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Research Article

IN VITRO GERMINATION OF F_1 SEEDS OF COWPEA VARIETIES CULTIVATED UNDER *IN SITU* UV-B RADIATION

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ABSTRACT
The present study is to evaluate the viability of seeds from F_1 generation harvested from *in situ* control and supplementary UV-B irradiated (UV-B = 2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) cowpea varieties *viz.* GOWMATHI, FOLA and NS-634 by germinating in culture media, in an attempt to screen the seeds for germplasm conservation. F_1 progenies from GOWMATHI, FOLA and NS-634 parents grown under normal sunlight germinated. UV-B irradiated GOWMATHI and NS-634 seeds failed to germinate. However, F_1 seeds of UV-B stressed FOLA germinated, proving that it is suitable for surviving in elevated ultraviolet-B environment.

Keywords: *Cowpea, F_1 Seeds, In Vitro Germination, Three Varieties, Ultraviolet-B*

INTRODUCTION
Man-made emissions of CFCs (chlorofluorocarbons) and green house gases through global warming either by direct or indirect method of ozone destruction, cause a significant decrease in ozone level in the stratosphere, thereby allowing more amount of harmful ultraviolet-B (UV-B) radiation into the surface of the Earth. Elevated level of UV-B radiation (280-320 nm) in the sunlight directly disturbed the plants by decreasing photosynthesis (Kulandaivelu *et al.*, 1989; Sullivan *et al.*, 1994; Rajendiran, 2001), inhibiting plant growth (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004; Kokilavani and Rajendiran, 2014a; Rajendiran *et al.*, 2015a), reducing fruit harvest (Kokilavani and Rajendiran, 2014b; Rajendiran *et al.*, 2015a) and by suppressing 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). UV-B radiation also severely affected stomatal development in leaf epidermis (Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014d; Kokilavani and Rajendiran, 2015a; Kokilavani and Rajendiran, 2015b) and in cotyledonary epidermis (Rajendiran *et al.*, 2015b; Rajendiran *et al.*, 2015c). To gather additional information on the impact of UV-B radiation on F_1 progenies, an *in vitro* experiment was conducted with the seeds harvested from *in situ* control and UV-B exposed cowpea parents.

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, India. 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}. Seeds (F_1 generation) were harvested from both unstressed and supplementary UV-B stressed parent crops grown in the *in situ* condition. The seeds were germinated in culture media to evaluate their viability.

The seeds of three varieties of cowpea used for *in vitro* culture 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 culture medium. The final wash was given with aqueous sterilized solution of (0.1%) ascorbic acid. The surface sterilized seeds were dipped in 90% ethanol for a short period (40 seconds). The seeds were inoculated horizontally on MS medium for culture initiation. Different concentration and combination of cytokinins (6-benzyl amino purine – BAP and Kinetin ranging from 0.1 to 5.0 mgl^{-1}) and auxins (IAA - Indole acetic acid ranging from 0.1to 1.0 mgl^{-1}) were incorporated in the medium for inducing bud breaking dormancy. These cultures were incubated at 28±2˚C in the dark for 2-3 days and subsequently kept under diffused light (22 µ mol m^{-2} s^{-1} SFP- spectral flux photon) for 8 to 10 days. The light was provided by fluorescent tubes and incandescent bulbs. Temperature was maintained by window air conditioners. Positive air pressure was maintained in the culture rooms, in order to regulate temperature and to maintain aseptic conditions. The cultures were regularly monitored and the germination was recorded till 7 DAI (days after inoculation). The plant tissue culture media generally comprise of inorganic salts, organic compounds, vitamins, gelling agents like agar-agar. All the components were dissolved in distilled water except growth regulators. Auxins were dissolved in 0.5N NaOH or ethanol and cytokinins were dissolved in dilute 0.1N HCl or NaOH. For the present study MS basal medium (Murashige and Skoog, 1962) was used as nutrient medium. MS basal medium was used either as such or with certain modification in their composition. Sucrose and sugar cubes were added as a source of carbohydrate. The pH of the media was adjusted to 5.8±2 with 0.5N NaOH or 0.1N HCl before autoclaving. The medium was poured in the culture vessels. Finally the medium was steam sterilized by autoclaving at 15 psi pressure at 121˚C for 15 minutes.

Chemical Composition of MS Medium (Murashige and Skoog, 1962)

	Constituents		Quantity (mgL^{-1})
Macronutrients			
	NH_4NO_3	1650	
	KNO_3		1900
	$CaCL_2.2H_2O$		440
	$MgSO_4.7H_2O$		370
	KH_2PO_4	170	
	Na.EDTA		37.23
	$FeSO_4.7H_2O$		27.95
Micronutrients			
	KI		0.83
	H_3BO_3		6.20
	$MnSO_4.4H_2O$		22.30
	$ZnSO_4.7H_2O$		8.60
	$Na_2MoO_4.2H_2O$		0.25
	$CuSO_4,5H_2O$		0.025
	$CoCl_2.6H_2O$		0.025
	Meso-Inositol		100
	Glycine		2.0
	Thiamine. HCl		0.1
	Nicotinic acid		0.5
	Pyridoxine. HCl	0.5	
	Sucrose (%w/v)	3 %	
	pH		5.8

Preparation of MS Medium

Approximately 90 % of the required volume of the deionized-distilled water was measured in a container of double the size of the required volume. Dehydrated medium was added into the water and stirred to dissolve the medium completely.

The solution was gently heated to bring the powder into solution. Desired heat stable supplements were added to the medium solution. Deionized-distilled water was added to the medium solution to obtain the final required volume. The pH was adjusted to required level with NaOH or HCl. The medium was finally dispensed into culture vessels. The medium was sterilized by autoclaving at 15 psi (pounds per square inch) at 121˚C for appropriate period of time.

Photography

The culture tubes were photographed in daylight using a Sony digital camera fitted with appropriate close-up accessories.

Dendrogram

At least three replicates were maintained for all treatments and control. The experiments were repeated 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, dendrograms were draw to derive the interrelationship between them and presented in Table 1 and Plate 3.

RESULTS AND DISCUSSION

GOWMATHI, FOLA and NS-634 varieties of cowpea grown under *in situ* condition suffered heavily under elevated UV-B radiation as indicated by the stunted appearance of the crops in comparison to the controls (Plate 1).

To test the viability for germplasm conservation, seeds of F_1 generation harvested from *in situ* control and supplementary UV-B irradiated cowpea varieties were germinated in culture media. The F_1 seeds harvested from *in situ* grown control and supplementary UV-B exposed cowpea varieties recorded varied responses when germinated on culture media.

The F_1 seeds from *in situ* grown control parents *viz.,* GOWMATHI, FOLA and NS-634 germinated under *in vitro* culture (Plate 2). However only one variety of F_1 seeds harvested from *in situ* supplementary UV-B irradiated parent *viz.,* FOLA germinated on culture medium (Plate 2). Rajendiran *et al.,* (2014) observed similar results during *in vitro* germination of F_1 seeds harvested from ten varieties of cowpea grown under *in situ* supplementary UV-B radiation.

The varieties GOWMATHI and NS-634 as one group yielded 100 % similarity between them as only the control F_1 seeds of these two varieties germinated in culture media. FOLA remained separated from the group with 50 % similarity index as its F_1 seeds from both control and UV-B stressed parents responded to *in vitro* germination (Table 1; Plate 3).

Table 1: The similarity indices in the *in vitro* germination of seeds of F_1 generation harvested from *in situ* grown three varieties of *Vigna unguiculata* (L.) Walp. after supplementary UV-B exposure

Varieties	GOWMATHI	FOLA	NS-634
GOWMATHI	100%	50%	100%
FOLA	50%	100%	50%
NS-634	100%	50%	100%

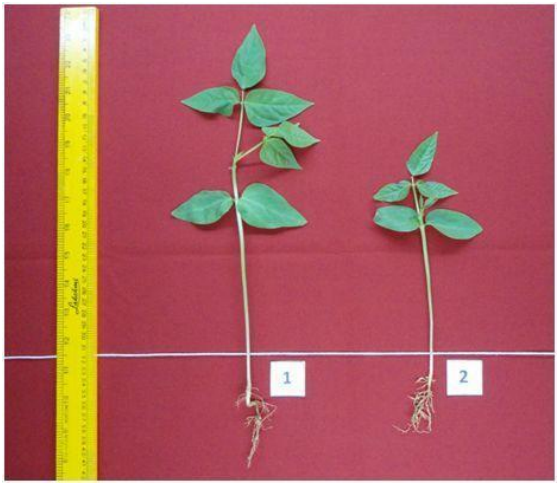

Figure 1: GOWMATHI 15 DAS 30 DAS

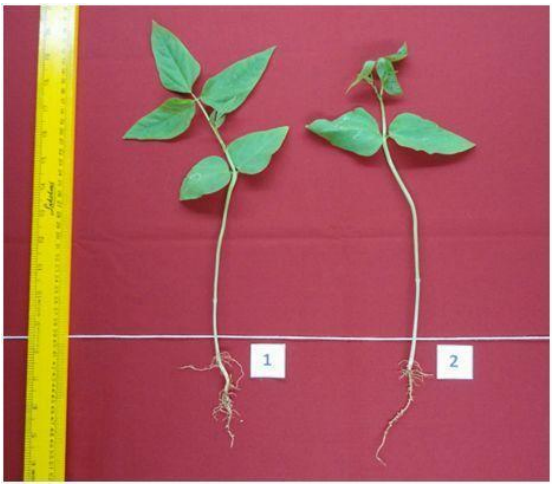

Figure 2: FOLA 15 DAS 30 DAS

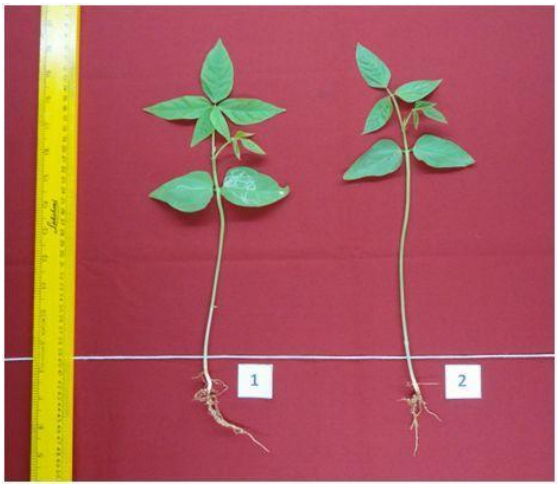

Figure 3: NS-634 15 DAS 30 DAS

Plate 1: The control and supplementary UV-B stressed plants of three varieties of *Vigna unguiculata* (L.) Walp. on 15 and 30 DAS (days after seed germination) (1: Control, 2: UV-B)


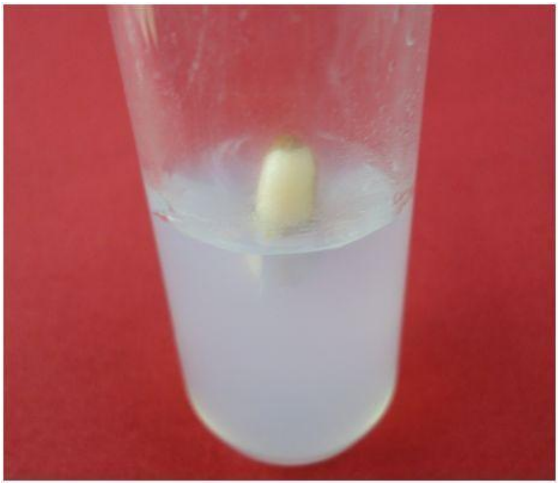

Figure 1: GOWMATHI **Control** **UV-B**


Figure 2: FOLA **Control** **UV-B**


Figure 3: NS-634 **Control** **UV-B**

Plate 2: *In vitro* germination of seeds of F_1 generation harvested from *in situ* control and ultraviolet-B (UV-B) irradiated *Vigna unguiculata* (L.) Walp. parent crops on 7 DAI (days after inoculation)

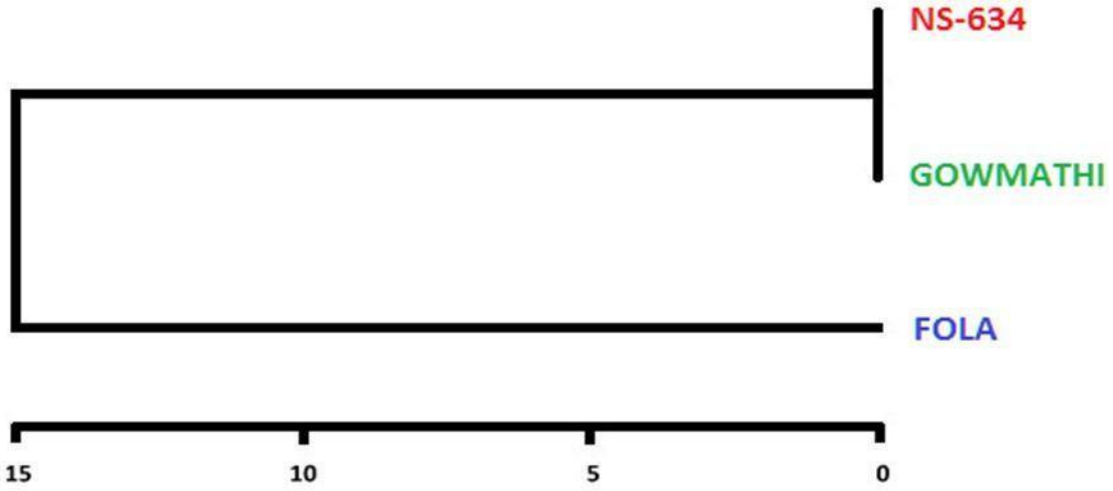

Plate 3: Dendrogram showing the interrelationship between the three varieties of *Vigna unguiculata* (L.) Walp. in the *in vitro* germination of seeds of F_1 generation harvested from *in situ* control and UV-B irradiated parents

As the F_1 seeds of UV-B irradiated GOWMATHI and NS-634 failed to respond *in vitro* germination and only progenies of FOLA from UV-B exposed parents germinated, the present experiment recommends FOLA variety of cowpea to be suitable for cultivation in UV-B enhanced environment.

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