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Research Article

EPICUTICULAR WAX DEPOSITION IN THE LEAVES OF COWPEA VARIETIES GROWN UNDER *IN SITU* SUPPLEMENTARY UV-B RADIATION

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ABSTRACT
Epicuticular wax pattern in the leaves of three varieties of cowpea (*Vigna unguiculata* (L.) Walp. Hepper) *viz.* GOWMATHI, FOLA and NS-634 grown under *in situ* ultraviolet-B (UV-B) radiation was analysed through thin layer chromatography. Fully developed third trifoliate leaves from the top of 30 DAS (days after seed germination) cowpea 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 radiation stimulated epicuticular wax production by 66.84 % in GOWMATHI, 66.77 % in FOLA and 69.27 % in NS-634 compared to cowpea varieties grown in normal condition. Wax samples from GOWMATHI, FOLA and NS-634 under control and UV-B exposed conditions resolved into five components. Three major ones corresponded to alkanes, aldehydes and secondary alcohols, while monoesters and diketones appeared as two minor spots.

Keywords: *Cowpea, Epicuticular Wax, Three Varieties, Ultraviolet-B*

INTRODUCTION
The green house gases accumulating around the earth, increases in thickness and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, there by cooling the stratosphere.

Colder than normal temperatures act to deplete ozone layer, allowing enormous ultraviolet-B (UV-B) radiation into earth's surface, affecting the plants in various ecosystems.

The response of leaves to the forecasted increase in climate stress occurrence is considered to be the key issue in global climate change.

Foliar 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 the wax deposit is believed to play a major part in maintaining the water balance of plants during different seasons.

All the experiments with ultraviolet-B (UV-B) radiation (280-320 nm) were concentrated on plant growth (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004; Kokilavani and Rajendiran, 2014a; Rajendiran *et al.*, 2015), fruit harvest (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 a variety of crop plants, with very little investigations directed towards UV-B induced changes in leaf epicuticular wax deposition in plants. As the leaves are the organs that are directly exposed to UV-B radiation, an assessment of the foliar epicuticular wax pattern becomes necessary.

The present work deals with the comparison of wax composition in the leaves of *in situ* UV-B exposed cowpea varieties.

MATERIALS AND METHODS

Cowpea (*Vigna unguiculata* (L.) Walp.) belonging to the family Fabaceae which is a nitrogen fixing grain legume was chosen for the study. Viable seeds of the three varieties of cowpea *viz.* GOWMATHI, FOLA and NS-634 (Namdhari Seeds) 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 experiment.

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 cowpea 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 cowpea 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 cowpea, dendrogram was draw to derive the interrelationship between them and presented in Table 2 and Plate 2.

RESULTS AND DISCUSSION

GOWMATHI, FOLA and NS-634 varieties of cowpea grown under *in situ* ultraviolet-B radiation increased epicuticular wax secretion by 66.84, 66.77 and 69.27 % compared to the respective controls (Table 1).

All the wax samples of three varieties of cowpea grown under control conditions resolved into five components (Table 1; Plate 1).

The trend seen in control samples of cowpea varieties continued in UV-B irradiated leaf samples also, as the epicuticular wax resolved into five components. Out of the five spots formed, three spots appeared as major ones and two were minor spots. The three major spots appearing at Rf 0.96, 0.42 and 0.26 corresponded to alkanes, aldehydes and secondary alcohols respectively. Two minor spots which occurred at Rf 0.64 and 0.54 were identified as monoesters and diketones respectively (Table 1; Plate 1). Similar results were reported by Rajendiran (2001) in green gram exposed to UV-B radiation and soil drench with triadimefon alone and in combination.

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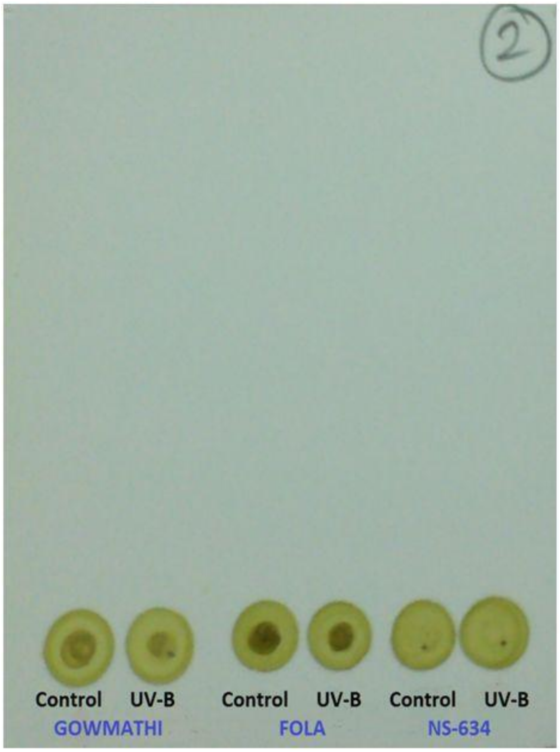

Figure 1: Spotted on activated silica gel

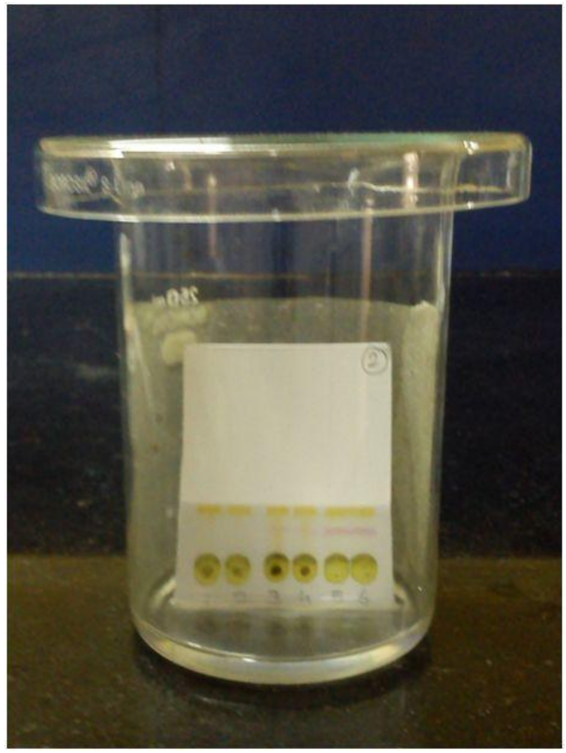
Figure 2: Developed in benzene as solvent

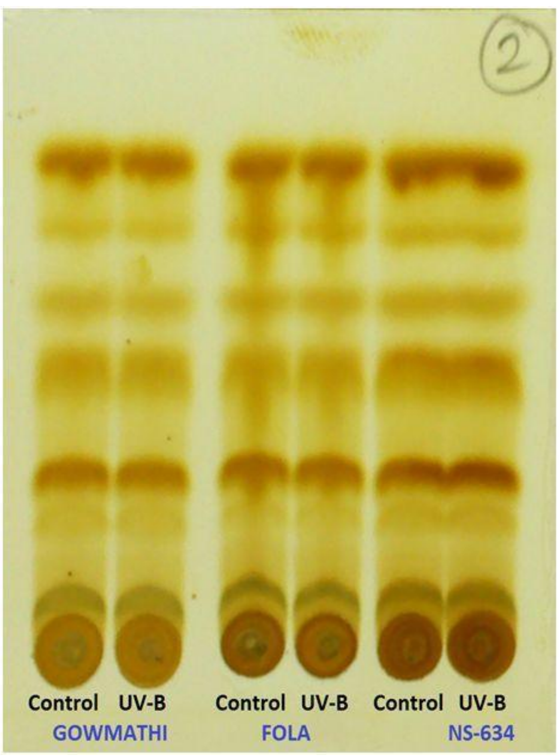

Figure 3: Stained with iodine vapour

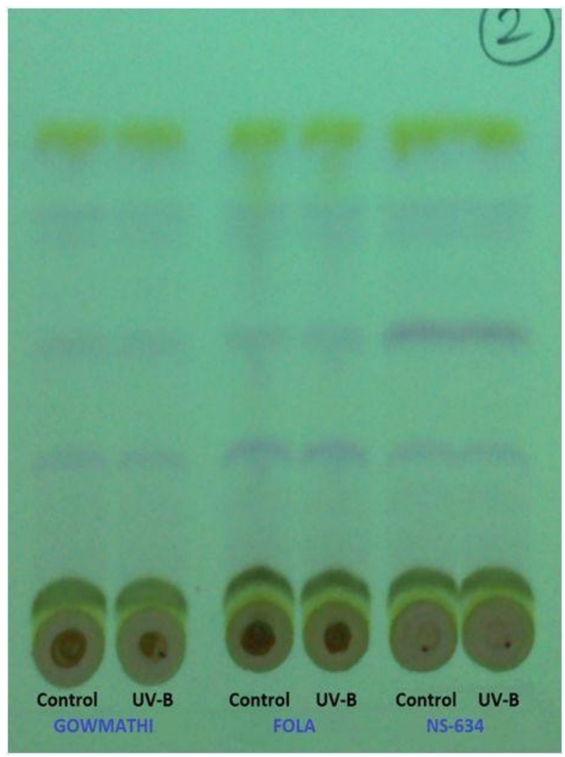

Figure 4: Under ultraviolet light

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

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

Varieties	Treatment	Rf values					Wax content µg g^{-1} fw
		Spot 1	Spot 2	Spot 3	Spot 4	Spot 5	
GOWMATHI	Control	0.96 (+)	0.64 (F)	0.54 (F)	0.42 (+)	0.26 (+)	3.92
	UV-B	0.96 (+)	0.64 (F)	0.54 (F)	0.42 (+)	0.26 (+)	6.54
FOLA	Control	0.96 (+)	0.64 (F)	0.54 (F)	0.42 (+)	0.26 (+)	3.37
	UV-B	0.96 (+)	0.64 (F)	0.54 (F)	0.42 (+)	0.26 (+)	5.62
NS-634	Control	0.96 (+)	0.64 (F)	0.54 (F)	0.42 (+)	0.26 (+)	3.71
	UV-B	0.96 (+)	0.64 (F)	0.54 (F)	0.42 (+)	0.26 (+)	6.28

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

Rf value	***Components***
0.96	*Alkanes*
0.64	*Monoesters*
0.54	*Diketones*
0.42	*Aldehydes*
0.26	*Secondary alcohols*

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

Varieties	GOWMATHI	FOLA	NS-634
GOWMATHI	100%	100%	100%
FOLA	100%	100%	100%
NS-634	100%	100%	100%

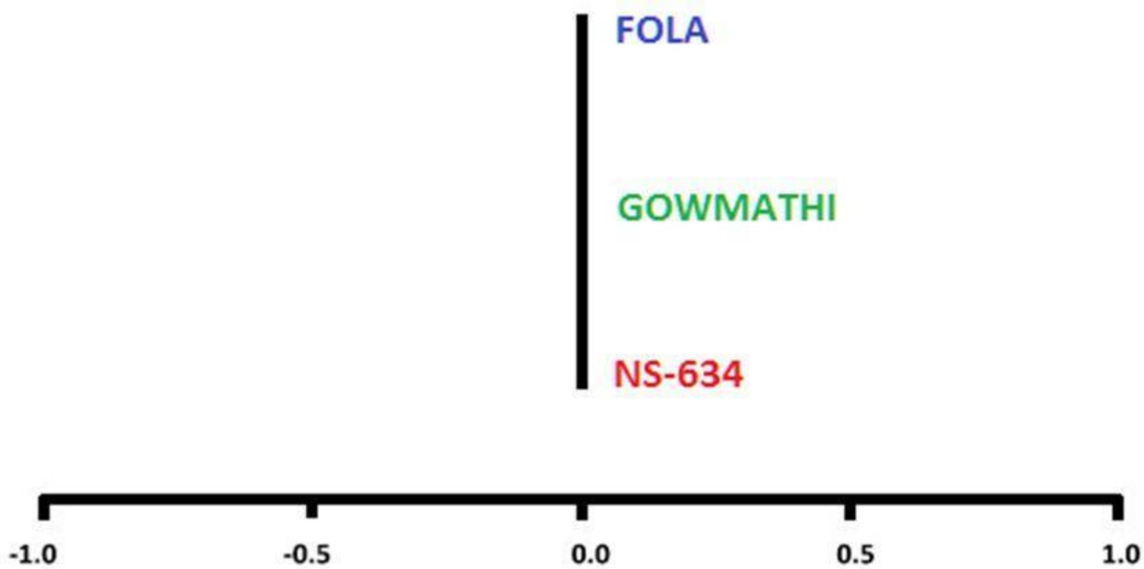

Plate 2: Dendrogram showing the interrelationship between three varieties of *Vigna unguiculata* (L.) Walp. in leaf epicuticular wax pattern under control and supplementary UV-B exposed conditions – *In situ*

The epicuticular wax pattern assessed through dendrogram in three varieties of cowpea under control and UV-B exposed conditions failed to show any difference between them. This was due to similar quantity of epicuticular wax production and identical wax pattern resolved on thin layer chromatography plates by control and UV-B irradiated leaves of cowpea varieties (Table 2; Plate 2). As a result, GOWMATHI, FOLA and NS-634 varieties of cowpea recorded 100 % similarity between them and remained as one group without forming clusters in the dendrogram.

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