

BRIEF COMMUNICATION

Effect of growth regulators and ethylmethane sulphonate on growth, and chlorophyll, sugar and proline contents in *Dracaena sanderiana* cultured *in vitro*A. JUNAID^{1,2}, A. MUJIB^{1*} and M.P. SHARMA¹*Cellular Differentiation and Molecular Genetics Section, Department of Botany, Hamdard University, New Delhi-110062, India¹**Plant Tissue Culture and Agriculture Research Laboratory, Technical Institute of Dubai, P.O. Box 19099, Dubai, United Arab Emirates²***Abstract**

A high efficient four step protocol (callus initiation, regeneration, shoot elongation and rooting) for *in vitro* propagation of *Dracaena sanderiana* Sander *ex* Mast was developed. Callusing was achieved from nodal stem segment explants treated with various concentrations of ethylmethane sulphonate (EMS) on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D; 1.5 g m⁻³). A significant increase in callus induction percentage and biomass production was noticed from lower EMS treated lines (ET₁ and ET₂) comparatively to control and other (ET₃, ET₄ and ET₅) lines. Calli of ET₁ line showed high regeneration potential on MS medium with N⁶-benzylaminopurine (BAP; 1.75 g m⁻³). Length of microshoots, which was reduced by EMS, restored by addition of gibberellic acid (GA₃; 0.4 g m⁻³). A marked increase in rooting with increasing EMS concentration was noticed on MS medium fortified with 3-indolebutyric acid (IBA; 1.5 g m⁻³).

Additional key words: auxins, cytokinins, gibberellic acid, *in vitro* mutagenesis, lucky bombo.

Dracaena sanderiana Sander *ex* Mast (lucky bombo) belongs to family *Agavaceae*. It is distributed in tropical and subtropical open lands of Africa and India. Despite medicinal and ornamental importance of *Dracaena* species, not much work has been done in *in vitro* conditions and they are mostly propagated vegetatively (Junaid *et al.* (2008). However, the vegetatively propagated plants accumulate several bacterial, fungal, viral and mycoplasmal diseases. *In vitro* mutagenesis can overcome these problems. It includes the rapid vegetative multiplication of plants after treatments with various mutagenic agents (Maluszynski *et al.* 1995, Predieri 2001, Muthusamy *et al.* 2007). In the present investigation the effect of different concentrations of ethylmethane sulphonate (EMS) on *in vitro* grown nodal explant of *D. sanderiana* was studied together with optimization of growth regulator combination for their further growth. Different biochemical analyses were

also made in order to evaluate the biochemical differences between normal and EMS treated plantlets.

The explants employed for mutagenesis studied were nodal stem segment of *in vitro* grown *Dracaena sanderiana* Sander *ex* Mast. They were placed in 250 cm³ Erlenmeyer conical flask (Borosil, Mumbai, India) with 50 cm³ of filter-sterilized EMS solution. A range of EMS concentrations (0.0, 0.2, 0.4, 0.8, 1.2 and 1.6 cm³ m⁻³) were used and designated as control, ET₁, ET₂, ET₃, ET₄ and ET₅. Erlenmeyer flasks were agitated on rotatory shaker for 8 h at temperature of 25 ± 2 °C. Explants were removed from flasks inside the laminar hood, transferred to Murashige and Skoog (1962) medium (10 pieces per conical flask) with 1.5 g m⁻³ 2,4-dichlorophenoxyacetic acid (2,4-D). Calli induced (80 - 90 mg) were transferred for regeneration on MS medium supplemented with optimized concentration of N⁶-benzylaminopurine (BAP; 1.75 g m⁻³; data unpublished). The

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Abbreviations: BAP - N⁶-benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; GA₃ - gibberellic acid; IBA - indole-3-butyric acid; EMS - ethylmethane sulphonate; MS medium - Murashige and Skoog medium; ANOVA - analysis of variance; DMRT - Duncan's multiple range test.

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height of produced regenerants was reduced due to the EMS. Therefore, the shoots were sub-cultured on MS medium supplemented with a range of GA₃ concentrations (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 g m⁻³). Data were scored in terms of shoot development percentage, shoot number per nodal calli, shoot length and leaf number per shoot. Roots were induced directly by the use of single shoot on MS medium fortified with optimized concentration of IBA (1.5 g m⁻³; unpublished). Data were scored in terms of root development percentage, root number per shoot, root length and number of adventitious roots. The pH was adjusted to 5.6 - 5.8 before autoclaving. The media were sterilized in an autoclave for 15 min at 121 °C and cultures were incubated at 25 ± 2 °C under 16-h photoperiod (cool white fluorescent tubes, irradiance of 100 μmol. m⁻² s⁻¹). Chlorophyll and carotenoid contents were determined according to Wintermans and De Mots (1965). Total sugars were estimated by ninhydrin method according to Dey (1990). Proline content was determined by the procedure of Bates *et al.* (1973). The effects of growth regulators and EMS on morphogenesis and biochemical parameters were analyzed by one-way analyses of variance (ANOVA). Values are means of five replicates from two experiments, and they were separated using Duncan's multiple range test (DMRT) at *P* ≤ 0.05.

Of the various EMS concentrations used for

mutagenesis treatment of nodal stem before callus initiation; only lower EMS concentrations [ET₁ (0.2 cm³ m⁻³) and ET₂ (0.4 cm³ m⁻³)] showed a significant (*P* ≤ 0.05) increase in callus induction percentage and biomass production when compared to the control and higher EMS concentrations respectively. However, at increasing EMS concentration callus induction time was greatly affected which in ET₃ and ET₄ line start after two weeks of inoculation; while in ET₅ line callusing started 5th weeks onwards. A marked difference in morphological appearance of callus were noticed with increasing EMS concentrations. Callus of ET₅ line turned brown after one week of initiation and its regeneration was arrested. A similar differential caulogenic response after *in vitro* mutagenesis has been documented previously in other plant system (Vu Duc Quang *et al.* 1988, Svetleva and Crino 2005). After the successful callusing stage, calli of each line were transferred on MS medium fortified with BAP (1.75 g m⁻³). Regeneration occurred in most of the treated lines, but the regeneration potential varied considerably with EMS concentration (Table 1). We found that calli of ET₁ - ET₄ lines showed a high regeneration potential but a significant difference in shoot length was noticed when compared to control. Whereas shoot length decreased, the number of shoots increased, sometimes started to be uncountable due to rosette of microshoots with stunted shoot growth. These results

Table 1. Effect of EMS concentrations (0, 0.2, 0.4, 0.8, 1.2 and 1.6 cm³ m⁻³ for control, ET₁ - ET₅, respectively) on shoot multiplication and rooting. Data were scored after 7 weeks of culture ** indicates that shoots were not countable due to very tiny size. Means ± standard deviation of 5 replicates from 2 experiments. Means with in each column with common superscripts are not significantly different at *P* ≤ 0.05, according to DMRT.

EMS	Shoot multiplication				Rooting			
	shoot development [%]	shoot number [explant ⁻¹]	shoot length [cm]	leaf number	root development [%]	root number [shoot ⁻¹]	root length [cm]	number of adventitious roots
Control	84.8 ^b	5.3 ^b	7.5 ^a	7.9 ^a	84.3 ^d	14.7 ^c	5.3 ^b	32.3 ^b
ET ₁	90.3 ^a	21.9 ^a	5.2 ^b	4.9 ^b	87.1 ^c	16.3 ^c	5.4 ^{ab}	34.1 ^a
ET ₂	82.3 ^b	**	4.2 ^b	3.2 ^b	90.2 ^b	18.6 ^b	5.9 ^a	0.0 ^c
ET ₃	62.2 ^c	**	1.3 ^c	**	95.4 ^a	22.1 ^a	6.2 ^a	0.0 ^c
ET ₄	18.3 ^d	3.3 ^c	0.9 ^d	**	38.3 ^e	9.3 ^d	4.1 ^c	0.0 ^c
ET ₅	0.0 ^e	0.0 ^d	0.0 ^e	0.0 ^c	10.1 ^f	5.2 ^e	2.8 ^d	0.0 ^c

Table 2. Effect of EMS on contents of chlorophyll (*a+b*), carotenoids, sugars and proline [μmol g⁻¹ (f.m.)] in different tissues. Means ± standard deviation of 5 replicates from 2 experiments. Means within each column with common superscripts are not significantly different at *P* ≤ 0.05, according to DMRT.

EMS	Chl (<i>a+b</i>)	Carotenoids	Sugars callus	Sugars			Proline callus	Proline		
				shoot	leaf	root		shoot	leaf	root
Control	1.2 ^{cd}	0.9 ^c	23.6 ^b	30.1 ^c	33.1 ^c	27.4 ^c	6.1 ^f	6.8 ^e	7.2 ^d	6.9 ^e
ET ₁	1.6 ^a	1.1 ^{bc}	24.6 ^b	31.9 ^b	34.8 ^b	30.1 ^b	13.8 ^e	15.2 ^d	20.4 ^c	16.2 ^d
ET ₂	1.4 ^b	1.2 ^b	26.1 ^a	33.0 ^a	36.2 ^a	33.1 ^a	16.1 ^d	18.4 ^c	22.1 ^c	19.2 ^c
ET ₃	1.1 ^d	1.7 ^a	18.3 ^d	24.4 ^d	26.1 ^d	20.2 ^d	28.8 ^c	30.1 ^b	38.2 ^b	37.1 ^b
ET ₄	0.8 ^e	1.9 ^a	11.4 ^e	15.1 ^e	16.5 ^e	13.9 ^e	38.3 ^b	43.1 ^a	48.1 ^a	45.8 ^a
ET ₅	0.0 ^f	0.0 ^d	10.0 ^f	0.0 ^f	0.0 ^f	0.0 ^f	55.1 ^a	0.0 ^f	0.0 ^e	0.0 ^f

correspond with the previous report where in *Rosa hybrida* Ibrahim *et al.* (1998) found increased survival and regeneration of X-irradiated explants compared to untreated line. Also low doses of γ -radiation and N-nitroso-N²-ethylurea (ENU) on cultured maize favored callus growth and plant regeneration (Moustafa *et al.* 1989). Furthermore, the supplementation of MS medium with different concentrations of GA₃ (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 g m⁻³) helped to restore the normal length of produced microshoots. Of the various concentrations, 0.4 g m⁻³ GA₃ proved to be highly effective to restore the normal length of shoots (data not shown). Addition of GA₃ to the medium not only enhanced the shoot length but also favored the proliferation of healthier shoots. The promotive effect of GA₃ on internode elongation in culture was reported earlier in other plant species including *Saussurea lappa* (Arora and Bhojwani 1989), *Ocimum americanum*, *O. sanctum* and *O. basilicum* (Pattnaik and Chand 1996, Sahoo *et al.* 1997). The shoots were subculture every three weeks on fresh medium. This mode of multiplication ensured continuous supply of shoots for a longer period without any evidence of decline in morphogenetic potential. Single shoots were used for rooting and implanted on MS medium supplemented with 1.5 g m⁻³ IBA. Shoots of higher EMS concentrations showed a significant increase in root induction percentage, root number and length when compared to control. In addition, after 4 weeks of culture adventitious roots induced vigorously at lower EMS concentration, however, at higher concentrations they started to disappear and root development was accompanied with

callus formation at the basal root zone (Table 1). This promotion of rhizogenesis is in agreement with previous observation on olive or grapevine after low dose of γ -radiation (Al-Bashir 1995, Charbaji and Nabulsi 1999). The interactive effects of 1.75 g m⁻³ BAP, 0.4 g m⁻³ GA₃ and 1.5 g m⁻³ IBA were also studied and we found that calli induced at lower EMS level (ET₁ - ET₃) were capable to produce only shoots, only root and complete plantlets, however, in ET₄ - ET₅ only root formation was observed (data not shown).

Biochemical study has been carried out in treated and non-treated lines. The proline content increased significantly with increasing EMS concentrations (Table 2). Proline has been earlier shown to act as a compatible osmolyte and its increased production confirms osmotolerance in plants (Kavikishore *et al.* 1995, Nanjo *et al.* 1999). In lines, ET₁ and ET₂ a marginal increase in contents of photosynthetic pigments (chlorophyll *a+b* and carotenoids) and sugars was observed. This increase might suggest delay of senescence. However, their contents decreased with increasing EMS concentration. The incidence of chlorophyll mutation induced by EMS was mentioned already by Natrajan and Upadhyya (1964). The effect of mutation on chlorophyll and starch was studied by Joseph *et al.* (2004) and they found less chlorophyll in cassava.

In summary, low EMS concentrations on *in vitro* developed nodal stem of *D. sanderiana* significantly increased callus induction and biomass production and GA₃ restored the length of microshoots. Plant growth regulators and EMS influenced morphological and biochemical characteristics.

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