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Research Article

SCREENING OF F_1 SEEDS OF BLACK GRAM VARIETIES GROWN UNDER *IN SITU* UV-B RADIATION FOR *IN VITRO* GERMINATION

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ABSTRACT
The ability of progenies of 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}) black gram varieties *viz.* VAMBAN-3, NIRMAL-7 and T-9 was tested by germinating the harvested seeds under *in vitro* condition. VAMBAN-3 and T-9 seeds both from control and UV-B stressed parents germinated. NIRMAL-7 seeds harvested from UV-B exposed parents germinated, while the control failed. All the varieties of black gram are fit to survive in the UV-B enhanced environment.

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

INTRODUCTION
The ozone layer has the capability to absorb almost 97-99% of the harmful ultraviolet radiations from the sunlight. Man-made emissions of CFCs (chlorofluorocarbons) and other chemicals used in refrigeration, aerosols and cleansing agents may cause a significant decrease in ozone level in the stratosphere, thereby letting through more of the harmful ultraviolet-B (UV-B) radiation. Enhanced ultraviolet-B radiation (280-320 nm) causes long term devastating effects on the biosphere. In response to UV-B rays plants show decreased photosynthesis (Kulandaivelu *et al.*, 1989; Sullivan *et al.*, 1994; Rajendiran, 2001), stunted plant growth (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004; Kokilavani and Rajendiran, 2014a; Rajendiran *et al.*, 2015a), reduced yield (Kokilavani and Rajendiran, 2014b; Rajendiran *et al.*, 2015a) and suppressed 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 induces anomalies in leaf epidermis (Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014d; Kokilavani and Rajendiran, 2015a; Kokilavani and Rajendiran, 2015b) and cotyledonary epidermis (Rajendiran *et al.*, 2015b; Rajendiran *et al.*, 2015c). In view of the adverse effects of UV-B radiation on plants, an *in vitro* experiment was conducted with the seeds harvested from normal and UV-B stressed black gram parent crops to test the behaviour of F_1 progenies.

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, 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 ($UV\text{-}B_{BE}$) 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 tee varieties of black gram 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 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 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, dendrograms were draw to derive the interrelationship between them and presented in Table 1 and Plate 3.

RESULTS AND DISCUSSION

VAMBAN-3, NIRMAL-7 and T-9 varieties of black gram grown under *in situ* condition suffered heavily under elevated UV-B irradiation as indicated by the reduction in size of the plants compared to the respective controls (Plate1).

The seeds of F_1 generation harvested from *in situ* control and supplementary UV-B irradiated black gram varieties when germinated under *in vitro* condition exhibited varied responses. Unstressed NIRMAL-7 F_1 seeds failed to respond *in vitro* germination, while seeds from UV-B irradiated parents germinated with abnormalities (Plate 2, Figure 2).

However, F_1 seeds from varieties VAMBAN-3 and T-9 harvested from *in situ* grown control and UV-B exposed parents responded to *in vitro* culture (Plate 2, Figure 1, 3). Similar results were reported by Rajendiran *et al.,* (2014) during *in vitro* germination of F_1 seeds harvested from ten varieties of cowpea grown under *in situ* supplementary UV-B radiation.

The varieties VAMBAN-3 and T-9 as one group yielded 100 % similarity as both the control as well as UV-B stressed F_1 seeds of these two varieties responded to *in vitro* germination. NIRMAL-7 remained separated from other varieties of black gram as its control F_1 seeds did not germinate in culture media (Table 1; Plate 2).

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 mungo* (L.) Hepper after supplementary UV-B exposure

Varieties	VAMBAN-3	NIRMAL- 7	T-9
VAMBAN-3	100%	50%	100%
NIRMAL-7	50%	100%	50%
T-9	100%	50%	100%

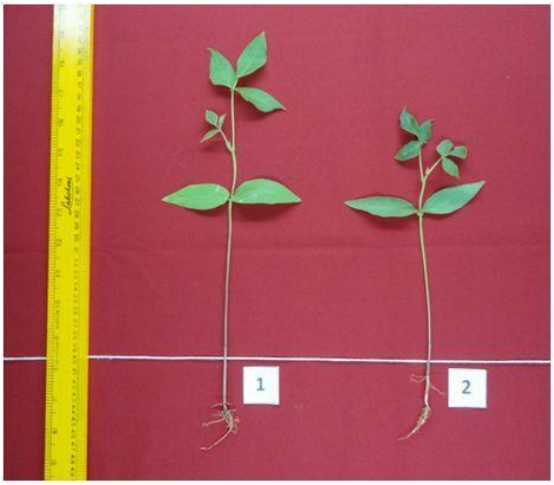


Figure 1: VAMBAN-3 15 DAS

30 DAS

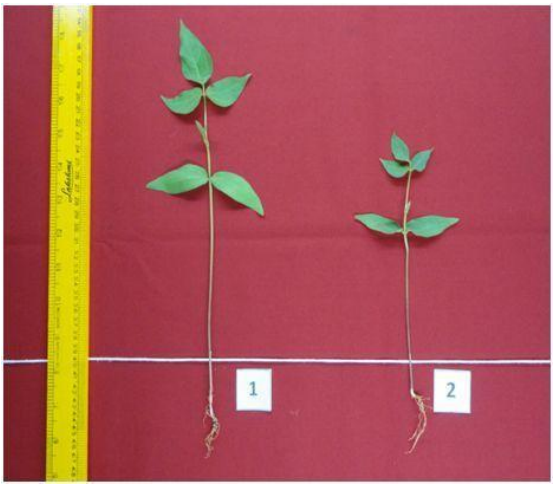


Figure 2: NIRMAL-7 15 DAS

30 DAS

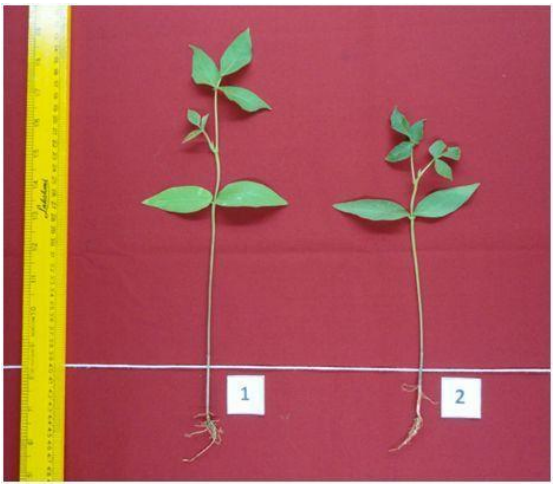


Figure 3: T-9 15 DAS

30 DAS

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

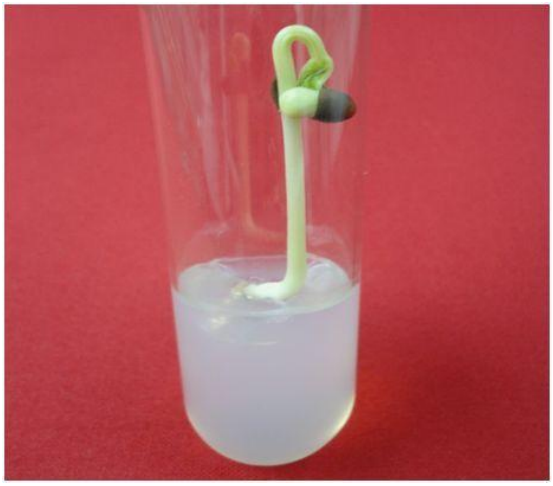
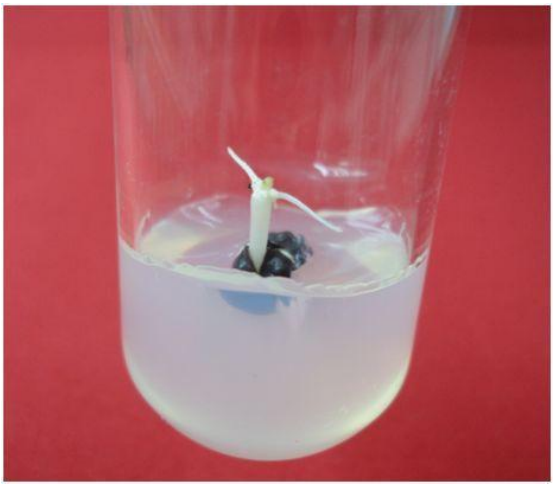


Figure 1: VAMBAN-3 Control

UV-B

Figure 2: NIRMAL-7 Control



UV-B

Figure 3: T-9 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 mungo* (L.) Hepper parent crops on 7 DAI (days after inoculation)

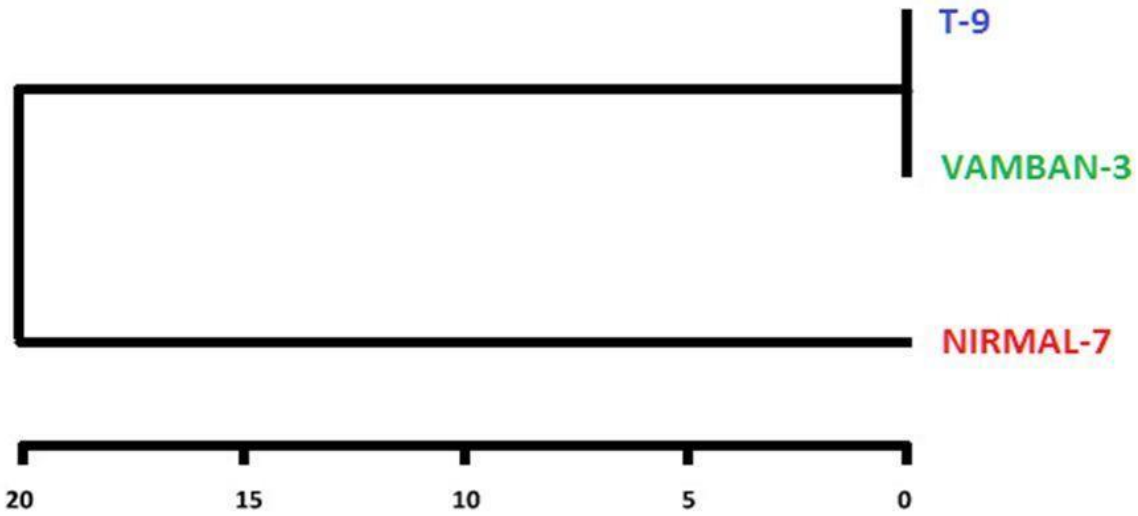


Plate 3: Dendrogram showing the interrelationship between the three varieties of *Vigna mungo* (L.) Hepper in the *in vitro* germination of seeds of F_1 generation harvested from *in situ* control and UV-B irradiated parents

The present study suggests that out of the three varieties of black gram taken for screening, the progenies of two varieties *viz.,* VAMBAN-3 and T-9 possessed more survival value as their F_1 seeds harvested from supplementary UV-B irradiated *in situ* grown parents germinated under *in vitro* condition.

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