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Effect of UV-B (280-320 nm) radiation in *IN-VITRO* culture from callus explant of black gram cultivars

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Abstract

The *in vitro* regeneration was carried out in black gram (*Vigna mungo*) under UV-B irradiation for the first time. Callus induction was tried with leaf explants harvested from control and UV-B irradiated three cultivars of *Vigna* mungo (Vamban 1, Vamban 2 and Vamban 3) to study their viability for Callus suspension cells. UV-B delayed callus induction and depressed biomass accumulation. However, the suspension cells were 100% viable in all the cultivars of *Vigna mungo* under the control conditions. The cultivar Vamban 2 (V2) is the more sensitive one to the enhanced UV-B radiation treatment. The suspension cells which received 10 and 15 min of enhanced UV-B radiation only 53% and 38% cells were viable, respectively.

Keywords: Ultraviolet-B, *Vigna mungo*, six cultivars, Callus Initiation

Introduction

The ozone layer in the stratosphere protects life on earth from exposure to dangerous levels of ultraviolet light. It does so by filtering out harmful ultraviolet radiation from the sun. When ozone-degrading chemicals are emitted, they mix with the atmosphere and eventually rise to the stratosphere. Chlorine and bromine catalyzes the destruction of ozone. This destruction is occurring at a more rapid rate than ozone can be created through natural processes. The degradation of the ozone layer leads to higher levels of ultraviolet radiation reaching Earth's surface. This in turn can lead to a greater incidence of skin cancer, cataracts, and impaired

immune systems, and is expected also to reduce crop yields, diminish the productivity of the oceans, and possibly to contribute to the decline of amphibious populations that is occurring around the world. Ultraviolet-B (UV-B) radiation (280-320 nm) is a dangerous atmospheric stress (Caldwell *et al.* 1983) as it was found to affect foliar epidermis (Bornman and Vogelmann, 1991), suppress photosynthesis (Jayakumar *et al* 2004; Periyakaruppiah *et al* 2012) and inhibit nodulation and nitrogen metabolism (Amudha *et al* 2005; Vijayalakshmi and Rajendiran 2014) in all sensitive crops. In this context, in vitro screening methods have to be developed to select suitable crop varieties that can survive in UV-B irradiation and also to conserve callus cells. The present study was effect of UV-B irradiation in *in-vitro* suspension culture from Callus explants of *Vigna mungo.*

Materials and Methods

The impact of UV-B damage and photorepair in the selected cultivars of *V.mungo* (Vamban (V) 1, 2 and 3) was studied using tissue culture techniques viz. suspension cultures. For callus induction Murashige and Skoog (1962) (MS) medium supplemented with various auxins and cytokinins alone or in composition at different concentration (0.25, 0.5, 1, 2 mg l-1) was used for callus intiation. pH of the media was adjusted to 5.8 with 0.1 N NaOH and then agar 7.0 g l-1 was added to solidify the medium and sterilized. The temperature of the growth room was maintained at 25 ±2°C. A 16 h light period/day was maintained with light intensity of 36 µmol m-2 using a fluorescent light. Induced calli were transferred periodically to freshly prepared culture medium: MS supplemented with 0.5 mg l-1 2, 4-D, 0.5 mg l-1 BA, 3% sucrose and 0.85% of agar. The stock cultures were sub-cultured every four weeks. Cell viability in suspension culture was determined following by the method of 2, 3, 5-Triphenyl tetrazolium chloride (TTC) assay (Zapata *et al* 1991). One gram of 25 days old suspension cultures each derived from the control and UV-B irradiated callus cultures was transferred into micro centrifuge tubes they were spun down for 1min at 9000 rpm. The supernatant was discarded and the pellet was gently suspended. It was incubated at room temperature for 12-20 days. Then the cells suspension cultures were observed and photographed using a phase contrast microscope. Living cells reduced the reagent to insoluble in red formazan activity. Cell viability was calculated as per the formula given below.

$$\text{Cell viability} = \frac{(\text{L.C} - \text{D.C})}{\text{Total number of cells}} \text{X100}$$

Where, L.C = Number of living cells in the sample

D.C= Number of dead cells in the sample.

Results and Discussion

Cell culture was established by transferring the callus from MS medium supplemented with 0.5 mg l-1KN and 1.0 2,4-D mg l-1 into an Erlenmeyer flask containing liquid medium and kept in a gyratory shaker. Cell suspension was transferred into a Petri-dish and they were exposed to enhanced UV-B. After exposure they were transferred to the solid medium in a Petri-dish. Enhanced UV-B exposure leads to the reduction in the number of calli development. Number of cells transforming into calli was reduced by 38% over control in V2 cultivar, 86% of calli from V3 cultivar, survived after being exposed to UV-B radiation (Table1).

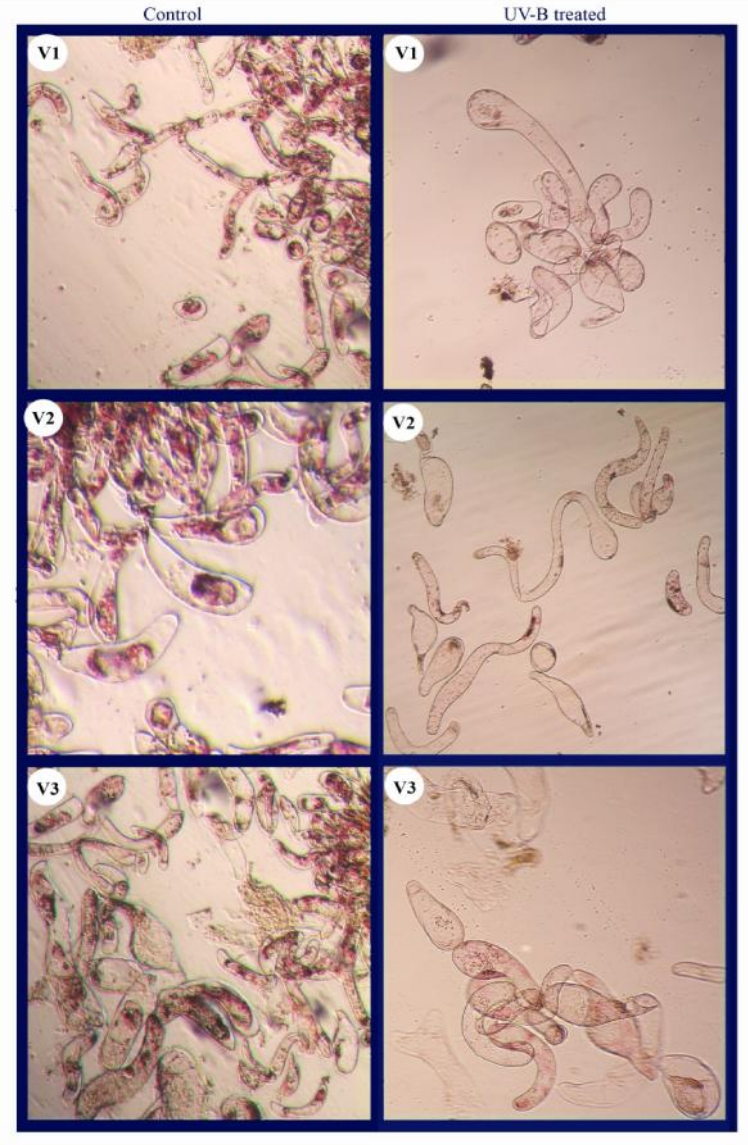
Table 1. Influence of enhanced UV-B radiation treatment on the cell viability of *V.mungo* cultivars. (Results are mean ± SE of three replicates)

Cultivar	UV-B treatment on cells (minutes)	Percentage (%) of viable cells
V 1	0(Control) 5 10 15	100 85±4.8 73±5.0 58±5.2
V 2	0(Control) 5 10 15	100 72±5.3 53±2.8 38±4.0
V 3	0(Control) 5 10 15	100 86±3.8 74±5.8 57±5.0

Calli grown under white light with supplementary UV radiation were also compact and dense but yellow green. The formation of chlorophyll containing cells proceeded faster in these calli than in the control culture; the difference was especially evident in the first half of the growth. In the

culture irradiated with UV during growth, the accumulation pattern of phenolic compounds was clearly different. The largest total content of soluble phenolic compounds was noted by the end of the growth in Tea plant (Zagoskina *et al.* 2005). Enhanced UV-B exposure leads to the reduction in the number of callus cells. The suspension cells were 100% viable in all the cultivars of *Vigna mungo* under the control conditions. The cultivar Vamban 2 (V2) is the more sensitive one to the enhanced UV-B radiation treatment. The suspension cells which received 10 and 15 mins of enhanced UV-B radiation only 53% and 38% cells were viable, respectively.

Figure: Phase contrast microscopic observation of suspension of culture cells *Vigna mungo* cultivars (Vamban 1, 2 and 3) stained with TTC.



Conclusion

In conclusion, clearly UV-B irradiation could play a role in callus explants. Marginal viable in callus cells under enhanced UV-B radiation conditions as observed in *Vigna mungo* cultivar Vamban 3 may be of great significance from *in vitro* propagation and more sensitive in V2 cultivar.

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