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Research Article

IN VITRO REGENERATION OF APICAL SHOOT EXPLANTS FROM *IN SITU* GROWN UV-B STRESSED *OCIMUM SANCTUM* L

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ABSTRACT
The *in vitro* regeneration of apical shoot explants 15 and 30 DAS (days after seed germination) harvested from *in situ* UV-B irradiated *Ocimum sanctum* L. plants was reported for the first time. Leaves proliferated from apical shoot explants of both control and UV-B irradiated *Ocimum sanctum* L. plants at both the ages. Leaf initiation was delayed in both 15 DAS as well as 30 DAS old UV-B exposed explants. The proliferation in control apical shoot explants was erect while it took a rosette shape in UV-B irradiated explants. 15 DAS old apical shoot explants of UV-B stressed plants accumulated more fresh and dry biomass than that of control. However the regenerated 30 DAS old explants of *in situ* UV-B irradiated plant weighed less than control. 15 DAS old apical shoot explants obtained from UV-B stressed *Ocimum sanctum* L. plant responded quickly to *in vitro* regeneration indicating that they are best suited for UV-B stressed habitat.

Keywords: *In situ UV-B, Tulsi, Apical Shoot Explants, in vitro Regeneration*

INTRODUCTION
The ozone layer acts like an umbrella, protecting plants and animals from much of the sun's harmful ultraviolet radiation. Thinning of ozone layer due to human activities, allows enormous ultraviolet-B (UV-B) radiation (280-320 nm) into earth's atmosphere which is a dangerous atmospheric stress (Caldwell *et al.,* 1983). It destroys foliar epidermis (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b), inhibits photosynthesis (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and disturbs nodulation and nitrogen metabolism (Rajendiran and Ramanujam, 2006; Rajendiran and Ramanujam, 2003; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014) in sensitive plants. The present work was carried out for the first time to regenerate the apical shoot explants of *Ocimum sanctum* L harvested from *in situ* supplementary UV-B irradiated and normal plants in culture media.

MATERIALS AND METHODS
The *Ocimum sanctum* L. plants, obtained from Pondicherry Agro Service and Industries Corporation Limited (PASIC), Pondicherry, 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 ($UV\text{-}B_{BE}$) 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 $UV\text{-}B_{BE}$ 10 kJ m^{-2} d^{-1}. Apical shoot explants (3 cm length from top of canopy) were harvested from 15 and 30 DAS (days after seed germination) crops that received supplementary UV-B irradiation and sunlight separately in the *in situ* condition.
Apical shoot 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).

Apical shoot 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 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 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

The culture tubes with apical shoot explants were photographed in daylight using a Sony digital camera fitted with appropriate close-up accessories.

RESULT AND DISCUSSION

Proliferation of leaves occurred in apical shoot explants of both control and UV-B irradiated *Ocimum sanctum* L. plants irrespective of the age of explants (Table 1; Plate1). However, leaf initiation was delayed by one day in UV-B exposed 15 DAS old explants and by 4 days in UV-B exposed 30 DAS old explants compared to that of the normal explants (Table 1; Plate 1). The control apical shoot explants of both the ages grew taller (Plate 1, Figure 1, 3) while the UV-B exposed explants exhibited rosette shape (Plate 1, Figure 2, 4).

The regenerated 15 DAS old apical shoot explant of *in situ* UV-B stressed *Ocimum sanctum* L. accumulated 52.53 % more fresh biomass and twice the dry biomass than that of control (Table 1). The proliferated 30 DAS old explant of *in situ* UV-B irradiated plant weighed less by 7.09 % in fresh biomass and by 13.69 % in dry biomass compared to normal counterpart (Table 1).

Figure 1: Control (15 DAS old)

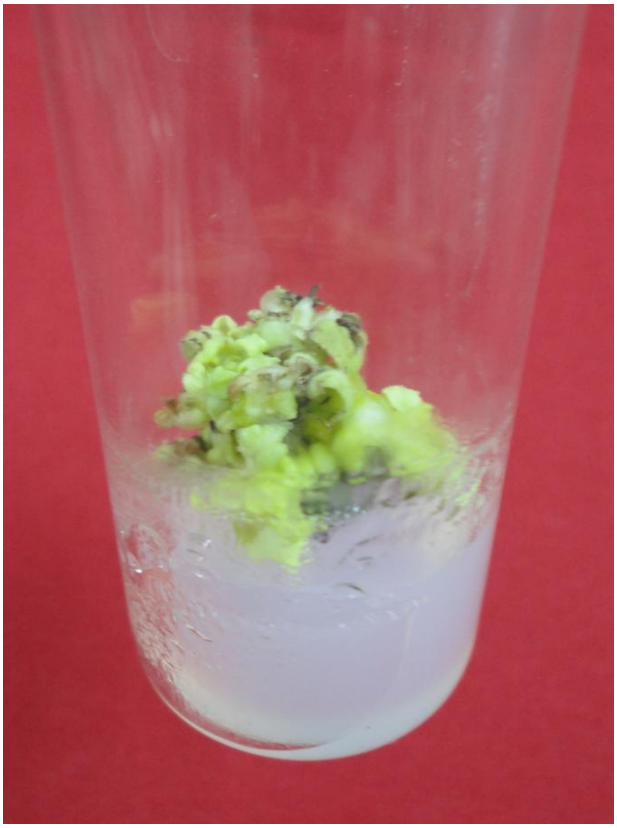

Figure 2: UV- B (15 DAS old)

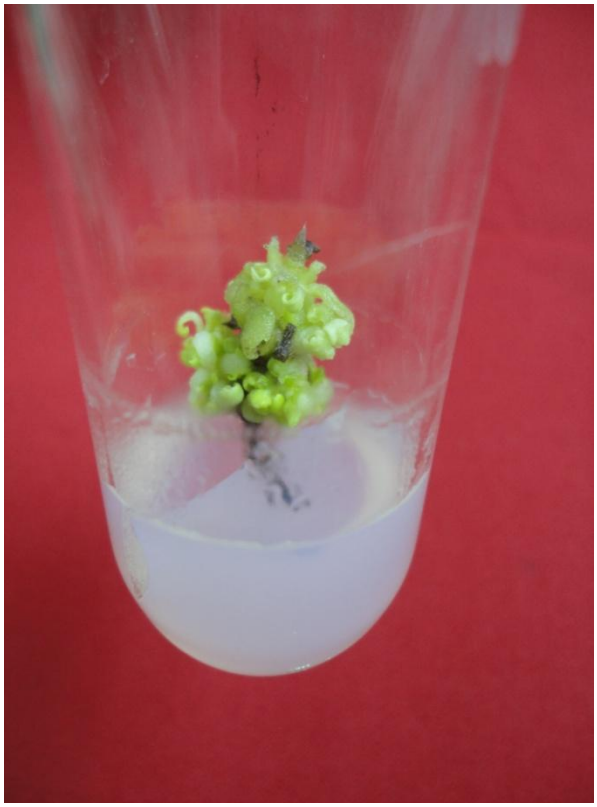

Figure 3: Control (30 DAS old)

Figure 4: UV-B (30 DAS old)

Plate 1: *In vitro* regeneration of apical shoot explants harvested from *in situ* grown normal and supplementary ultraviolet-B (UV-B) irradiated *Ocimum sanctum.* (DAS: Days after seed germination)

Table 1: Characteristics of regenerated apical shoot explants of 30 DAI (days after inoculation) *Ocimum sanctum* L. from control and supplementary UV-B exposed plants

Age of explant	Treatment	Time taken for initiation (d)	Fresh weight (g)	Dry weight (g)
15 DAS	Control	24	0.613	0.096
	UV-B	25	0.935	0.198
30 DAS	Control	22	0.564	0.884
	UV-B	26	0.524	0.763

Considering the parameters taken for the study, the apical shoot explants from 15 DAS old UV-B stressed *Ocimum sanctum* L. plant responded quickly to *in vitro* regeneration indicating that they are the best explants for germplasm conservation and for propagating in UV-B enhanced environment.

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