatography.

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

Varieties	**Treatment**	**Rf values**			**Wax content**
		Spot 1	**Spot 2**	**Spot 3**	**µg g^{-1} fw**
VAMBAN-3	Control	0.74 (+)	0.54 (F)	0.42 (+)	3.56
	UV-B	0.74 (+)	0.54 (F)	0.42 (+)	5.33
NIRMAL-7	Control	0.74 (+)	0.54 (+)	0.42 (F)	3.85
	UV-B	0.74 (+)	0.54 (+)	0.42 (F)	5.89
T-9	Control	0.74 (+)	0.54 (+)	0.42 (+)	3.21
	UV-B	0.74 (+)	0.54 (+)	0.42 (+)	5.62

(+) : Major spot
(F) : Minor spot

Rf value	***Components***
0.74	*Monoketones*
0.54	*Diketones*
0.42	*Secondary alcohols*

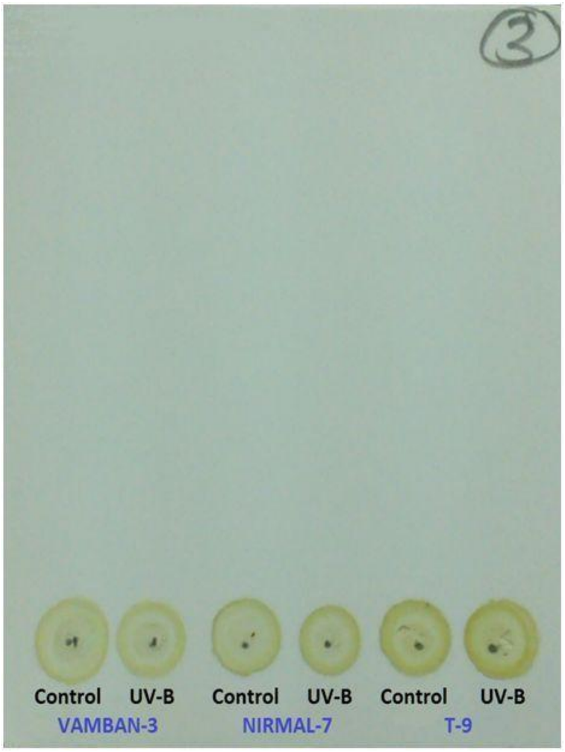

Figure 1: Spotted on activated silica gel

Figure 2: Developed in benzene as solvent

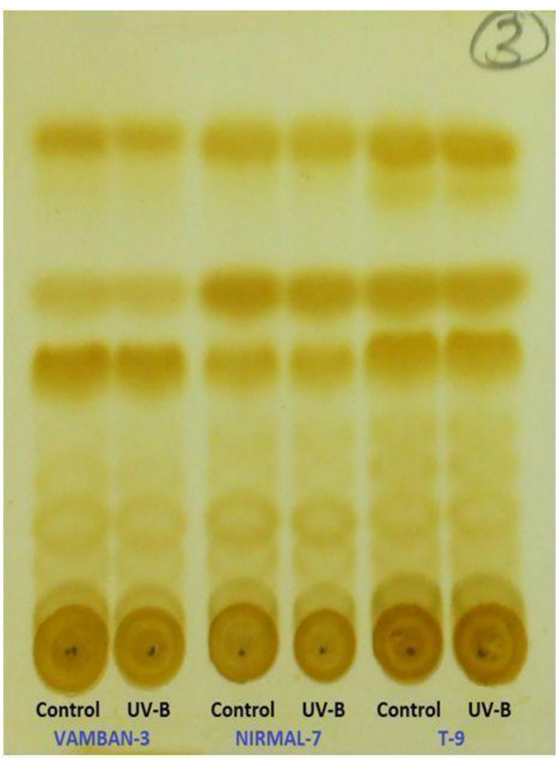

Figure 3: Stained with iodine vapour

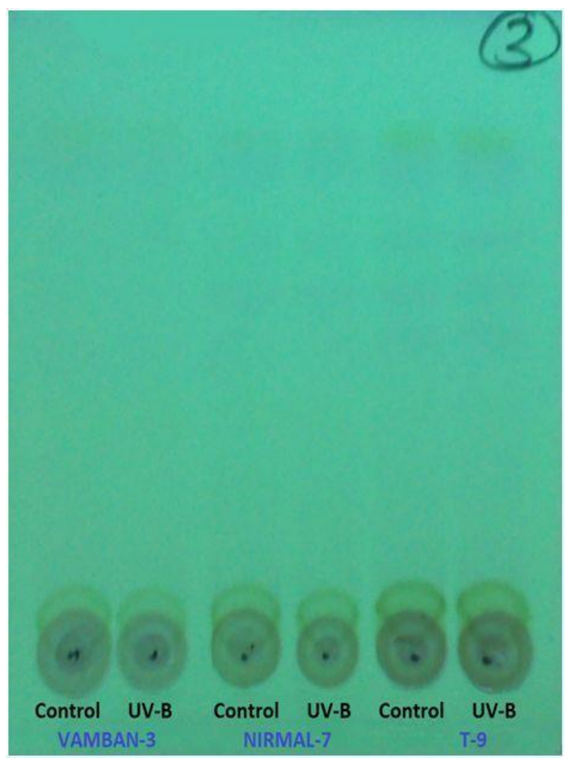

Figure 4: Under ultraviolet light

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

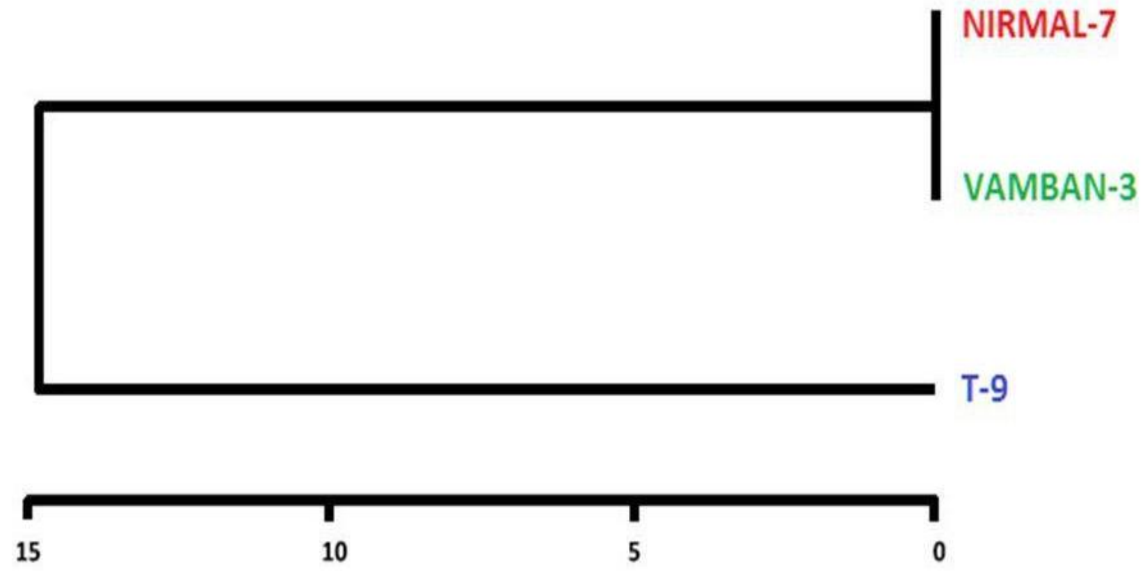

Plate 2: Dendrogram showing the interrelationship between three varieties of *Vigna mungo* (L.) Hepper in leaf epicuticular wax pattern under control and supplementary UV-B exposed conditions

The three components appeared as either major or minor spots at Rf 0.74, 0.54 and 0.42 which corresponded to monoketones, diketones and secondary alcohols respectively (Table 1; Plate 1). VAMBAN-3, NIRMAL-7 and T-9 after UV-B exposure failed to show any difference in the wax composition in comparison with their respective control samples. Epicuticular wax harvested from UV-B exposed and normal leaves of T-9 variety resolved monoketones, diketones and secondary alcohols as three major spots. In control and UV-B irradiated VAMBAN-3 variety of black gram the epicuticular wax at Rf 0.54 which resolved into diketones, appeared as a minor spot (Table 1; Plate 1). In NIRMAL-7 variety, Rf 0.42 corresponding to secondary alcohols also appeared as a minor spot both in control and UV-B stressed crops (Table 1; Plate 1). Similar disparity in the resolution of epicuticular wax components was reported by Rajendiran (2001) in green gram leaves treated with UV-B and triadimefon alone and their combination.

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

Varieties	VAMBAN-3	NIRMAL-7	T-9
VAMBAN-3	100%	33.33%	66.67%
NIRMAL-7	33.33%	100%	66.67%
T-9	66.67%	66.67%	100%

The epicuticular wax pattern assessed through dendrogram in three varieties of black gram under control and UV-B exposed conditions exhibited differences with each other. Even though all the three varieties of black gram resolved into three components, there were differences in the formation of major and minor spots between them. As a result epicuticular wax pattern of VAMBAN-3 and NIRMAL-7 formed separate groups with a minimum similarity value of 33.33 % between them (Table 2; Plate 2). On the other hand, due to the occurrence of all major spots in its epicuticular wax T-9 variety remained alone in the cluster having 66.67 % similarity with the other two varieties of black gram.

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REFERENCES

Arulmozhi D and Rajendiran K (2014a). Effect of supplementary ultraviolet-B radiation on nodulation and nitrogen metabolism in *Lablab purpureus* L. var. Goldy. *International Journal of Advanced Biological Research* **4**(3) 343-346.

Arulmozhi D and Rajendiran K (2014b). Supplementary ultraviolet-B induced reduction in nodulation and nitrogen metabolism in hyacinth bean. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 73-77.

Arulmozhi D and Rajendiran K (2014c). Effect of elevated ultraviolet-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. COFC-8. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(2) 184-188.

Bhat TM and Kudesia R (2011). Evaluation of Genetic Diversity in Five Different Species of Family Solanaceae using Cytological Characters and Protein Profiling. *Genetic Engineering and Biotechnology Journal* **2011** 1-8.

Everstt B (1985). *Clustering Analysis* (John Wiley and Sons, New York).

Koch K and Ensikat HJ (2008). The hydrophobic coatings of plant surfaces: epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. *Micron* **39**(7) 759-72.

Kokilavani V and Rajendiran K (2014a). Influence of elevated ultraviolet-B radiation on the morphology and growth of ten varieties of cowpea. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 171-189.

Kokilavani V and Rajendiran K (2014b). Effect of supplementary UV-B radiation on the yield of ten varieties of cowpea. *International Journal of Geology, Earth and Environmental Sciences* **4**(3) 65-73.

Kokilavani V and Rajendiran K (2014c). Ultraviolet-B induced reduction in nodulation in ten varieties of cowpea. *International Journal of Innovative Research and Review* **2**(4) 77-82.

Maskay N. 1998. Single linkage clustering. In: *Encyclopedia of Biostatistics*, edited by Armintage P and Cotton T (Wiley, New York) **5** 4121- 4122.

Rajendiran K (2001). Amelioration of Ultraviolet-B radiation impacts in green gram by Triadimefon. PhD. Thesis, Pondicherry University.

Rajendiran K and Ramanujam MP (2003). Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum* **46** 621-624.

Rajendiran K and Ramanujam MP (2004). Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. *Biologia Plantarum* **48** 145-148.

Rajendiran K and Ramanujam MP (2006). Interactive effects of UV-B irradiation and triadimefon on nodulation and nitrogen metabolism in *Vigna radiata* plants. *Biologia Plantarum* **50**(4) 709-712.

Rajendiran K, Vidya S, Gowsalya L and Thiruvarasan K (2015). Impact of supplementary UV-B radiation on the morphology, growth and yield of *Vigna mungo* (L.) Hepper var. ADT-3. *International Journal of Food, Agriculture and Veterinary Sciences* **5**(2) 104-112.

Steinmuller D and Tevini M (1982). Some affects of enhanced UV-B radiation on surface lipids of *Cucumis sativus* cotyledons. *Gesellschaft fuer Strahlen- und Umweltforschung, Muenchen* **5** 93-101.

Sudaroli Sudha J and Rajendiran K (2013a). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Sesbania grandiflora* (L.) Pers. *International Journal of Science and Nature* **4**(4) 664-667.

Sudaroli Sudha J and Rajendiran K (2013b). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. c.v. BCP-25. *International Journal of Food, Agriculture and Veterinary Sciences* **3**(3) 77-81.

Sudaroli Sudha J and Rajendiran K (2014a). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. COVU-1. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 224-230.

Sudaroli Sudha J and Rajendiran K (2014b). Ultraviolet-B induced reduction in nodulation and nitrogen metabolism in *Vigna mungo* (L.) Hepper var. T-9. *Global Journal of Bioscience and Biotechnology* **3**(4) 370-373.

Sudaroli Sudha J and Rajendiran K (2014c). Ultraviolet-B induced reduction in nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. CO-1. *Global Journal of Bioscience and Biotechnology* **4**(3) 10-14.

Vijayalakshmi R and Rajendiran K (2014a). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Cyamopsis tetragonoloba* (L.) Taub. var. PNB. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 78-82.

Vijayalakshmi R and Rajendiran K (2014b). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Phaseolus vulgaris* L. cv. Prevail. *International Journal of Advanced Biological Research* **4**(3) 339-342.

Vijayalakshmi R and Rajendiran K (2014c). Effect of elevated ultraviolet-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. CW-122. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(2) 189-193.