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Research Article

IN VITRO GERMINATION OF F_1 SEEDS HARVESTED FROM *IN SITU* UV-B STRESSED GREEN GRAM VARIETIES

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ABSTRACT
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}) green gram varieties *viz.* CO-8, NVL-585 and VAMBAN-2 was evaluated by germinating in culture media, in an attempt to screen the seeds for germplasm conservation. F_1 progenies from CO-8, NVL-585 and VAMBAN-2 both from control and UV-B stressed parents responded to *in vitro* germination suggesting that all the three varieties of green gram are suitable for cultivation in the increasing solar ultraviolet-B radiation flux in future.

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

INTRODUCTION
The ozone layer which has the capability to absorb the harmful ultraviolet-B (UV-B, 280-320 nm) radiations from the sunlight was destroyed directly by man-made emissions of CFCs (chlorofluorocarbons) and indirectly by green house gases through global warming. Enhanced UV-B radiation penetrating into the Earth's surface causes serious effects on 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 yield (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 rays were also reported to penetrate the plant tissues causing stomatal abnormalities 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). This *in vitro* experiment was conducted with the seeds harvested from *in situ* normal and UV-B irradiated green gram varieties to evaluate the impact of UV-B stress on the viability of F_1 progenies.

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, 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 three varieties of green 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 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, dendrograms were draw to derive the interrelationship between them and presented in Table 1 and Plate 3.

RESULTS AND DISCUSSION

Under *in situ* elevated UV-B irradiation, CO-8, NVL-585 and VAMBAN-2 varieties of green gram showed stunted growth compared to the respective controls (Plate1). The seeds of F_1 generation harvested from *in situ* control and supplementary UV-B irradiated green gram varieties when germinated under *in vitro* condition exhibited varied responses. All the three varieties of F_1 green gram seeds harvested from *in situ* grown unstressed CO-8, NVL-585 and VAMBAN-2 varieties germinated under *in vitro* culture (Plate 2). The trend observed with control seeds continued with supplementary UV-B irradiated F_1 seeds also, as all the UV-B stressed green gram varieties responded to *in vitro* germination. 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.

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 radiata* (L.) Wilczek after supplementary UV-B exposure

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

CO-8, NVL-585 and VAMBAN-2 varieties of green gram as one group yielded 100 % similarities between them as both the control and UV-B stressed F_1 seeds of these three varieties germinated well in culture media (Table 1). As a result, the varieties failed to produce any cluster in the dendrogram (Table 1; Plate 3).

Figure 1: CO-8 **15 DAS**

30 DAS

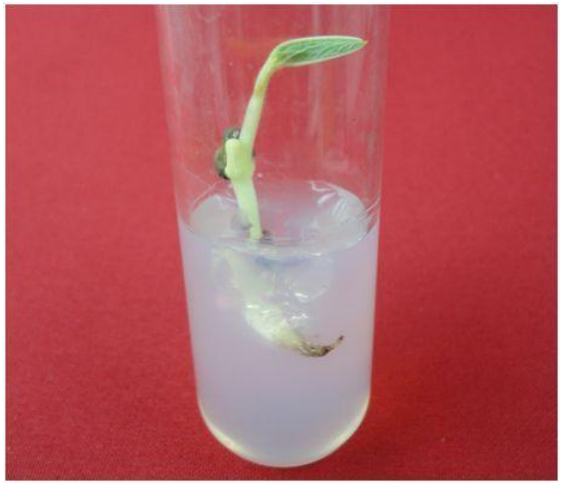
Figure 2: NVL-585 **15 DAS**

30 DAS

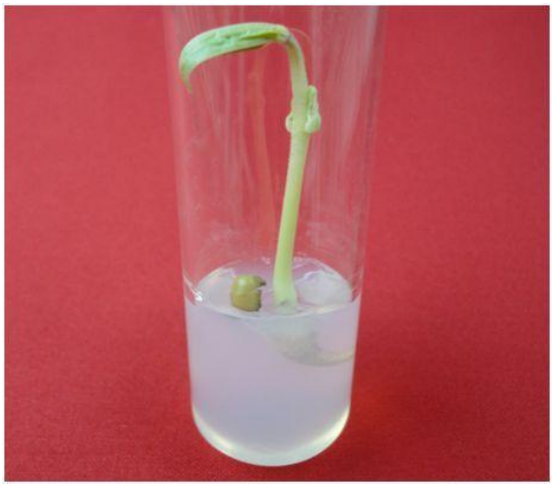
Figure 3: VAMBAN-2 **15 DAS**

30 DAS

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



7 DAI Figure 1: CO-8 **Control** **UV-B**



7 DAI Figure 2: NVL-585 **Control** **UV-B**

7 DAI Figure 3: VAMBAN-2 **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 radiata* (L.) Wilczek parent crops on 7 DAI (days after inoculation)

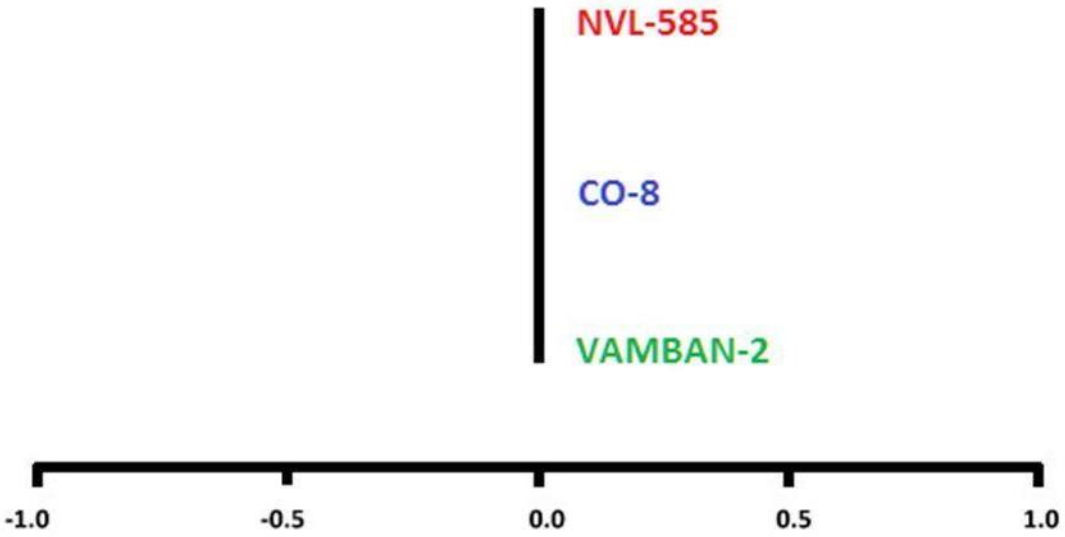


Plate 3: Dendrogram showing the interrelationship between the three varieties of *Vigna radiata* (L.) Wilczek in the *in vitro* germination of seeds of F_1 generation harvested from *in situ* control and UV-B irradiated parents

It is concluded that CO-8, NVL-585 and VAMBAN-2 varieties of green gram taken for screening are fit to survive in elevated UV-B environment as the F_1 seeds harvested from both normal and supplementary UV-B irradiated *in situ* grown parents responded to *in vitro* germination.

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