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Research Article

IN VITRO SEED GERMINATION AND GROWTH OF THREE VARIETIES OF BLACK GRAM AFTER ULTRAVIOLET-B RADIATION

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Abstract

In vitro seed germination and growth of seedlings was tried with three varieties of black gram (*Vigna mungo* (L.) Hepper) viz. VAMBAN-3, NIRMAL-7 and T-9 after ultraviolet-B irradiation (UV-B = 2 hours once with 1 hour recovery time @ 12.2 kJ m⁻² d⁻¹; ambient = 10 kJ m⁻² d⁻¹). Unstressed and UV-B stressed VAMBAN-3 and T-9 seeds both in dry and wet conditions responded to *in vitro* germination. Unstressed NIRMAL-7 failed to germinate under *in vitro* culture. UV-B stressed NIRMAL-7 responded to *in vitro* culture. UV-B irradiation enhanced seedling height at both dry and wet conditions in VAMBAN-3 followed by NIRMAL-7 compared with controls. Root and shoot length of UV-B stressed VAMBAN-3 and NIRMAL-7 performed five to six times better than control. Root and shoot length of T-9 was reduced (2.61 to 8.69 %) below control after UV-B exposure. UV-B stressed VAMBAN-3 under dry and wet exposure accumulated three to six times more plant biomass over controls. UV-B stressed NIRMAL-7 and T-9 dry seeds accumulated less plant biomass by 70.86 % and 12.39 % respectively than their controls. NIRMAL-7 and T-9 under dry UV-B exposure produced two times more leaves than control.

Key words: Black gram; *In vitro* seed germination; three varieties; Ultraviolet-B.

Introduction

Observation, measurement and documentation of heritable plant traits in the biosphere are the primary tasks of germplasm characterization. But rapid climate change through global warming, acid rain and increased flux of ultraviolet radiation pose a threat to germplasm conservation as the stored explants may find it difficult to adapt to a changing or totally transformed habitat. Prior to selection of germplasm of crops, a series of experiments need to be conducted to test the viability of explants on culture media after an imposed stress like ultraviolet-B radiation. Ultraviolet-B (UV-B) radiation (280-320 nm) present in the sunlight is a dangerous atmospheric stress (Caldwell *et al.*, 1998) that damages the epidermal layer of leaves (Kokilavani and Rajendiran 2013; Kokilavani and Rajendiran 2014a; Kokilavani and Rajendiran 2014b; Kokilavani and Rajendiran 2014c; Kokilavani and Rajendiran 2014d; Kokilavani and Rajendiran 2014f; Kokilavani and Rajendiran 2014g; Kokilavani and Rajendiran 2014h; Kokilavani and Rajendiran 2014j; Kokilavani and Rajendiran 2014k; Kokilavani and Rajendiran 2014l; Kokilavani and Rajendiran 2014m;

Kokilavani and Rajendiran 2014n; Kokilavani and Rajendiran 2015a; Kokilavani and Rajendiran 2015b) causes aberrations in cotyledonary epidermis (Rajendiran *et al.*, 2015c; Rajendiran *et al.*, 2015d), disturbs photosynthesis (Kulandaivelu *et al.*, 1989; Sullivan *et al.*, 1994; Rajendiran 2001) causes injuries and reduction in plant growth (Rajendiran and Ramanujam 2003; Rajendiran and Ramanujam 2004; Kokilavani and Rajendiran 2014o; Rajendiran *et al.*, 2015b; Rajendiran *et al.*, 2015k; Rajendiran *et al.*, 2015n; Rajendiran *et al.*, 2015p; Rajendiran *et al.*, 2015q) reduces fruit yield (Mark and Tevini 1997; Rajendiran and Ramanujam 2004; Kokilavani and Rajendiran 2014e; Rajendiran *et al.*, 2015m; Rajendiran *et al.*, 2015p; Rajendiran *et al.*, 2015q) and disturbs nodulation and nitrogen metabolism (Rajendiran and Ramanujam 2006; Sudaroli Sudha and Rajendiran 2013a; Sudaroli Sudha and Rajendiran 2013b; Kokilavani and Rajendiran 2014i; Sudaroli Sudha and Rajendiran 2014a; Sudaroli Sudha and Rajendiran 2014b; Sudaroli Sudha 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; Rajendiran *et al.*, 2015i; Rajendiran *et al.*, 2015o) in a variety of sensitive legumes. The present work aims at identifying the variety of black gram that can withstand UV-B radiation and to test the seeds through *in vitro* culture methods for germplasm conservation and regeneration.

Materials and Methods

In vitro UV-B radiation

Black gram (*Vigna mungo* (L.) Hepper), the nitrogen fixing grain legume belonging to the family Fabaceae 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. Ultraviolet-B (UV-B) radiation was provided by one UV-B lamp (Philips TL 20W/12 Sunlamps, The Netherlands) which was suspended horizontally over the seeds. UV-B dose was maintained by adjusting the distance (30 cm) between seeds and the lamp. The lamp was wrapped with cellulose diacetate filters (0.076 mm) to filter UV-C radiation (< 290 nm). UV-B exposure to seeds was given only once for two hours duration with one hour recovery time in between. Seeds received a biologically effective UV-B dose (UV-B_{BE}) of 12.2 kJ m⁻² d⁻¹. The control seeds were exposed to sunlight for same duration receiving UV-B_{BE} 10 kJ m⁻² d⁻¹ with one hour recovery time in between (Caldwell 1971).

Experimental design

Black gram seeds were divided into two lots - one for growing under normal ambience (control) and another for receiving ultraviolet-B (UV-B). Each lot was again subdivided into two groups, where, one received treatment in dry condition (dry seeds) while the other received treatment in wet condition (wet seeds) after soaking in water over night.

In vitro culture with seeds

Seeds after appropriate aseptic treatment were used for *in vitro* culture. Seeds 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% HgCl₂ for 4-5 minutes and washed 6 to 8 times with autoclaved water under Laminar Air Flow Cabinet (Technico Systems, Chennai). 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 to initiate germination. 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 0.1 to 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. Subsequently these were 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 growth parameters were recorded after 15 DAI (days after inoculation). The experiments were carried out with three replicates per treatment.

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 modifications 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 (pounds per square inch) pressure at 121°C for 15 minutes.

Measurement of plant growth

Three seedlings from each treatment were carefully uprooted on 15 DAI and their axial growth (root and shoot length and plant height) and fresh biomass were measured. They were then dried in an oven at 80° C for 48 h and weighed again for dry mass measurements. Alongside, morphological and developmental abnormalities if any, caused by UV-B radiation were also recorded. Assessment of growth of three varieties of black gram was recorded on 15 DAI and calculations were done using standard methods. The leaf area (the leaflets from all the nodes) was determined at various stages using Area meter (Analytical Development Corporation, UK, model AM100). The total leaf area per plant was obtained by summing up the area of the leaves from all the nodes of the plant. Leaf area index (LAI) (Williams, 1946), specific leaf weight (SLW) (Pearce *et al.*, 1968) and shoot / root ratio (Racey *et al.*, 1983) were calculated using the following formulae.

$$\text{LAI} = \frac{\text{Leaf area of the plants (cm}^2\text{)}}{\text{Ground area occupied (cm}^2\text{)}}$$

$$\text{SLW} = \frac{\text{Leaf dry weight (g)}}{\text{Leaf area (m}^2\text{)}}$$

$$\text{S/R ratio} = \frac{\text{Shoot weight (g)}}{\text{Root weight (g)}}$$

Culture media

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.

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Chemical composition of MS medium (Murashige and Skoog 1962)

Constituents	Quantity (mgL ⁻¹)
Macronutrients	
NH ₄ NO ₃	1650
KNO ₃	1900
CaCl ₂ .2H ₂ O	440
MgSO ₄ .7H ₂ O	370
KH ₂ PO ₄	170
Na.EDTA	37.23
FeSO ₄ .7H ₂ O	27.95
Micronutrients	
KI	0.83
H ₃ BO ₃	6.20
MnSO ₄ .4H ₂ O	22.30
ZnSO ₄ .7H ₂ O	8.60
Na ₂ MoO ₄ .2H ₂ O	0.25
CuSO ₄ .5H ₂ O	0.025
CoCl ₂ .6H ₂ O	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 at 121°C for appropriate period of time.

Photography

The culture tubes with seeds and seedlings 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 Tables 5, 6 and Plates 5, 6.

Results and Discussion

Standardisation of culture media for seed germination

For the standardisation of culture media, seeds of NIRMAL-7 variety of black gram kept under control condition were used (Plate 1). The seeds were inoculated on MS medium for culture initiation containing different concentration and combination of cytokinins (6-benzyl amino purine - BAP = 2.0 mgL⁻¹ and Kinetin = 0.1, 0.25 and 0.5 mgL⁻¹) and auxins (IAA - Indole acetic acid = 1.0 mgL⁻¹). The combination of cytokinins (6-benzyl amino purine - BAP = 2.0 mgL⁻¹ and Kinetin = 0.25 mgL⁻¹) and auxins (IAA - Indole acetic acid = 1.0 mgL⁻¹) was found to be best suited for initiating seed germination (Plate 1) and used for *in vitro* culturing of seeds of all varieties of black gram (Plate 2 to 4)

In vitro germination of seeds and growth of seedlings

The seeds of unstressed and UV-B stressed black gram varieties *viz.*, VAMBAN-3, NIRMAL-7 and T-9, both in dry and wet conditions responded to *in vitro* germination (Table 1 to 2; Plate 2 to 4). UV-B stressed dry seeds of VAMBAN-3 and NIRMAL varieties responded well to *in vitro* germination with root growth performing better than

control multiple times. UV-B exposure reduced root length significantly by 8.69 % on 15 DAI in T-9. The trend seen in dry seeds continued with wet seeds also. UV-B stressed VAMBAN-3 and NIRMAL achieved manifold growth than their respective controls, while T-9 recording a reduction by 2.61 % below control (Table 1 to 2). UV-B stressed dry seeds of VAMBAN-3 and NIRMAL enhanced shoot growth by five to six times more than that of controls, while T-9 variety under UV-B exposure was 27.12 % taller than control. UV-B exposure in VAMBAN-3 and NIRMAL wet seeds reduced shoot length by 11.84 to 34 % on 15 DAI, while T-9 recorded four times longer shoot. Overall, the height of the seedling was enhanced by UV-B irradiation at both dry and wet conditions in VAMBAN-3 and NIRMAL varieties of black gram compared with control (Table 1 to 2; Plate 2 to 4). However the plant growth in UV-B stressed T-9 both in dry and wet treatments showed little reduction in plant height than the controls. The S / R ratio was decreased by UV-B stress on 15 DAI by 6.36 % to 58.02 % in dry seed exposure and by 55.45 % to 61.44 % in wet seeds. However, UV-B stressed T-9 dry seeds showed enhanced S / R ratio by 39.87 % over control (Table 1). Biomass accumulation in root was enhanced by UV-B irradiation by more than 100 % in both dry and wet seed treatments of all varieties of black gram with an exception in T-9 where the accumulation was below control by 7.03 % in wet seed UV-B exposure (Table 1 to 2). Application of UV-B increased shoot biomass by more than 100 % in VAMBAN-3 both in dry and wet treatments and in dry NIRMAL-7. However, shoot biomass was suppressed by 12.39 % to 77 % below control in UV-B exposed dry seeds of NIRMAL-7 and T-9. The trend observed in shoot biomass pattern was reflected at the whole seedling level too with accumulation of shoot biomass by more than 100 % in VAMBAN-3 both in dry and wet treatments and in dry NIRMAL-7, followed by biomass suppression by 8 to

70 % below control in UV-B exposed dry seeds of NIRMAL-7 and T-9.

Enhancement in root biomass content from more than 100 % in VAMBAN-3 and NIRMAL on 15 DAI reaching a minimum of 20 % in T-9 was caused by UV-B treatment in dry seed treatments. Even though over 100 % dry biomass accumulation took place in VAMBAN-3 under dry UV-B and NIRMAL-7 performing well to level with control, T-9 recorded 40 % less value than the controls. UV-B exposure suppressed dry weight of shoot by 46.34 % on 15 DAI below control in dry seed treatment of NIRMAL-7 and enhanced it by 58.62 % to 71.43 % in VAMBAN-3 and T-9 respectively. In wet seed treatment T-9 performed on par with control, while VAMBAN-3 and NIRMAL-7 recorded less dry biomass by 51.42 % and 58.53 % respectively. After UV-B stress, the plant biomass was accumulated by 97 % in VAMBAN-3 and by 48.72 % in T-9 above their respective controls while NIRMAL-7 recorded 25 % reduction on 15 DAI after dry seed exposure. The value of UV-B exposed T-9 wet seeds was very near to control, while the other two varieties of black gram showed reductions compared to the respective controls. The major damage was with VAMBAN-3 wet seeds which recorded 93 % reduction (Table 1 to 2; Plate 2 to 4). Seeds failing to germinate in culture tubes and reduction in growth of the seedlings by UV-B stressed seeds were also reported by Rajendiran *et al.*, (2014c) in ten varieties of cowpea. Similar results were also obtained during *in vitro* germination of F₁ seeds harvested from *in situ* UV-B stressed black gram (Thiruvarasan and Rajendiran 2015), cowpea (Gowsalya and Rajendiran 2015) and green gram varieties (Vidya and Rajendiran 2015). Even leaf and stem explants excised from *in situ* UV-B irradiated cowpea varieties showed variations during *in vitro* regeneration (Rajendiran *et al.*, 2014a and Rajendiran *et al.*, 2014b).

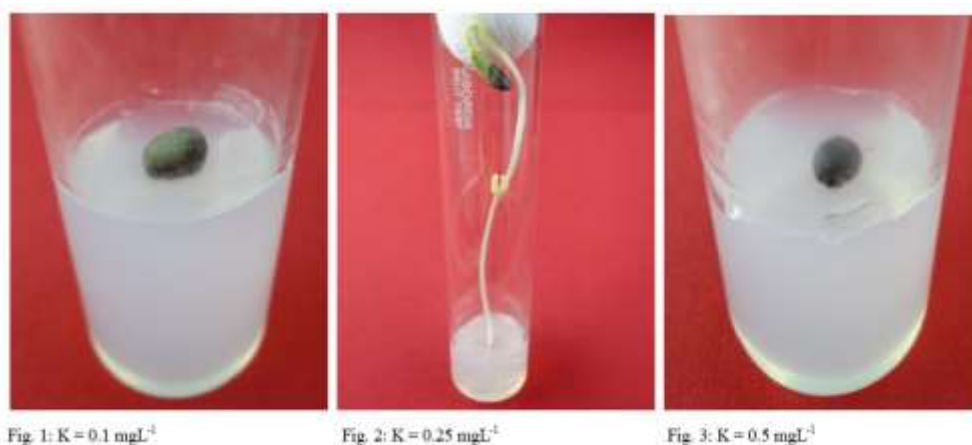


Plate 1: Standardisation of Kinetin (K) concentration in culture media for *in vitro* seed germination and seedling growth using *Vigna mungo* (L.) Hepper var. NIRMAL-7 control seeds. (7 DAI - Days after inoculation)

Table 1: Changes in growth parameters of three varieties of 15 DAI *Vigna mungo* (L.) Hepper in control and UV-B irradiated dry seeds - *In vitro*.

Varieties	Treatment	Root length (cm)	Shoot length (cm)	Shoot / root ratio	Root fresh wt. (g)	Shoot fresh wt. (g)	Plant fresh wt. (g)	Root dry wt. (g)	Shoot dry wt. (g)	Plant dry wt. (g)
VAMBAN-3	Control	0.9	3.2	3.55	0.013	0.057	0.070	0.001	0.035	0.036
	UV-B	13.0	19.3	1.48	0.123	0.529	0.652	0.011	0.060	0.071
NIRMAL-7	Control	0.8	4.4	5.50	0.007	0.144	0.515	0.002	0.082	0.084
	UV-B	15.4	20.9	1.35	0.295	0.524	0.819	0.019	0.044	0.063
T-9	Control	11.5	17.7	1.53	0.128	0.382	0.510	0.010	0.029	0.039
	UV-B	10.5	22.5	2.14	0.154	0.559	0.816	0.012	0.046	0.058

Table 2: Changes in growth parameters of three varieties of 15 DAI *Vigna mungo* (L.) Hepper in control and UV-B irradiated wet seeds - *In vitro*.

Varieties	Treatment	Root length (cm)	Shoot length (cm)	Shoot / root ratio	Root fresh wt. (g)	Shoot fresh wt. (g)	Plant fresh wt. (g)	Root dry wt. (g)	Shoot dry wt. (g)	Plant dry wt. (g)
VAMBAN-3	Control	0.9	3.2	3.55	0.013	0.057	0.070	0.001	0.035	0.036
	UV-B	10.7	13.2	1.23	0.085	0.184	0.269	0.008	0.017	0.025
NIRMAL-7	Control	0.8	4.4	5.50	0.007	0.144	0.515	0.002	0.082	0.084
	UV-B	3.0	2.9	1.3	0.011	0.033	0.044	0.002	0.034	0.035
T-9	Control	11.5	17.7	1.53	0.128	0.436	0.555	0.010	0.029	0.039
	UV-B	11.2	15.6	1.39	0.119	0.382	0.510	0.006	0.029	0.035

Foliage of seedlings

UV-B stressed NIRMAL-7 and T-9 under dry condition had 100 % more number of leaves than their controls (Table 3 to 4; Plate 2 to 4). However, there was only half the number of leaves in VAMBAN-3 under UV-B dry seed exposure. Under UV-B stressed wet seed treatment all the three varieties equalled with their respective controls. UV-B irradiation reduced the total leaf area both under dry and wet seed treatments by 62.39 % to 84.21 %

in VAMBAN-3 and T-9. UV-B stressed NIRMAL-7 which had more leaf area (74.47 %) under dry exposure showed only little enhanced leaf area (3 %) over control under wet seed treatment. The LAI was reduced by UV-B exposure to a larger extent by 52 % to 74 % both under dry and wet seed treatments in VAMBAN-3. NIRMAL-7 had three times more leaf area index in dry treatment while it showed only 3 % enhancement in wet UV-B treatment. T-9 after UV-B exposure levelled with control in dry

condition, while in wet seed treatment there was 65.45 % reduction. The SLW in UV-B irradiated NIRMAL-7 seedlings under dry and wet conditions decreased by 14.28 % to 66.66 %. However, VAMBAN-3 and T-9 under dry and wet conditions showed enhancements up to 54 %. UV-B stress enhanced the fresh weight of leaves by 50 % to more than 100 % when compared to that of control in VAMBAN-3 and T-9 under dry seed exposure, with NIRMAL-7 showing 50 % reduction (Table 3 to 4). UV-B when applied to wet seeds increased the fresh weight of foliage by 15 % to more than 100 % compared to control. UV-B exposure decreased the dry weight of leaves by 5 % to 10 % below control in NIRMAL-7 under dry seed exposure. UV-B irradiation to wet seeds showed greater enhancement in dry weight of foliage by 75 % to more than 100 % compared to control in the other two varieties

(Table 3 to 4). UV-B induced suppression of foliage was reported by Rajendiran *et al.*, (2015a) in *Amaranthus dubius* Mart. Ex. Thell., Rajendiran *et al.*, (2015e) in *Macrotyloma uniflorum* (Lam.) Verdc., Rajendiran *et al.*, (2015f) in *Momordica charantia* L., Rajendiran *et al.*, (2015g) in *Spinacia oleracea* L., Rajendiran *et al.*, (2015h) in *Trigonella foenum-graecum* (L.) Ser., Rajendiran *et al.*, (2015i) in *Benincasa hispida* (Thunb.) Cogn. and Rajendiran *et al.*, (2015j) in *Portulaca oleracea* L. seedlings after short term UV-B exposure to dry and wet seeds. Similar results were also reported by Thiruvarasan and Rajendiran (2015) in black gram, Gowsalya and Rajendiran (2015) in cowpea and Vidya and Rajendiran (2015) in green gram varieties during *in vitro* germination of F₁ seeds harvested from *in situ* UV-B stressed crops.

Table 3: Changes in foliage of three varieties of 15 DAI *Vigna mungo* (L.) Hepper in control and UV-B irradiated dry seeds - *In vitro*.

Varieties	Treatment	Number of leaves	Total leaf area (cm ²)	Leaf area index	Specific leaf weight (g ⁻²)	Fresh weight of foliage (g)	Dry weight of foliage (g)
VAMBAN-3	Control	2	2.758	0.243	0.003	0.006	0.001
	UV-B	1	0.435	0.114	0.004	0.009	0.002
NIRMAL-7	Control	2	2.964	0.235	0.006	0.020	0.019
	UV-B	4	5.068	0.630	0.002	0.017	0.013
T-9	Control	2	3.088	0.303	0.001	0.009	0.004
	UV-B	4	1.161	0.305	0.009	0.035	0.011

Table 4: Changes in foliage of three varieties of 15 DAI *Vigna mungo* (L.) Hepper in control and UV-B irradiated wet seeds - *In vitro*.

Varieties	Treatment	Number of leaves	Total leaf area (cm ²)	Leaf area index	Specific leaf weight (g ⁻²)	Fresh weight of foliage (g)	Dry weight of foliage (g)
VAMBAN-3	Control	2	1.267	0.157	0.007	0.006	0.001
	UV-B	2	0.290	0.076	0.020	0.044	0.006
NIRMAL-7	Control	2	0.897	0.098	0.021	0.020	0.019
	UV-B	2	0.924	0.101	0.018	0.023	0.018
T-9	Control	2	1.254	0.110	0.031	0.019	0.010
	UV-B	2	0.145	0.038	0.048	0.046	0.021



1 DAI Fig. 1: Control

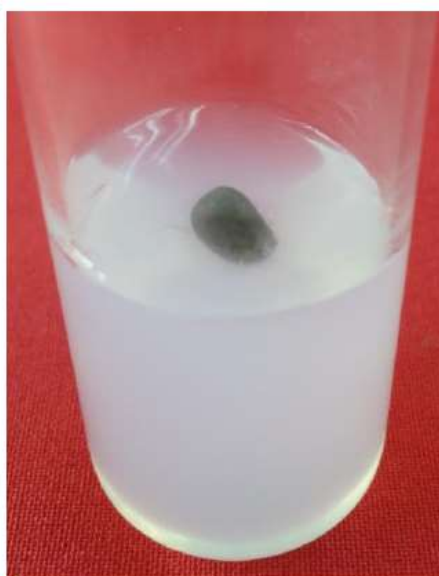


Fig. 2: UV-B on dry seed



Fig. 3: UV-B on soaked seed



7 DAI Fig. 4: Control



Fig. 5: UV-B on dry seed



Fig. 6: UV-B on soaked seed



15 DAI Fig. 7: Control



Fig. 8: UV-B on dry seed



Fig. 9: UV-B on soaked seed

Plate 2. *In vitro* seed germination and growth of *Vigna mungo* (L.) Hepper var. VAMBAN-3 in control and UV-B irradiated dry and soaked seeds. (DAI - Days after inoculation)



1 DAI Fig. 1: Control

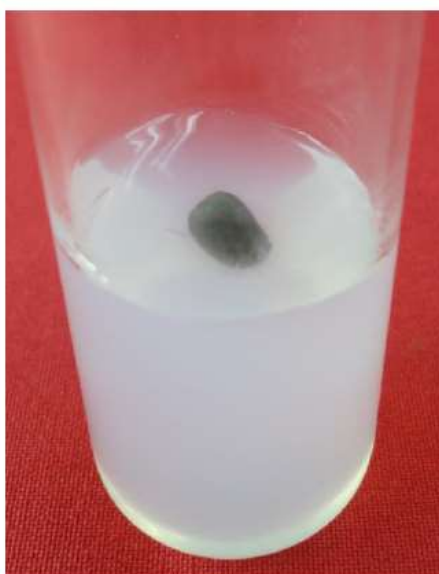


Fig. 2: UV-B on dry seed



Fig. 3: UV-B on soaked seed



7 DAI Fig. 4: Control



Fig. 5: UV-B on dry seed



Fig. 6: UV-B on soaked seed



15 DAI Fig. 7: Control



Fig. 8: UV-B on dry seed



Fig. 9: UV-B on soaked seed

Plate 3. *In vitro* seed germination and growth of *Vigna mungo* (L.) Hepper var. NIRMAL-7 in control and UV-B irradiated dry and soaked seeds. (DAI - Days after inoculation)



1 DAI Fig. 1: Control



Fig. 2: UV-B on dry seed



Fig. 3: UV-B on soaked seed



7 DAI Fig. 4: Control



Fig. 5: UV-B on dry seed



Fig. 6: UV-B on soaked seed



15 DAI Fig. 7: Control



Fig. 8: UV-B on dry seed



Fig. 9: UV-B on soaked seed

Plate 4: *In vitro* seed germination and growth of *Vigna mungo* (L.) Hepper var. T-9 in control and UV-B irradiated dry and soaked seeds. (DAI - Days after inoculation)

Dendrogram

Growth parameters in dry seeds

The growth parameters studied in three varieties of black gram under *in vitro* culture, after exposure of dry seeds to UV-B radiation, showed variations in germination of seeds, plant height, number of leaves, total leaf area, fresh weight, dry weight, and relative growth rate on 15 DAI. The similarity index between VAMBAN-3 and T-9 was the least with a value of 45.5 %. These two varieties remained as one group and showed affinity towards NIRMAL-7 with similarity indices ranging from 52.5 to 59.2 % (Table 5; Plate 5).

Table 5. The similarity indices in growth parameters of three varieties of *Vigna mungo* (L.) Hepper in control and UV-B exposed dry seeds - *In vitro*.

Varieties	VAMBAN-3	NIRMAL-7	T-9
VAMBAN-3	100%	59.2%	45.5%
NIRMAL-7	59.2%	100%	52.5%
T-9	45.5%	52.5%	100%

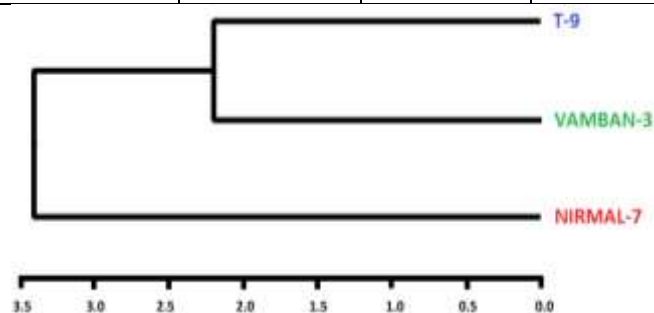


Plate 5: Dendrogram showing the interrelationship between the three varieties of *Vigna mungo* (L.) Hepper in growth parameters in control and UV-B irradiated dry seeds - *In vitro*.

Table 6: The similarity indices in growth parameters of three varieties of *Vigna mungo* (L.) Hepper in control and UV-B exposed wet seeds - *In vitro*.

Varieties	VAMBAN-3	NIRMAL-7	T-9
VAMBAN-3	100%	56.5%	45.5%
NIRMAL-7	56.5%	100%	41.5%
T-9	45.5%	41.5%	100%

Growth parameters in wet seeds

Three varieties of black gram under *in vitro* culture, after exposure of wet seeds to UV-B radiation, also showed variations in germination of seeds, plant height, number of leaves, total leaf area, fresh weight, dry weight, and relative growth rate on 15 DAI. The similarity index of 56.5 % brought together VAMBAN-3 and NIRMAL-7 varieties as one group. T-9 remained alone in the cluster showing similarity indices of 41.5 to 45.5 % with the

group members *viz.*, VAMBAN-3 and NIRMAL-7 varieties of black gram (Table 6; Plate 6).

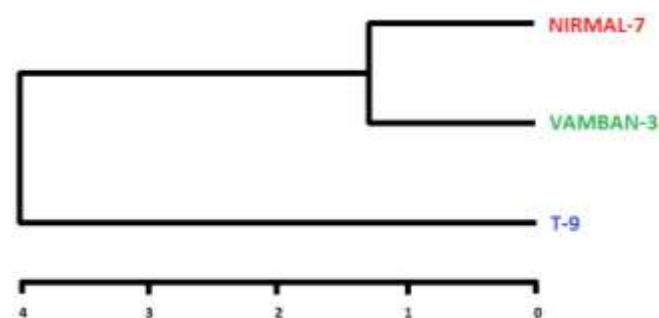


Plate 6: Dendrogram showing the interrelationship between the three varieties of *Vigna mungo* (L.) Hepper in growth parameters in control and UV-B irradiated wet seeds - *In vitro*.

Conclusion

The present investigation recommends the seeds of all the three black gram varieties *viz.*, VAMBAN-3, NIRMAL-7 and T-9 for germplasm conservation for regenerating in UV-B stressed habitat. However, the NIRMAL-7 variety of black gram was found to be more comfortable under UV-B irradiation as the seedlings established well in culture medium even after UV-B exposure.

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