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Research Article

EVALUATION OF ULTRAVIOLET-B STRESSED EXPLANTS OF *MACROTYLOMA UNIFLORUM* (LAM.) VERDC. FOR *IN VITRO* PROPAGATION

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ABSTRACT
The *in vitro* regeneration was tried in horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) after *in situ* UV-B with seeds, stem and leaf explants (third from top of canopy) harvested on 30 days after seed germination from *in situ* control and supplementary ultraviolet-B irradiated (UV-B = 2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) horse gram to study their viability for germplasm storage. UV-B reduced the growth parameters in horse gram at 15 and 30 DAS of growth. On prolonged exposure to UV-B the leaves exhibited various kinds of abnormalities. Both unstressed and UV-B stressed seeds both in dry and wet conditions responded *in vitro* germination. UV-B radiation enhanced growth of seedlings over control in dry condition. UV-B irradiation suppressed height of seedlings at wet condition compared with control. Plant height responded well equaling control after dry UV-B exposure to seeds. UV-B stressed wet seeds accumulated plant biomass by 12.16 % over control. Axillary bud regeneration and callus induction did not occur both in control as well as in UV-B irradiated stem explants. Callus induction did not occur both in control and UV-B stressed horse gram leaf explants. From this study it is evident that out of the explants of horse gram taken for screening, the seeds are considered to be best choice for germplasm conservation and cultivation in UV-B elevated environment in future.

Keywords: *Ultraviolet-B, Horse Gram, In Situ Growth, Seeds, Leaf Explants, Stem Explants, In Vitro Regeneration*

INTRODUCTION
Due to increases in ozone depleting substances (ODS) as well as thickness of green house gases around the earth released by human activities ozone depletion continues to occur. The heat that has to escape the troposphere and enter the stratosphere was not allowed, making the stratosphere cooler. Too much coldness in this layer favours ozone depletion and as a result, the UV-B radiation will increase, affecting the ecosystems. Elevated ultraviolet-B (UV-B) radiation (280-320 nm) is a dangerous atmospheric stress (Caldwell *et al.*, 1983; Jordan, 1997; Caldwell *et al.*, 1998) as it affects foliar epidermis (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000a; Rajendiran and Ramanujam, 2000b; Rajendiran, 2001; Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b), suppresses photosynthesis (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and inhibits nodulation and nitrogen metabolism (Rajendiran and Ramanujam, 2006; Rajendiran and Ramanujam, 2003; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Sudaroli and Rajendiran, 2014; 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 sensitive plants. Hence screening methods have to be developed to select the best varieties of crops that are suitable for surviving in elevated UV-B environment and to conserve their germplasms. On this line, the present work is an attempt for the first time to find out the horse gram that can tolerate supplementary UV-B irradiation and to identify the germplasm of the crop for conservation and regeneration through tissue culture method.

MATERIALS AND METHODS
Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) was chosen for the study. Viable seeds of horse gram were procured from local farmers in Pondicherry. 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 5th day after sowing.

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}.

The responses of horse gram in control and supplementary UV-B irradiation under *in situ* condition were assessed in terms of growth on 15 and 30 DAS.

Supplementary 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).

The filters were changed periodically to maintain uniform optical properties. 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).

Seeds, nodal shoot segments (stem explants) and leaf discs (leaf explants) after appropriate aseptic treatment were used for *in vitro* culture. The explants 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 explants were dipped in 90% ethanol for a short period (40 seconds).

The seeds stem and leaf explants were inoculated vertically 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 0.1 to 1.0 mgl^{-1}) were incorporated in the medium for inducing bud breaking. 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 and callus proliferation were recorded after 15 DAI (days after inoculation) and 30 DAI. 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 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 (mg l^{-})
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

Plants grown under *in situ* condition and *in vitro* cultures tubes were photographed in daylight using a Sony digital camera fitted with appropriate close-up accessories.

RESULT AND DISCUSSION

In situ Studies

There were fewer leaves only (60 %) under UV-B stress on 15 as well as 30 DAS, but plants under normal ambience had more number of leaves (Table 1 to 2; Plate 1). Supplementary UV-B irradiation reduced the total leaf area throughout the growth period, the maximum being 60.32 % on 30 DAS in horse gram. The LAI was reduced by UV-B exposure to 11.73 % on 15 DAS and the maximum being 31.77 % below control on 30 DAS in horse gram. The SLW in UV-B irradiated reduced by 8.69 % on 15 DAS and increased by 11.52 % on 30 DAS. UV-B stress reduced the fresh weight of leaves by 41.13 both on 15 DAS as well as on 30 DAS. The dry weight of foliage decreased by 22 % on 15 DAS and by 48.07 % on 30 DAS of UV-B exposed plants when compared with controls (Table 1 to 2; Plate 1). Reductions in leaf area and mass were observed in the field-grown sweetgum plants exposed to elevated UV-B radiation (Sullivan *et al.,* 1994) and *in situ* pot-grown ten varieties of cowpea (Kokilavani and Rajendiran,

2014a; 2014b, 2014c, 2014d, 2014e, 2014f, 2014g, 2014h, 2014i, 2014j, 2014k, 2014l, 2014m, 2014n, 2014o). Britz and Adamse (1994) opine that changes in the leaf area and dry mass indicated that cell elongations as well as cell contents were affected and inhibitions in the growth of the plants are part of general UV-B effects.

UV-B exposed leaves on the long run exhibited various kinds of abnormalities (Plate 2). The leaves became generally pale which at times occurred in patches. The yellowing intensified and became discretely chlorotic. Browning developed in patches indicating necrosis of the underlying tissues during later stages. Necrotic lesions appeared in older leaves which have received UV-B over a long time. The trifoliate leaves on UV-B exposure had reduced number of leaflets, exhibited bronzing and became silvery and brittle. Varied abnormalities occurred in black gram (Kokilavani and Rajendiran, 2013), cucumber (Kokilavani and Rajendiran, 2014b) and several varieties of cowpea grown under *in situ* UV-B irradiation (Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b; Kokilavani and Rajendiran, 2014c; Kokilavani and Rajendiran, 2014e; Kokilavani and Rajendiran, 2014f; Kokilavani and Rajendiran, 2014g; Kokilavani and Rajendiran, 2014j; Kokilavani and Rajendiran, 2014k; Kokilavani and Rajendiran, 2014l; Kokilavani and Rajendiran, 2014m; Kokilavani and Rajendiran, 2014n; Kokilavani and Rajendiran, 2014o; Kokilavani and Rajendiran, 2015a; Kokilavani and Rajendiran, 2015b).

UV-B exposure reduced root length significantly by 24.57% on 15 DAS and by only 5.49 % on 30 DAS (Table 3 to 4; Plate 1). Shoot length of UV-B stressed plants decreased by 7.92 % within 15 DAS and continued so till 30 DAS with 27.01 % reduction. The S / R ratio was increased by UV-B stress by 35.74 % in horse gram on 15 DAS. However, the reduction was only 24.85 % below control on 30 DAS. Fresh weight of roots increased with age in all treatments. The biomass accumulation in root was inhibited by UV-B treatment by 41.20 % on 15 DAS, the maximum reduction being on 30 DAS (51.78 %). A general decrease of 30.27 % in shoot fresh weight of UV-B treated plants was observed with the maximum sensitivity shown by horse gram was on 30 DAS of growth with 37.78 % reduction. The trends observed in root and shoot biomass pattern were reflected at the whole plant level too with inhibitions at UV-B, by 31.71 % on 15 DAS with the maximum reduction of 39.10 % on 30 DAS. A gradual reduction in the root biomass content starting from 16.97 % on 15 DAS and reaching 32.55 % on 30 DAS was caused by UV-B treatment. UV-B exposure suppressed dry weight of shoot by 13.10 % on 15, reaching a maximum of 43.83 % on 30 DAS over control. Plant dry weight after UV-B stress fell below control by 13.75 % on 15 DAS and 7.26 % on 30 DAS.

Inhibition of growth indicated by reductions in root and shoot length and biomass content due to UV-B stress were apparent at all stages. Such inhibitions are characteristic of UV-B stressed legumes as in *Vigna unguiculata* (Kulandaivelu *et al.,* 1989), *Phaseolus vulgaris* (Mark and Tevini, 1997), *Vigna mungo* (Rajendiran and Ramanujam, 2000a) and *Vigna radiata* (Rajendiran and Ramanujam, 2003) and ten varieties of cowpea (Kokilavani and Rajendiran, 2014o). The stunting of UV-B stressed plants is attributed to destruction of endogenous IAA whose photo-oxidative products may be inhibitory (Kulandaivelu *et al.,* 1989; Tevini and Teramura, 1989) as indicated by a decrease in IAA content concomitant with a corresponding increase in IAA oxidase activity in rice leaves (Huang *et al.,* 1997). The relative growth rate (RGR) increased in UV-B irradiated plants by 7.26 % above control on 15 DAS. However, RGR showed maximum increase by 40.84 % on 30 DAS (Table 3 to 4). Similar inhibitions of RGR by UV-B were observed by Jain *et al.,* (1999) in mungbean and in ten varieties of cowpea (Kokilavani and Rajendiran, 2014o).

In vitro Studies

The seeds of unstressed horse gram responded *in vitro* germination. The UV-B stressed dry and wet seeds of horse gram also responded well as they germinated profusely under *in vitro* condition**.** Only UV-B stressed dry seeds responded well to *in vitro* germination (Table 5 to 6; Plate 3). UV-B stressed horse gram seedlings under dry and wet conditions had similar number of leaves compared with the controls. UV-B irradiation reduced the total leaf area both under dry and wet seed treatments. It was lowered in UV-B irradiated dry seeds by 57.77 % and after wet seed treatment by 4.05 % when compared to control (Table 5 to 6; Plate 3). The LAI was increased by UV-B exposure by 5.28 % under dry seed treatment.

Same trend was recorded after wet seed treatment which showed a reduction by 38.05 %. The SLW in UV-B irradiated seedlings under dry conditions decreased by 33.33 %, where as it showed a decrease by 38.46 % under wet condition. UV-B stress decreased the fresh weight of leaves by 13.51 % when compared to that of control under dry seed exposure. UV-B when applied to wet seeds reduced the fresh weight of foliage only by 5.36 % compared to control. The trend observed in fresh weight continued with dry weight of foliage also. UV-B exposure decreased the dry weight of leaves by 71.28 % below control under dry seed exposure. UV-B irradiation to wet seeds also showed reduction in dry weight of foliage by 67.43 % compared to control (Table 5 to 6; Plate 3).

Figure 1: On 15 DAS

Figure 2: On 30 DAS

Plate 1: The control and supplementary UV-B stressed plants of *Macrotyloma uniflorum* (Lam.) Verdc. (1: Control, 2: UV-B)

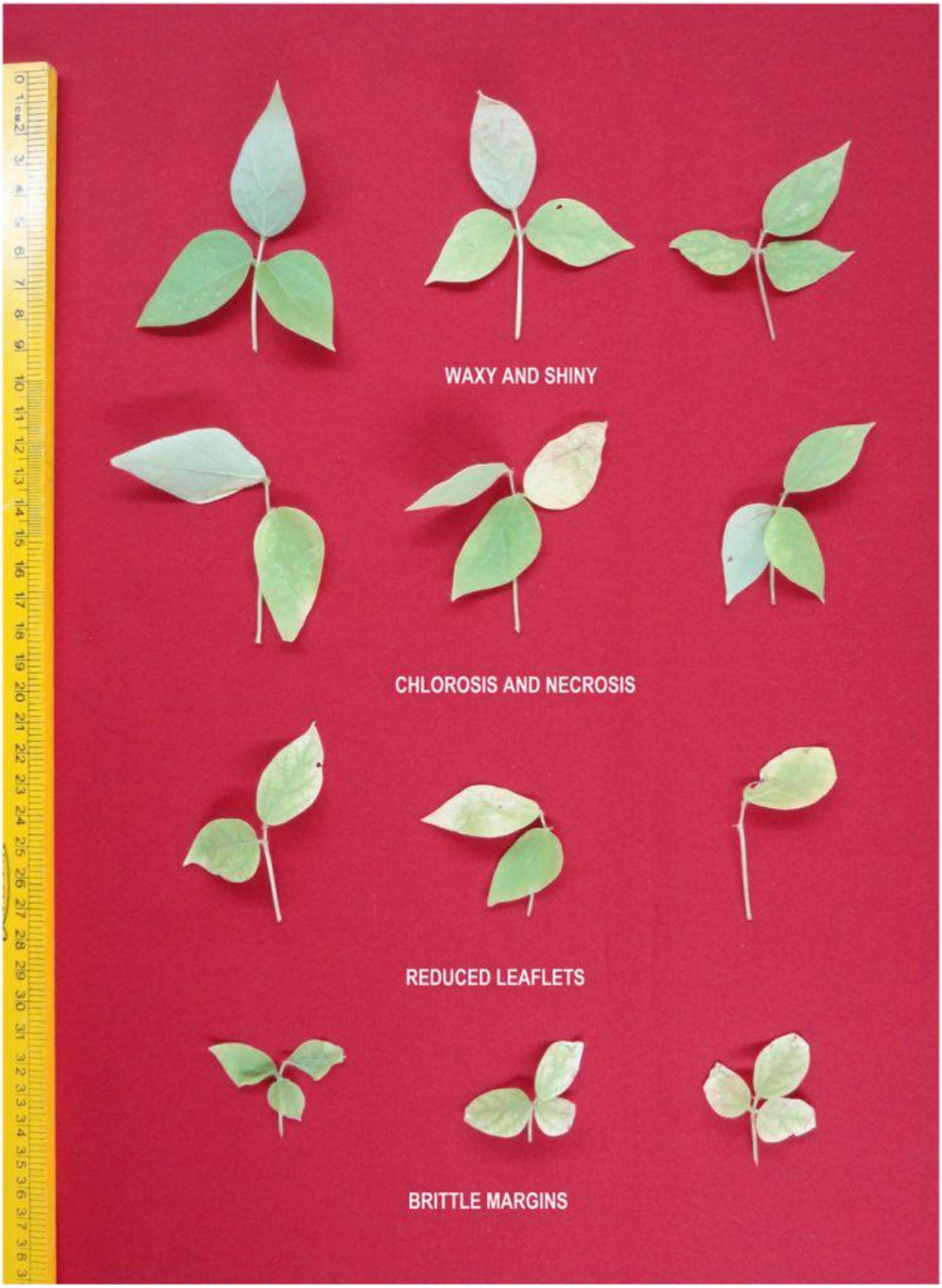


Plate 2: Types of foliar injury caused by elevated UV-B radiation in *Macrotyloma uniflorum* (Lam.) Verdc. on 30 DAS

1 DAI Figure 1: Control

Figure 2: UV-B on dry seed

Figure 3: UV-B on soaked seed

7 DAI Figure 4: Control

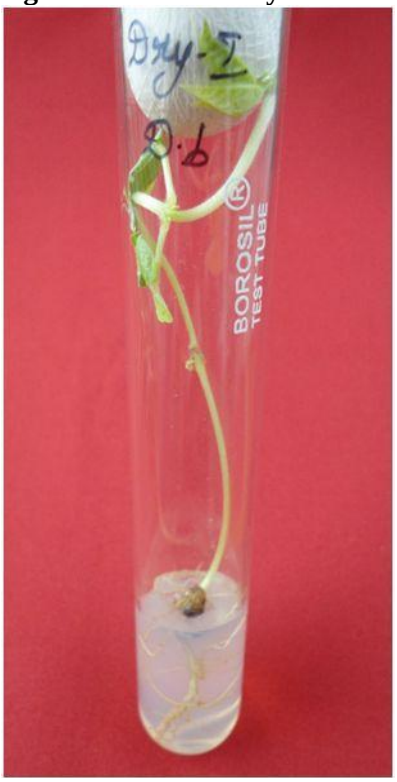


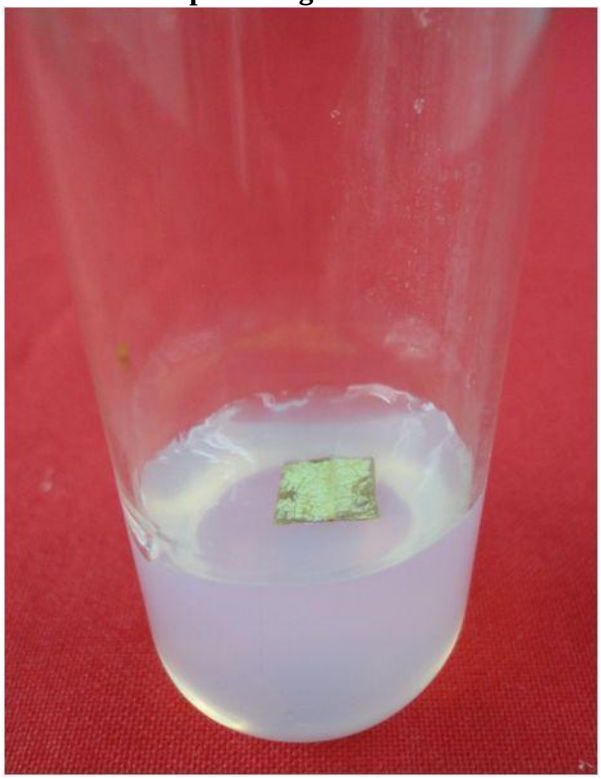
Figure 5: UV-B on dry seed

Figure 6: UV-B on soaked seed

Plate 3: *In vitro* seed germination and growth of *Macrotyloma uniflorum (Lam.) Verdc*. in control and UV-B irradiated dry and soaked seeds (DAI - Days after inoculation)

7 DAI: Stem explants Figure 1: Control

Figure 2: UV- B



7 DAI: Leaf explants Figure 3: Control

Figure 4: UV- B

Plate 4: *In vitro* callus proliferation from stem and leaf explants of control and UV-B irradiated *Macrotyloma uniflorum (Lam.) Verdc*. plants. (DAI - Days after inoculation)

Table 1: Changes in foliage of 15 DAS *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated plants– *In situ*

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)
Control	3	99.30	0.035	0.004	0.358	0.045
UV-B	2	47.37	0.031	0.004	0.239	0.035

Table 2: Changes in foliage of 30 DAS *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated plants– *In situ*

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)
Control	5	295.78	0.034	0.005	0.683	0.137
UV-B	3	117.34	0.023	0.005	0.402	0.071

Table 3: Changes in growth parameters of 15 DAS *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated plants– *In situ*

Treatment	Plant height (cm)	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)	Relative growth Rate
Control	19.63	5.7	13.9	6.65	0.066	0.440	0.507	0.021	0.107	0.129	-0.05
UV-B	17.06	4.3	12.8	9.02	0.039	0.307	0.346	0.018	0.093	0.111	-0.06

Table 4: Changes in growth parameters of 30 DAS *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated plants – *In situ*

Treatment	Plant height (cm)	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)	Relative growth Rate
Control	31.03	9.1	21.1	9.10	0.086	0.785	0.872	0.034	0.199	0.233	- 0.572
UV-B	23.96	8.6	15.4	6.84	0.042	0.488	0.531	0.023	0.112	0.135	- 0.805

Table 5: Changes in foliage of 15 DAI *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated dry seeds– *In vitro*

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)
Control	2	13.06	0.350	0.003	0.182	0.039
UV-B	2	5.51	0.368	0.002	0.158	0.011

Table 6: Changes in foliage of 15 DAI *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated soaked seeds – *In vitro*

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)
Control	2	13.06	0.350	0.003	0.182	0.039
UV-B	2	13.72	0.244	0.002	0.173	0.012

Table 7: Changes in growth parameters of 15 DAI *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated dry seeds – *In vitro*

Treatment	Plant height (cm)	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)	Relative growth Rate
Control	19.7	8.2	11.5	1.70	0.171	0.292	0.463	0.026	0.037	0.064	-0.02
UV-B	20.1	7.6	12.6	1.12	0.161	0.584	0.745	0.021	0.024	0.046	-8.51

Table 8: Changes in growth parameters of 15 DAS *Macrotyloma uniflorum* (Lam.) Verdc. in control and UV-B irradiated soaked seeds – *In vitro*

Treatment	Plant height (cm)	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)	Relative growth Rate
Control	19.7	8.2	11.5	1.70	0.171	0.292	0.463	0.026	0.037	0.064	- 0.02
UV-B	17.5	7.5	10	1.78	0.187	0.333	0.520	0.018	0.032	0.051	- 0.01

UV-B exposure reduced root length significantly by 7.31 % on 15 DAI. Unstressed plants showed profuse root growth on 15 DAI (days after inoculum). UV-B stressed wet seeds of horse gram responded to *in vitro* germination, but the root length reduced by 8.5 % on 15 DAI. UV-B stressed dry seeds of horse gram exhibited an increase in shoot growth of the seedlings by 9.56 % on 15 DAI. UV-B exposure in wet seeds showed reduction in shoot length of the seedlings by 13.04 % on 15 DAI. Unstressed wet seeds showed profuse shoot growth on 15 DAI. Overall, the height of the seedling was enhanced by UV-B irradiation at dry conditions by 2.53 %. However, at wet seed treatment the plant height was reduced by 11.16 % below control seedlings on 15 DAI. The S / R ratio was decreased by UV-B stress on 15 DAI by 33.84 % in dry seed exposure and increased by 4.42 % in wet seed treatment (Table 7 to 8; Plate 3).

Biomass accumulation in root was inhibited by UV-B irradiation by 6.23 % in dry seed treatments with an exception in wet seed treatment where the accumulation was 13.72 % more than control (Table 7 to 8; Plate 3). A general decrease of 99.40 % in shoot fresh weight after UV-B exposure to dry seeds was observed on 15 DAI. However, there was an increase in shoot biomass by 13.72 % in UV-B irradiated horse gram wet seeds on 15 DAI.

Fresh biomass of the seedlings increased by 60.68 % in dry seed treatment and by 12.16 % in wet seed UV-B irradiation when compared with the 15 DAI unstressed seedlings. Reduction in the root biomass content by 16.79 % on 15 DAI was recorded in seedlings grown after dry seed treatment. The effect was severe in the case of wet seed UV-B treatment in which the seedlings showed 30.15 % reduction on 15 DAI. UV-B exposure suppressed dry weight of shoot by 34.92 % on 15 DAI under control in dry seed treatment, whereas after wet treatment it decreased by only 12.96 %. After UV-B stress, plant dry mass fell below control by 27.50 % and 20 % on 15 DAI after dry and wet seed exposures respectively. The relative growth rate (RGR) of seedlings was lowered in UV-B irradiated dry seeds by 61.62 % and after wet seed treatment by 14.86 % when compared to control seedlings. Similar results were reported by Rajendiran *et al.*, (2014a, 2014b) after experimenting with the *in vitro* regeneration of UV-B stressed seeds in ten varieties of cowpea.

Regeneration of axillary buds as well as callus induction were not observed in horse gram both in control stem explants as well as in stem explants harvested from *in situ* supplementary UV-B irradiated crops (Plate 4, Figure 1 to 2). Similar experiments were carried out by Rajendiran *et al.*, (2014c) with *in vitro* regeneration of stem explants harvested from *in situ* grown UV-B stressed ten varieties of cowpea.

The trend recorded with stem explants was observed with leaf explants also as the callus induction did not occur both in control leaf explants as well as in leaf explants harvested from *in situ* supplementary UV-B irradiated crops (Plate 4, Figure 3 to 4). This is in accordance with the report of Rajendiran *et al.*, (2014d) who worked *in vitro* regeneration of leaf explants harvested from *in situ* grown UV-B stressed ten varieties of cowpea.

Considering all the parameters studied *viz.*, growth under *in situ* condition and the responses of seeds and explants under *in vitro* culture, it is concluded that out of the explants of horse gram taken for screening, the seeds are best suited for germplasm conservation and regeneration.

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