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RESEARCH ARTICLE

Influence of enhanced uv-b radiation in *in-vitro* propagation of black gram cultivars

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ABSTRACT

Callus induction was tried with leaf explants harvested from control and UV-B irradiated six cultivars of *Vigna mungo* (Vamban 1, Vamban 2, Vamban 3, Vamban 4, Vamban 5 and Vamban 6) to study their callus fresh weight and dry weight. Callus induction was recorded in Vamban 1, Vamban 2, Vamban 3, Vamban 4, Vamban 5 and Vamban 6 both from control and UV-B leaf explants harvested. UV-B delayed callus induction and depressed biomass accumulation. However, the callus fresh weight and dry weight were 100% compare to UV-B treated 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 fresh weight and dry weight decreased by under UV-B treated plants 14%, 14%, 14%, 14%, 16% and 17% in the cultivar V1,V3, V4, V5, V6 and V2 respectively.

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; Rajendiran and Ramanujam, 2004; Selvakumar *et al.,* 2008; Periyakaruppiah *et al.,* 2015) 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 leaf explants of *Vigna mungo.*

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MATERIALS AND METHODS

Growth of leaf calli of *V.mungo* cultivars was measured in terms of fresh and dry weight. Fresh weights of calli/explants were taken after removing the excess moisture on the surface using a blotting paper. Dry weight of callus was determined by drying the calli (1g) in a hot air oven at 60ºC for 24 h. Growth was studied by determining the fresh weight and dry weight of callus after 25, 35 and 45 days of culturing. Dry weight of callus was determined after drying in a vacuum oven at 60ºC until constant weight (Keskin and Kunter, 2008) for callus induction Murashige and Skoog (1962) (MS) medium supplemented with various auxins viz. Indole 3-acetic acid (IAA), indolebutyric acid (IBA), 2,4-dichlorophenoxy- acetic acid (2,4-D) and cytokinins viz. kinetin (KN), 6-benzyladenine purine (BAP) 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.

RESULTS AND DISCUSSION

Our studies showed that the greenish and yellow friable calli were observed in all the cultivars when MS medium was supplemented with 0.5 mg l-1 KN and 0.5 mg l-1 BAP. Enhanced UV-B exposure leads to the reduction in the fresh weight of the calli in all the cultivars for 25th, 35th and 45th days of growth. The UV-B enhanced 15 min of exposure cultivar Vamban 4, a marginal reduction of only 14% in fresh weight of callus over that of the control calli among all the six cultivars on 45th day after inoculation. Enhanced UV-B radiation treatment decreased the calli fresh weight and dry weight in all the cultivars of *Vigna mungo* compared with control plants. Significant decrease in calli fresh and dry weight in the UV-B exposed plants calli was observed in all the six cultivars of *Vigna mungo*. Fresh weight of the calli was decreased by 14%, 14%, 14%, 14%, 16% and 17% in the cultivar V1,V3, V4, V5, V6 and V2 respectively, after 45 days of callus growth when they were irradiated with enhanced UV-B for 15 min (Table 1). Reduction in dry weight of the calli cultivars was recorded as 6%, 6%, 7%, 8%, 8% and 19% for V3, V4, V6, V1, V5 and V2 respectively, than that of the controls after 45 days of in vitro callus growth upon receiving enhanced UV-B irradiation for 15 min. Similar reports shows that the growth rates of tea callus cultures are characteristically very low (Zagoskina *et al.,,* 1990),the callus weight for both control and UV-B treatments increased significantly in the period from the 25th to 35th days of culturing. Callus was initiated after six days in control Vamban 4 cultivar of *Vigna mungo* at the cut end of explants. In V1, V3, V5 and V6 cultivars, callus initiation was observed after 7-9 days under control conditions, while in control V2, callus initiation occurred after eight days (Table 1, Figure 1). In the V2 cultivar the calli was appeared to be brown and in unviable condition in the UV-B exposed calli on 35th day after initiation (Figure 2). During further culturing the biomass increased only in the control callus. Hence, UV radiation facilitated earlier on completion of the growth cycle and promoted the transition of the culture to the stationary growth. 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 plants (Zagoskina *et al.,* 2005; Nagarajan *et al.,* 2008; Benjamin *et al.,* 2010; Periyakaruppiah *et al.,* 2012a; Periyakaruppiah *et al.,* 2012b; Periyakaruppiah *et al.,* 2014). 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 (Periyakaruppiah *et al.,* 2016).

Table 1. Influence of enhanced UV-B irradiation treatment on the Callus fresh weight and dry weight of cultivars of *V.mungo.* Values are expressed on unit fresh weight. Figures in parentheses are percentage values with reference to respective control. Mean ± SE, n=10

Cultivar	Duration of UV-B treatment (minutes)	Callus fresh weight(F.W) and Dry weight (D.W) (g/l) Growth period (Days)					
		25		35		45	
V1		F.W	D.W	F.W	D.W	F.W	D.W
	0(Control)	51.96(100)	4.77(100)	95.07(100)	11.16(100)	127.02(100)	18.81(100)
	5	50.28(97)	4.68(98)	87.81(92)	11.1(99)	125.28(99)	18.33(97)
	10	47.49(91)	4.26(89)	81.75(86)	10.86(97)	114.6(90)	18.06(96)
	15	41.16(79)	4.14(87)	79.56(84)	10.74(96)	108.6(86)	17.37(92)
V2	0(Control)	48.83(100)	4.35(100)	91.02(100)	10.9(100)	125.32(100)	16.46(100)
	5	47.28(97)	4.23(97)	83.34(92)	10.81(99)	122.35(98)	16.34(99)
	10	44.47(91)	4.12(95)	78.75(87)	10.31(95)	109.54(87)	15.16(92)
	15	43.16(88)	4.11(94)	76.54(84)	10.24(94)	103.53(83)	13.36(81)
V3	0(Control)	49.96(100)	4.93(100)	95.75(100)	11.3(100)	127.43(100)	18.76(100)
	5	50.97(102)	4.82(98)	87.92(92)	11.25(100)	125.53(99)	18.52(99)
	10	47.99(96)	4.38(89)	81.83(85)	10.78(95)	114.36(90)	18.34(98)
	15	41.23(83)	4.36(88)	79.71(83)	10.75(95)	109.64(86)	17.65(94)
V4	0(Control)	52.28(100)	4.96(100)	97.07(100)	12.6(100)	131.02(100)	19.63(100)
	5	51.22(98)	4.91(99)	89.81(93)	12.53(99)	129.28(99)	19.37(99)
	10	48.41(93)	4.78(96)	83.75(86)	11.98(95)	118.65(91)	19.33(98)
	15	42.16(81)	4.64(94)	81.56(84)	11.94(95)	112.67(86)	18.54(94)
V5	0(Control)	51.34(100)	4.77(100)	95.31(100)	12.86(100)	128.02(100)	18.99(100)
	5	50.23(98)	4.53(95)	87.43(92)	11.74(91)	127.34(99)	18.67(98)
	10	47.27(92)	4.37(92)	81.75(86)	11.34(88)	116.65(91)	18.06(95)
	15	41.12(80)	4.34(91)	79.54(83)	11.33(88)	110.63(86)	17.56(92)
V6	0(Control)	51.93(100)	4.79(100)	95.56(100)	11.19(100)	127.65(100)	18.85(100)
	5	50.25(97)	4.72(99)	87.76(92)	11.12(99)	125.26(98)	18.34(97)
	10	47.42(91)	4.28(89)	81.87(86)	10.83(97)	114.65(90)	18.32(97)
	15	41.11(79)	4.16(87)	79.34(83)	10.43(93)	107.76(84)	17.54(93)

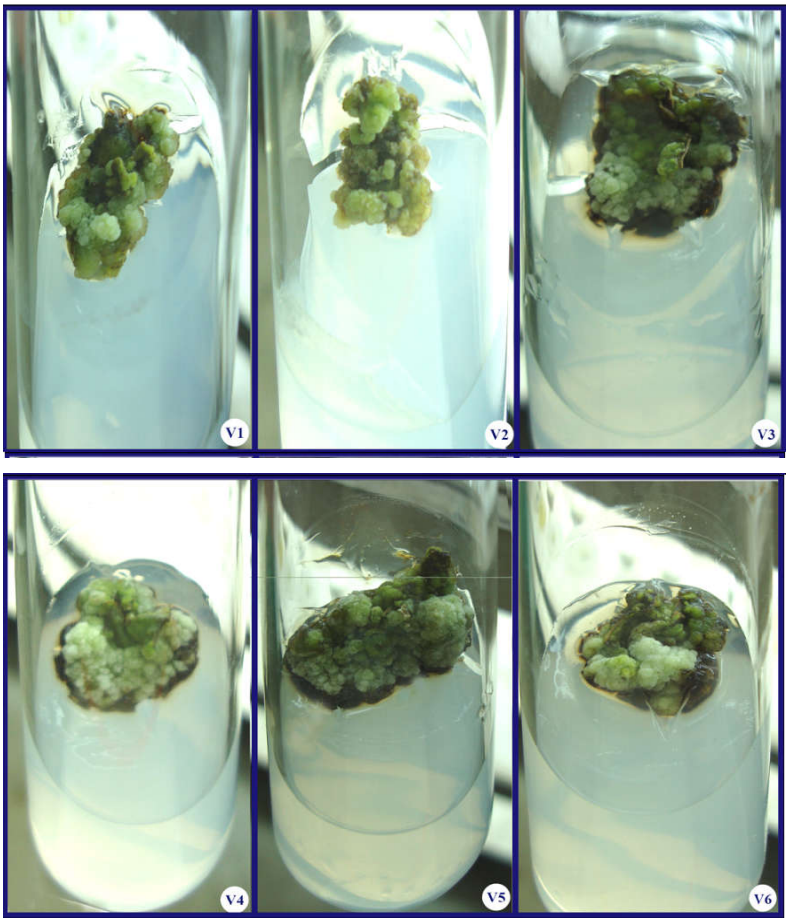

Figure 1. Initiation of calli in *Vigna mungo* cultivars (Vamban 1, 2, 3, 4, 5 and 6; V1, V2, V3, V4, V5 and V6) from leaf explants in MS medium supplemented with 0.5 mg l-1 KN and 0.5 mg l-1 BAP; 35 day old culture

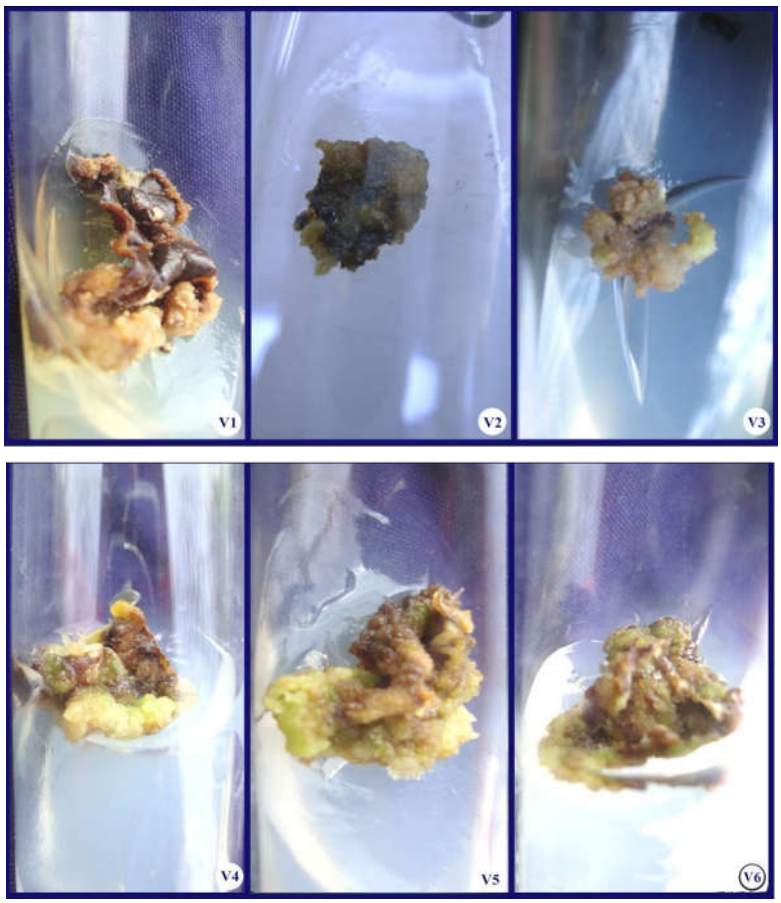

Figure 2. Initiation of calli in *Vigna mungo* cultivars (Vamban 1, 2, 3, 4, 5 and 6; V1,V2,V3,V4, V5 and V6) from leaf explants in MS medium supplemented with 0.5 mg l-1 KN and 0.5 mg l-1 BAP; 35 day old culture (UV-B treated)

Conclusion

The *in vitro* culture is a very attractive technique for the cultivation of callus mass, particularly fresh and dry weight of *Vigna mungo* cultivars. In conclusion, clearly UV-B irradiation could play a role in leaf explants. Marginal decrease in plant biomass under enhanced UV-B radiation conditions as observed in *Vigna mungo* cultivar Vamban 3 and 4 may be of great significance from *in vitro* propagation.

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