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In vitro Plant Regeneration from Shoot tip Explants of *Exacum travancoricum* Beedi

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Abstract

Shoot tip explants of *Exacum travancoricum,* an endangered herb mainly known for ornamental purpose grown on MS with 4.44 μM BAP and 1.34 μM NAA showed better growth response and produced 29.3 ± 0.3 shoots per explant with an average length of 4.6 ± 0.1 cm after 35 days. Roots were induced after transfer to half strength of MS supplemented with 2.46 μM IBA produced 4.8 ± 0.62 roots with an average height of 3.6 ± 0.10 cm after 30 days. The rooted plantlets were transferred for hardening, 80 per cent of plantlets survived and resumed growth in the mixture of soil, vermiculite and farm yard manure (1 : 1 : 1).

Introduction

Exacum travancoricum Beddi (Gentianaceae) is a woody, stunted, much branched ornamental herb (Gamble and Fischer 1956). Recently IUCN has categorized *E. travancoricum* under critically endangered plants (Davis 1995). It is an endemic species to southern Western Ghats (8 -10º 30′ N) and 80% of population have declined due to habitat loss and at present only less than 250 mature individuals are naturally distributed in scattered populations in less than 250 km^2 (Gopalan and Henry 2000). Henry et al. (1984) recommended employing both *in situ* and *ex situ* cultivation methods for conservation and sustainable use of *E. travancoricum* plant species for ornamental purposes. Moreover, *Exacum* propagation by stem cutting is rather slow and time consuming (Elangomathavan et al. 2006). Earlier, *in vitro* micropropagation of *E. travancoricum* was reported using nodal explants and use of thidiazuron and auxin was found to be beneficial for shoot multiplication (Elangomathavan et al. 2006, Kannan et al. 2007).

In vitro plant regeneration from shoot tip explants has not been reported so far. The present study aims at developing a simple, rapid, economical, and high-frequency regeneration protocol from shoot tip explants of *E. travancoricum* for a large scale propagation.

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Material and Methods

Healthy *E. travancoricum* Beedi plants were collected from Thirunelveli hills, Tamil Nadu, India and raised in pots containing soil and farm yard manure (1 : 1) under greenhouse conditions at the Plant Biotechnology Division.

Shoot tip explants were collected from 60 days old plants and processed for aseptic culture. Explants were surface sterilized by cleaning thoroughly under running tap water for 20 min, washed with a solution of Tween 20 (2 drops in 100 ml of water) for 1 min, and again washed with sterile distilled water. The cleaned explants were finally treated with 0.1% $HgCl_2$ (w/v) for 3 - 4 min under aseptic conditions and again washed five times with sterile distilled water to remove traces of $HgCl_2$.

After surface sterilization, shoot tip explants were trimmed to 0.6 - 0.8 cm and inoculated on MS supplemented with different concentrations and combinations of BAP (1.11, 2.22, 4.44, 6.66 and 8.88 μM) and NAA (0.54, 1.34, 2.69 and 5.36 μM) for shoot initiation. Shoot number and length were recorded after 35 days in culture. The proliferated shootlets 4.5 - 5.0 cm length was transferred to half strength of MS supplemented with IBA (0.49, 0.98, 2.46, 4.92 and 12.30 μM) for root development. Root number and length were recorded after 30 days in culture.

For *ex vitro* establishment, well rooted plantlets were rinsed thoroughly with sterile water to remove residual nutrient from the plant body and transplanted to plastic pots containing a mixture of red soil, vermiculite and farm yard manure in a 1 : 1 : 1. One hundred rooted plantlets were transferred to greenhouse under 60 - 70% humidity. The plantlets were fertigated with 1/10 diluted MS basal medium on alternative days for two weeks. After 15 days, the fully acclimatized plantlets were transferred to pots (6 cm dia) containing red soil, vermiculite and farmyard manure in 1 : 1 : 1. Established plantlets were then transferred to bigger pots (14 cm diam).

MS containing 3% sucrose was used for all *in vitro* culture. The pH of the medium was adjusted to 5.6 ± 0.2 prior to adding 0.9% agar (Himedia - CR 301), media were autoclaved at 121°C for 15 min. Cultures were maintained at 25 ± 1°C under 16/8 h light and dark cycle. The lighting was provided using white cool fluorescent tubes of 40 $\mu Mol/m^2/s^2$. The plant growth regulators were filter-sterilized using 0.2 μm filter (Minisart®, Sartorius) prior to addition to culture media.

Results and Discussion

Multiple shoots developed from shoot tip explants cultured on MS supplemented with BAP (1.11 - 8.88 µM) and NAA (0.54 - 5.36 µM). Initiation of multiple shoots in most of the treatments was observed within three weeks of culture. Higher number of multiple shoot proliferation from shoot tip explants was observed in MS containing 4.44 µM BAP and 1.34 µM NAA showed better growth response (80%) and produced 29.3 ± 0.3 shootlets with an average length of 4.6 ± 0.1cm after 35 days of culture (Table 1, Fig. 1A,B,C,D,E). This synergistic combination of auxin and cytokinin on organogenic differentiation has been well explained in plant tissue culture (Baskaran and Jayabalan 2005, Gururaj et al. 2007, Janarthanam and Seshadri 2008).

Table 1. Shoot proliferation from shoot tip explants of *Exacum travancoricum.*

Hormones (µM)		Shoot induction (%)	Number of shoots per explant	Shoot length (cm)
BAP	NAA			
2.22	0.54	20.0 ± 0.0	1.67 ± 0.58	1.2 ± 0.5
	1.34	23.3 ± 1.6	2.00 ± 1.0	1.4 ± 0.1
	2.69	33.3 ± 5.7	3.00 ± 0.0	1.6 ± 0.0
	5.36	16.7 ± 7.6	1.67 ± 0.6	1.2 ± 0.2
4.44	0.54	35.0 ± 5.0	3.67 ± 1.5	2.9 ± 0.1
	1.34	**80.0 ± 5.0**	**29.3 ± 0.3**	**4.6 ± 0.1**
	2.69	45.0 ± 8.7	4.00 ± 0.0	3.0 ± 0.1
	5.36	40.0 ± 0.0	2.67 ± 1.2	2.3 ± 0.2
6.66	0.54	33.3 ± 5.7	1.7 ± 1.15	1.9 ± 0.5
	1.34	45.0 ± 0.0	3.67 ± 1.5	2.6 ± 0.1
	2.69	35.0 ± 5.0	3.3 ± 0.20	2.2 ± 0.2
	5.36	35.0 ± 5.0	2.0 ± 0.5	1.9 ± 0.2
8.88	0.54	15.0 ± 0.0	1.67 ± 0.6	1.3 ± 0.25
	1.34	18.3 ± 2.9	2.00 ± 0.0	1.4 ± 0.2
	2.69	28.3 ± 2.8	2.33 ± 1.2	1.8 ± 0.1
	5.36	16.7 ± 5.7	1.6 ± 2.0	1.4 ± 0.0

Results represent mean ± SD of three replicated experiments. Data were recorded after 35 days of culture.

The combination of BAP at 4.44 µM, along with addition of NAA (0.54 - 5.37 µM) was effective for stimulating regeneration of shootlets. However, BAP at 4.44 µM and 1.34 µM of NAA was found to be most effective, capable of inducing 80% response, and produced 29.3 ± 0.3 shoots per explants. At higher concentration of BAP (8.88 µM) and NAA (5.36 µM), a gradual decrease in the number of shoots per explant was recorded (Table 1).

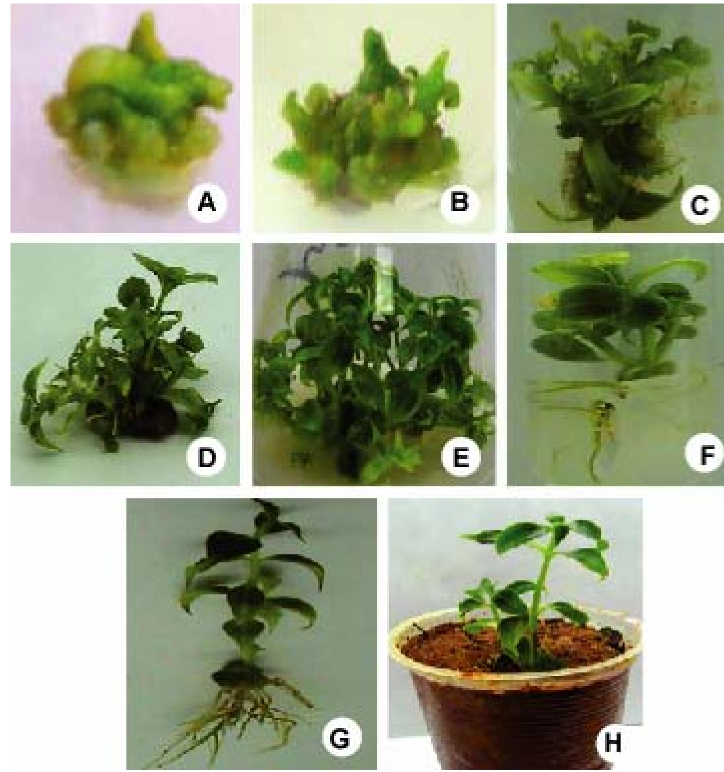

Fig. 1. Regeneration of multiple shoots from shoot tip explants of *Exacum travancoricum.* (A). Initiation of shoot from shoot tip explants after two weeks of culture. (B). Multiple shoot initiation after three weeks on MS containing 4.44 µM BAP and 1.34 µM NAA. (C, D). Proliferation of multiple shoots from shoot tip explants after four weeks. (E). Proliferation of multiple shoots from shoot tip explants at 35 days. (F). Rooted plants on MS containing 2.46 µM IBA after 30 days ready for transplantation. (G). A well established rooted plant. (H). Well established plants successfully transplanted to the plastic cups.

In the present study, development of shootlets from shoot tip explants were observed on MS medium containing BAP 4.44 µM and 1.34 µM NAA after 35 days in culture. In earlier study, the production of shoot buds from nodal explants of *E. travancoricum* showed the development of shoots (25.5, 26.9 and 15.8 and 17.1) both in second, third and fifth subcultures on MS supplemented with two different hormones BAP (13.32 µM) and Kn (13.8 µM) (Elangomathavan et al. 2006). The use of BAP and NAA for shoot development from shoot tip explants observed in this study is in agreement with earlier reports on organogenesis of dicotyledonous plants (Asamenew and Narayanaswamy 2000, Dhar and Joshi 2005).

Individual shoots from a multiple shoot complex were separated after 28 days of culture and transferred to half strength of MS supplemented with IBA (0.49 - 12.30 µM). The root induction was initiated after two weeks of culture, and

after four weeks, the root system was well developed (Fig. 1F,G). The maximum rooting response (80%) was achieved on medium supplemented with IBA (2.46 μM), with an average of 5.3 roots per shoot explant (Table 2). Earlier studies reported the use of higher concentration of IBA (14.7μM) for effective rooting in *E. travancoricum* (Elangomathavan et al. 2006). In the present study root induction was obtained with lower concentration of IBA.

Table 2. Root proliferation from *in vitro* shoots of *Exacum travancoricum*.

IBA (μM)	% response	Roots/ shoot	Root length (cm)
0.49	30.0 ±5.0	3.2 ± 0.2	3.2 ± 0.2
0.98	41.7 ±7.6	3.3 ± 0.17	3.3 ± 0.3
2.46	80.0± 0.0	5.3 ± 0.25	4.8 ± 0.2
4.92	55.0±5.0	3.3 ± 0.12	3.8 ± 0.1
12.30	36.6±2.8	2.0 ± 0.10	3.1 ± 0.1

Results represent mean ± SD of six replicated experiments. Data were recorded after 30 days of culture.

About 80 per cent of plantlets survival were observed after hardening of the regenerated E. *travancoricum* for three weeks. Seventy per cent of the plants transferred to pots survived and resumed growth (Fig. 1H). There was no detectable phenotypic variation among the acclimatized plants. In conclusion, the results showed the ability of the shoot tip explants to produce 29 shootlets without any intervening callus phase, where all the plantlets were uniform in height and growth. As no reports are available on regeneration of *E. travancoricum* from shoot tip explants, it is proposed this protocol for the first time to obtain more plantlets within a short period.

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