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Plant Regeneration through Micropropagation from Nodal Explants of Critically Endangered and Endemic Plant *Exacum travancoricum* Bedd

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Abstract

A rapid micropropagation protocol was established for *Exacum travancoricum* Bedd. The effect of two cytokinins viz. BA and kinetin were studied to evaluate the propagation of plants through nodal explants. MS medium supplemented with 13.32 μM BA induced early bud break and subsequent production of multiple shoots. Rooting of shoots occurred when cultured on 1/2 strength MS medium supplemented with 14.7 μM IBA. Rooted plants were acclimatized to greenhouse conditions. The propagated plants were transferred successfully to field with 68% success. As the plant was amenable to propagation *in vitro*, this can be employed as a tool for conservation of this critically endangered and endemic ornamental herb.

Key words: Endangered plant species, *Exacum travancoricum*, *in vitro* propagation, multiple shoots, nodal explants

Introduction

Almost all ornamental species have come from the wild and they have become very important commercial commodity in trade, with an expanding international market, as they satisfy the aesthetic sense of human beings. Southern part of the Western Ghats, India, with its unique flora of biogeographical interest, high diversity and endemism, have been selected as one of the Plant Diversity Centers in the Indian subcontinent as a step towards conservation of global biological diversity (Davis 1995). The rate of endemism and floristic richness are greatest in Tirunelveli and Travancore sections of Western Ghats where more than 150 strict endemics occur (Gopalan and Henry 2000). *Exacum travancoricum* Bedd. (Gentianaceae) a woody, stunted, much branched ornamental herb thrives well in 1200-1800 m altitude with Agasthiamalai (tail end of Southern Western Ghats, India) and its adjoining areas as geographical range of distribution. The very caespitose fleshly leaved *E. travancoricum* is a most distinct and one of the most beautiful plant species with violet coloured flowers (Figure 1 A). When in full bloom this plant gives a beautiful appearance and hence worth introducing in horticulture (Gamble and Fischer 1956).

Habitat clearance and over exploitation of plants for medicinal and horticultural value, fire, cattle razing and fuel wood cutting are the main threat factors affecting loss of biodiversity in this area (Davis 1995). It is an endemic species to Southern Western Ghats (8°-10° 30' N) and almost 80% of mature individuals have declined due to habitat loss as a result of human interferences and at present only less than 250 mature individuals are naturally distributed in less than five fragmented populations in less than 250 km^2 (Henry et al. 1987). Recently, the IUCN has categorized *E. travancoricum* under critically endangered plants category (Gopalan and Henry 2000).

Henry et al. (1984) recommended employing both *in situ* and *ex situ* cultivation methods for conservation and sustainable use *E. travancoricum* plant species for

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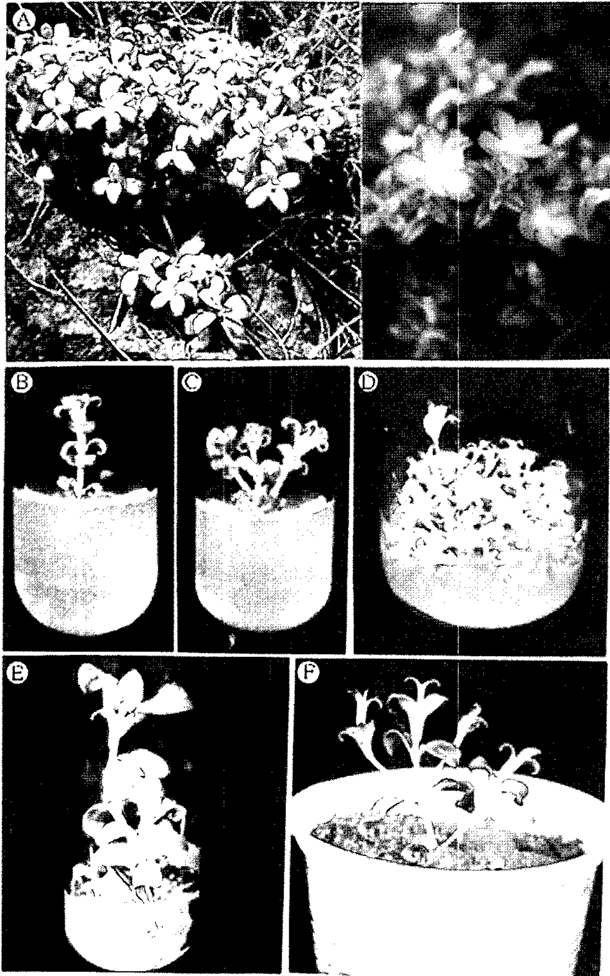

Figure 1. Micropropagation of *E. travancoricum*. (A) Mother plant with flowering, (B) Shoot initiation after 15 days of inoculation, (C) Multiple shoot initiation from nodal explant, (D) Mass multiplication of *E. travancoricum* shoots on third Subculture, (E) *In vitro* rooting, (F) Acclimatized plant in poly-cup.

ornamental purposes. For conservation, though a lot of methods are prescribed, in vitro propagation is a preferred method worldwide for quicker and faster multiplication of such wild ornamental plants, which may otherwise become rare, endangered and ultimately go extinct. The present paper describes our results on the *in vitro* plant regeneration in *E. travancoricuma* critically endangered endemic plant species, being introduced into culture for the first time.

Material and methods

Exacum travancoricum mother plants were collected from the Upper Kodayar hill (1800 m from MSL), Southern Western Ghats, Tamil Nadu, India and established in pots. Nodal explants measuring 0.7 to 1.0 cm collected from potted plants were washed well under tap water for 20 min. and surface sterilized by immersion in 0.5% (v/v) Labolene (Qualigens, India) and 0.1% (w/v) $HgCl_2$ solution for 2 min; washed five times with sterile distilled and implanted onto the medium. Unless otherwise mentioned MS medium (Murashige and Skoog 1962) supplemented with 3% (w/v)sucrose was used throughout the study. The medium was further supplemented with 6-benzyladenine (BA) (2.22-22.20 μM) and Kinetin (2.30-23.0 μM) individually. The medium was adjusted to pH 5.8, gelled with 0.8% (w/v) agar (Hi-Media, India) and autoclaved at 121°C for 20 min. The cultures were maintained at 25±2°C, under 16 h photoperiod (11.7 μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes) with 60-65% relative humidity. The cultures were grown for 45 days before data were collected. Eachtreatment consisted of 20 replicates and was repeated twice. For multiplication, the same media described above was used. After thirty-five days well grown multiple shoots (4-6 cm in height) were excised into single shoots and sub cultured onto the medium comprising BA (13.32 μM) and Kinetin (13.80 μM) with 35 days interval. After seven weeks, data was collected in terms of multiple shoot formation and average shoot height.

For rooting, 45 days old shoots (5-7 cm in height) were transferred to 1/2 strength MS medium fortified with various concentrations of auxins (IBA, IAA, NAA). Initially the cultures were incubated in dark condition for two weeks and later transferred to light. Data on the number of roots and average root length were recorded after 25 days of inoculation. Well-established plants were subsequently shifted to poly-cups containing soil, vermiculite and sand (1:1:1) mixture and covered with polythene bags. Acclimatized plants were shifted to pots and kept under green house.

Results and Discussion

When the nodal explants were implanted onto MS medium supplemented with either BA or Kinetin, significant number of shoots (>3) was obtained in all amendments when compared to control. Of the various BA concentrations tested, maximum number of shoots (21.4±1.8) wasobtained after 35 days with 75% response on MS medium fortified with 13.32 μM BA (Table 1; Fig. 1 B-D). A decline in number of multiple shoots observed when the BA concentration was altered above or below this concentration,could be attributed to the possible imbalances between the endogenously produced plant growth regulators and exogenous supplementation (Sudha and Seeni 1994). When compared, the performance of kinetin was poor than BA and the shoot proliferation was not much influenced by

Table 1. Shooting response of *E. travancoricum* nodal explants cultured on MS medium.

Cytokinins (μM)	Shooting response (%)	No. of shoots / explant *	Shoot height (cm)*
MS basal	-	-	-
BA			
2.22	54	2.36 0.50	3.8 0.95
4.44	65	4.68 0.60	5.5 0.84
8.88	68	9.65 0.65	7.4 1.28
13.32	75	21.4 1.80	6.2 1.43
22.20	70	5.75 1.20	3.4 0.85
Kinetin			
2.30	58	1.91 0.42	2.6 0.78
4.60	67	3.70 0.88	5.8 1.10
9.20	65	6.69 1.16	5.2 1.33
13.80	70	11.2 1.47	4.4 0.95
23.00	72	4.66 1.13	2.8 0.87

* Mean ± Standard Deviation.

the kinetin concentration in the medium. The number of shoots per explant was also quite less with all concentrations of kinetin. However, among kinetin treatments, shoot elongation was greatest for explants grown in medium supplemented with 9.2 μM followed by 4.6 μM kinetin. Maximum number of shoots (11.20±1.47) was obtained with 70% shooting response in 13.8 μM Kinetin followed by 9.2 μM kinetin. The stimulatory effect of cytokinins on multiple shoot induction has been reported earlier in a variety of plants viz. *Ocimum* spp. (Ahuja et al. 1982; Pattnaik and Chand 1996), *Hybanthus enneaspermus* (Prakash et al. 2001) and Orthosiphon spiralis (Elangomathavan et al. 2003) etc. Maximum shoot length (7.4±1.28 cm) was obtained in 8.88 μM BA. However, BA at high concentrations (13.32 and 22.20 μM) suppressed the shoot elongation resulting in shorter internodes (3-4 mm). These findings are in consonance with the results obtained in *Hedeoma multiflorum* (Koroch et al. 1997) and *Orthosiphon spiralis* (Elangomathavan et al. 2003).

When the shoots (3-5 cm length) were subcultured on MS medium supplemented with BA (13.32 μM) and Kinetin (13.8 μM) the number of multiple shoots increased up to third subculture and there was a decline at fourth subculture (Fig. 2). Of the two different hormone concentration supplemented, BA at 13.32 M produced maximum number of shoots in both second and third subcultures (25.50±2.54 and 26.9±1.79 shoots, respectively). Kinetin (13.80 μM) produced significant number of shoots in both second and third subcultures (15.8±2.75 and 17.1±1.10 shoots, respectively). Similar phenomena in shoot number increase during the first and subsequent subcultures have been described earlier in rose (Sauer et al. 1985), *Campanula isophylla* (Brandt 1992) and *Orthosiphon spiralis* (Elangomathavan et al. 2003). During fourth subculture, the multiple shoot induction reduced to 17.6±1.65 in 13.32 μM BA and 11.8±1.58 in 13.8 μM Kinetin. Similar results were reported in *Hedeoma multiflorum* (Koroch et al. 1997) and *Datura insignis* (Santos et al. 1990).

When the shoots were transplanted for rooting, 1/2 strength MS basal medium supplemented with various levels of auxins enhanced the root formation. Maximum number of roots (6.4±1.18) with 70% rooting response was observed in 1/2 MS + IBA 14.7 μM (Table 2; Fig. 1E). Neither roots nor basal callus were observed at high concentration (25.5 μM IBA) where the shoots did not grow further and remained dormant. These results are in consonance with the findings of Fracaro and Echeverrigaray (2001) on *Cunila galioides* and Kozlina *et al.* (1997) *on Fibigia triguetra*. Maximum root length was observed (4.8±1.36) in 14.7 μM IBA supplemented 1/2MS medium. After two weeks, the primary roots produced secondary and tertiary roots. However, when IAA and NAA were supplemented at various concentrations, they did not produce convincing results and resulted in production of basal callus.

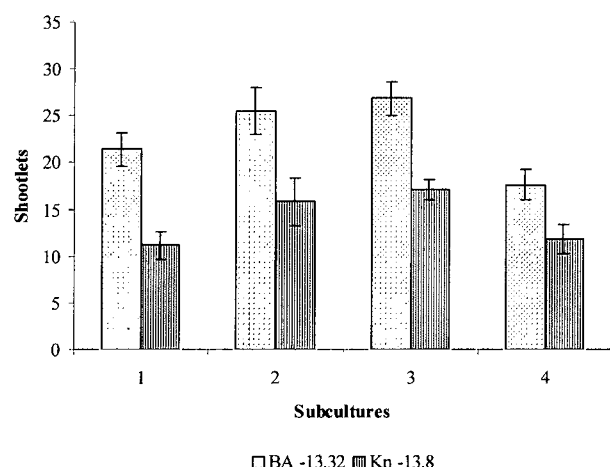

Figure 2. Effect of Cytokinins (μM) on multiple shoot production during sub culturing

For the acclimatization, the *in vitro* rooted plants were transferred to a sterile vermiculite:peat:soil mixture (1:1:1) base potting soil for indoor plants in polycups, irrigated with sterile water and maintained aseptically. They were maintained at 25±2°C under 60% relative humidity and were transferred and maintained in a greenhouse at 28°C under cool white fluorescent tubes (Philips TL 18W/133) providing ca. 27 mol $m^{-2}s^{-1}$ with a 16-h photoperiod (Figure1 F). The plants took 6-8 weeks to establish, the results of which could be seen by the emergence of new leaves from the plants. The micropropagated plants appeared morphologically uniform with elongated shoots, leaves expanded and turned green similar to mother plant. The plants were macromorphologically similar to the mother plant. Sixty-eight out of hundred plants were established successfully.

In conclusion, the present study describes an efficient reliable multiplication protocol for *in vitro* propagation of *E. travancoricum*. The important features necessary for the adaptation of *in vitro* propagation technology for large-scale propagation of plants viz. multiplication efficiency, good profuse rooting, establishment in soil and normal growth performance, observed in this study, will help in conserving this endemic horticulturally important plant. The protocol reported in this paper might help in its conservation and even for further commercial use.

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Table 2. Rooting response of *in vitro* grown shoots of *E. travancoricum* on 1/2 strength MS medium

IBA (μM)	Rooting response (%)	No. of roots / explant*	Root Length (cm) *
2.45	42	0.8 0.45	1.0 0.00
4.9	48	1.3 0.78	1.8 0.88
9.8	64	3.8 1.32	2.7 1.15
14.7	70	6.4 1.18	4.8 1.36
19.6	68	1.7 0.95	1.4 0.72
24.5	-	Basal callus	-

*- Mean Standard Deviation.
_No response.

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