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In Vitro Flowering and Shoot Multiplication from Nodal Explants of *Ceropegia bulbosa* Roxb. var. *bulbosa*

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ABSTRACT: A rapid micropropagation system was developed for *Ceropegia bulbosa* Roxb. var. *bulbosa*. First five nodes of ~ 1.0 cm each, harvested from young healthy shoots from garden raised plants were cultured on B_5 medium supplemented with different concentrations of BA and AdS each in combination with 0.05 mg/L NAA. Multiple shoot formation of upto 12 shoots was observed within one week in presence of BAP (3 mg/L) and NAA (0.05 mg/L). Shoots were multiplied by subculture on the same medium. Shoots of 3-4 cm length were rooted in medium supplemented with 2 mg/L IBA. The rooted plantlets were hardened and successfully established in pots at 70% success rate. *In vitro* flowering was observed at 0.5 mg/L BA+1 mg/L GA_3. Shoots transferred to the medium containing kinetin (0.05 mg/L) + IBA (2 mg/L) showed microtubers in 28 days while the shoots cultured in presence of 1 mg/L GA_3 +0.5 mg/L of BA showed 76% flowering leading to seed production and 65% of the seeds germinated in successive generations.

KEY WORDS: ***Ceropegia bulbosa* Roxb. var. *bulbosa*, Medicinal plant, Micropropagation, *In vitro* flowering.**

INTRODUCTION

Ceropegia (Asclepiadaceae) is a genus of climbers, herbs and rarely subshrubs distributed in tropical and subtropical Asia, Africa, Australia, Malaysia and in the Canary and Pacific islands (Anonymous, 1992; Nayar, 1985). The tuberous roots of many *Ceropegia* species are edible (Mabberley, 1987), whereas those of *C. bulbosa* and *C. candelabrum* have medicinal properties (Jain and Defilips, 1991). *Ceropegia bulbosa* var. *bulbosa* and *C. bulbosa* var. *lushii* are common varieties distributed throughout India. The fresh tubers of these species are usually boiled before they are eaten, to remove the bitterness. The bitter principle of the root is an alkaloid, Ceropegin (Mabberley, 1987).

The species of *Ceropegia* as a whole are under threat owing to either destructive collection or habitat degradation. They are not only genetically depleted but also they are scarcely available. Micropropagation of these through tissue culture may help in the multiplication and reestablishment of these species back to into the wild.

MATERIALS AND METHODS

Ceropegia bulbosa var. *bulbosa* plants were collected from the Kolli hills of the Eastern Ghats in Tamil Nadu and maintained in pots in the glasshouse until they were used for experiments. Young shoots of 2 cm length were excised from actively growing plants. The

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Abbreviations: NAA-∝-naphthaleneacetic acid, BA- 6-benzyladenine, AdS-Adenine sulphate.

defoliated shoots were washed in tap water for 30 minutes followed by a wash in distilled water, and surface decontamination in 0.1% $HgCl_2$ for 4 minutes, and 3 rinses in sterile distilled water. The nodal segments of ~ 1 cm were excised and inoculated on to B5 culture medium supplemented with combination of 1.0 - 5.0 mg/L BA and 0.05 mg/L NAA and 10 - 30 mg/L AdS and 0.05 mg/L NAA. Cultures were incubated at 25±2°C under 16 hr photoperiod of 3000-lux light intensity. Observation of shoot multiplication and growth were recorded at weekly intervals. After one week, shoots of above 3 cm length were harvested and subcultured on the same medium containing 3.0 mg/L BA and 0.05 mg/L NAA. Certain shoots were also tested for *in vitro* flowering in presence of kinetin and GA_3 (1 mg/L). Shoots of 3 cm length were transferred to medium containing different concentration of IBA. After 2 weeks, the rooted plants were deflasked and planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to a green house.

RESULTS AND DISCUSSION

Shoot multiplication

Shoot buds got initiated on nodal segments after 6 days of culture. The higher frequency (85%) formation of maximum number of shoots was observed in 3 mg/L BA in combination with 0.05 mg/L NAA. Initially 1 or 2 buds developed (Fig. a; Table 1), later upto 12 shoots of above 3 cm length were formed in node in two weeks. AdS in combination with 0.05 mg/L NAA was less effective than BA as it induced only upto 60% formation of 3 - 5 shoots. The superior activity of BA compared to other cytokinins is reported in many such plants i.e., *Gymnema sylvestre* (Komalavalli *et al*., 1997, 2000), *Holostemma annulare* (Sudha *et al*., 2000), *Hyptis suaveolens* (John Britto *et al.*, 2001a) and *Anisomeles indica* (John Britto *et al.*, 2001b). During the incubation additional 22 shoots were formed from nodal segments while some shoots (85%) continued to show the longitudinal growth (Fig. b; Table 2) without bud proliferation. Shoots transferred onto rooting medium produced rooting at the base in 10 days. The higher percentage (83%) rooting was achieved at 2 mg/L IBA (Fig. c; Table 3). Similar results were reported in *Anisomeles indica* L. by John Britto *et al.* (2001b). The rooted plantlets were transferred to plastic cups containing sterilized vermiculite for hardening (Fig. e).

Table 1. Multiple shoot formation of *Ceropegia bulbosa* Roxb. var. *bulbosa* on B_5 vitamins supplemented with AdS, BA and combination with NAA (0.05 mg/L) after 25 days.

Plant growth regulators		Multiple shoot formation	No. of Shoots /explant
BA	AdS	(%)	Mean ± SD
1	-	45	5.7 ± 2.91
2	-	60	7.8 ± 2.22
3	-	85	10.0 ± 1.41
4	-	65	8.4 ± 2.05
5	-	60	6.5 ± 2.71
-	10	30	4.9 ± 3.06
-	15	40	5.3 ± 3.02
-	20	60	7.1 ± 2.56
-	25	45	6.0 ± 2.87
-	30	35	4.6 ± 3.10

Fig. a. Shoot initiation from nodal explant of *Ceropegia bulbosa* var. *bulbosa*. b. High frequency of multiple shoot formation. c. Rooting from regenerated shoots. d. *In vitro* flowering. e. Hardened plantlets. f. F1 plantlets from the *in vitro* produced seeds.

Table 2. Subculture of BA and NAA combination plantlets production and successful rooting of *Ceropegia* spp.

Subcultures with 15 day intervals	No. of harvest	No. of shoot harvested	No.of plantlets successfully planted	% of plantlets production	No.of plantlets established/subculture (mean ±SD)
15	1	18	12	66.6	15.0±2.45
30	2	23	18	78.2	15.9±3.36
45	3	26	22	84.6	16.7±3.61
60	4	30	25	83.3	17.6±4.16
75	5	30	25	83.3	25.5±1.50
90	6	35	27	77.1	26.1±1.86

Table 3. Effect of IBA on root induction in *Ceropegia bulbosa* Roxb. var. *bulbosa* grown on B_5 medium supplemented with IBA after 10 days.

Growth regulator (IBA) mg/L	% of rooting	No. of roots/explant Mean ± SD
1	72	3.6 ± 0.45
2	83	5.3 ± 0.47
3	67	2.6 ± 1.25

In vitro tuber formation

It was interesting to note that 85 % of shoots transferred on to medium containing sucrose (4%), kinetin (0.05mg/L) and IBA (2 mg/L) showed tuber formation. The tubers so formed at 52% rate in presence of kinetin (0.05mg/L) and IBA (2 mg/L) were weighing 40 - 50 mg. Plantlets were transferred to the plastic cup containing sterilized vermiculite and half -strength B_5 medium for 15 days. The microtubers harvested from each shoots weighed >600 mg for individual shoots after harvesting. About 20 tubers were tested and it resulted in formation of microtubers with 70% regeneration during the next growth season.

In vitro flowering and seed formation

In vitro flowering was observed on B_5 medium containing 1 mg/L GA_3 + 0.5 mg/L BA (Fig. d). Flowers were formed after 25 days of culture transferred to the rooting medium. Stephen and Jayabalan (1998) and Thiruvengadam and Jayabalan (2001) have reported that a combination of NAA (0.15 mg/L) and GA_3 (0.5 mg/L) induced maximum flower buds in *Coriandum sativum* L. and *Vitex negundo* L., respectively. Both GA_3 and BA promote in the flowers of the entire species (*Ceropegia* sps.). After the flowering, seed formation occurred in 10 days and the seeds showed 65% of germination in soil condition and the seedlings grew well and showed normal growth in the green house (Fig. f).

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Ceropegia bulbosa Roxb. var. *bulbosa* 試管中開花與莖節之枝稍繁殖

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摘　　要

本研究提供 *Ceropegia bulbosa* Roxb. var. *bulbosa* 一種快速的微體培養系統。取自溫室栽種植珠之年青健康枝稍的前五節大約各 1 公分長的莖節，將之培養於 B_5 培養基中，其內含不同濃度之 BA 與 AdS，並均含有 0.05 mg/L 的 NAA。在含有 BAP (3mg/L)與 NAA (0.05 mg/L) 的培養基中，一星期內即可觀察到多至 12 個再生枝稍，並且在同一培養基的繼代培養中，芽可再增殖。約 3-4 cm 長之枝稍可在含有 2 mg/L 之 IBA 培養基中發根，此發根的小苗可移植於花盆中，成功率約為 70%。在 BA (0.5 mg/L)與 GA_3 (1 mg/L) 的培養基中可觀察到試管中開花。枝稍移植到含 kinetin (0.05 mg/L)與 IBA (2 mg/L) 的培養基中，28 天後可觀察到小塊莖，而枝稍培養於 GA_3 (1 mg/L)與 BA (0.5 mg/L) 的培養基中則有 76%的枝稍開花，並可形成種子，其種子萌芽率達 65%。

關鍵詞：*Ceropegia bulbosa* Roxb. Var. *bulbosa*，藥用植物，微體培養，試管中開花。

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