Research Article

In vitro multiplication of *Ceropegia bulbosa var bulbosa Roxb*

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Abstract

Ceropegia bulbosa var bulbosa Roxb is member of the family Asclepiadaceaewhich was described by William Roxburgh. It is very widely distributed, growing sporadically almost throughout India in red laterite soil. The leaves are subsessile to petiolate while *Ceropegia bulbosa var. bulbosa* has orbicular to ovate leaves. It cures the disorder Urolithiasis which is the development of stones in the urinary tract. This may lead to pain and bleeding. It is considered as the third most common affliction of the urinary tract. In most of the types of stones that are formed, the most frequent are calcium oxalate. As per clinical and epidemiological studies, calcium oxalate followed by calcium phosphate is the most commonly encountered crystalline components found in urolithiasis. Nodal explants from mature plant of this species were collected and cultured on MS medium supplemented with various concentrations (0.5, 1.0 and 3.0 mg l-1) of cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D) alone and in various combinations under controlled condition. Successful regeneration method was achieved by this method.

Keywords: *Ceropegia bulbosa var bulbosa* Roxb, Urolithiasis.

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INTRODUCTION

The genus *Ceropegia* was reported by 200 species distributed in the tropical and subtropical Asia, Africa, Australia and Malaysia and in the Canary and Pacific islands. In India 44 species of *Ceropegia* were found, out of them 27 species are endemic to the peninsular India which is distributed mainly in Western Ghats and most of them are enlisted under endangered category. *Ceropegia intermedia* are also endemic and endangered species of South India. Scanty population of this species is distributed in edges of moist deciduous forests in Tamilnadu (Thomas and Philip, 2005). As the species is a cross pollinating one, the seed grown progenies of *Ceropegia* are not true-to-type. Low seed germination rate and habitat destruction threatens its population in natural habitat. The vegetative propagation by root tubers and stem cuttings is very arduous. The tubers contain valuable constituents in many traditional Indian Ayurvedic drug preparations against many diseases, such as diarrhea and dysentery and they are edible also. The root tubers contain starch, sugar, gum, album in oids, fats and crude fiber and are valuable constituents in many traditional medicinal systems in India. Active principle of tuberous roots contains an alkaloid ceropegine which is active against diarrhoea and dysentery (Nadkarni, 1976). However it is needs to conserve Ceropegia through tissue culture. The Meristimatic tissue, apical shoot, Axillary bud Micropropagation is an effective alternative source for clonal propagation of Ceropegia.

MATERIAL AND METHODS

Preparation of Explants

Follicles of Ceropegia*bulbosa* were collected from Buldhana district and grow in the green house, Botanical garden, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Apical shoot, Axillary bud, node and Meristimatic tissue of *Ceropegia* were collected from two month old plants grown in the Botanical garden, department of Botany Dr. Babasaheb

Ambedkar Marathwada University, Aurangabad. All these explants were used from donor plants during present study. The explants were washed carefully in running tap water for 10 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol, $Hgcl_2$ (0.3 %) was used. Explants were surface sterilized for 5 minute by 0.3% mercuric chloride followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these explants were dissected into small pieces and treaded so that maximum part can be exposed to media.

Culture media

MS medium (Murashige and Skoog, 1962) was used for multiple shooting for apical shoot, Axillary bud, node explants of *Ceropegia bulbosa*. Axillary bud, apical shoot tip multiplication of shoots was examined using MS medium variously supplemented with BA, KIN, for rooting, half strength MS medium Supplemented with various concentrations of auxins IAA, IBA, and NAA were examined.

Culture conditions

MS medium contains with 3% sucrose and gelled with 3 gm/L solidified agent Clerigel, and the pH was adjusted to 5.8 after adding the growth regulators. The media were steam sterilized in an autoclave under 15 psi and 121° C. after the inoculation culture tubes and culture vessels were transfers to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes light and 25 ± ^{0}C temperature. At least ten cultures were raised for each treatment. Data were measured after 25days of five replicate for shoot multiplication and shoot length Mean (μ) values with the standard error (S.E.).

RESULTS AND DISCUSSION

Apical shoot, Axillary bud and nodal explants of *Ceropegia* grown on hormone free MS medium no effect on multiple shoots formation. MS media with different concentrations of BAP 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l and combination of IBA, NAA gives maximum average percentage of multiplication.

Effect of BAP shoots multiplication

Table 1: Effect of BAP and IAA for multiplication of different explant

Explant	Conc. of growth regulator (mg/L)		Shoot length (Mean± SE)	% of shoot formation
	BAP	IAA		
apical shoot tip	1.0	0.2	1.88± 0.073	30
	1.2	0.2	2.64± 0.129	32
	1.4	0.2	2.52± 0.122	37
	1.6	0.2	2.90± 0.149	51
	1.8	0.2	2.16± 0.160	49
	2.0	0.2	2.24± 0.172	47
axillary bud	1.0	0.2	1.70± 0.130	35
	1.2	0.2	2.04± 0.143	37
	1.4	0.2	2.14± 0.140	44
	1.6	0.2	2.62± 0.139	52
	1.8	0.2	1.76± 0.214	50
	2.0	0.2	1.82± 0.149	49

*After 25 days mean ± SE of 5 replicate

The presence investigation of an apical shoot tip, Axillary bud and nodal explant was essential for the development and formation of multiple shoots in *Ceropegia*. The two Cytokinin was tested viz. BAP and KIN respectively. BAP was more effective than KIN for multiplication. MS media containing 3% sucrose, 3 mg/L Clerigel and different concentration of BAP 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l alone with IBA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L, concentration of BAP with combination NAA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L gives average percentage of multiple shooting of *Ceropegia*.(Fig.1) Maximum average percentage of multiple shoot was recorded BAP 1.6 mg/L, with combination IBA 0.2 mg/L.(Fig.2)

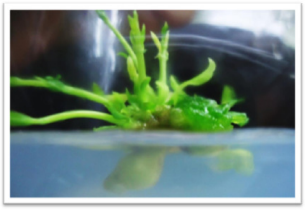

Figure 1: Multiple shoots formation along with callus

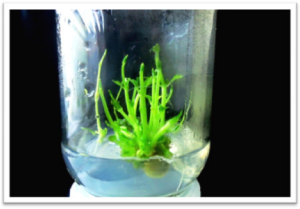

Figure 2: Multiple shoot formation along with tubers

Apical shoot tip and Axillary bud explant were inoculated on MS medium supplement with 3% sucrose, 2.5% Clerigel and various combinations of growth hormones as shown in the table No. 1 Maximum average shoot length and multiple shoot formation percentage of *Ceropegia* was recorded in 1.6 mg/L BAP combination with 0.2 mg/L IBA. Various combinations of IAA were added into the MS medium to achieve rooting. In vitro rhizogenesis was achieved by adding 0.5 mg/lit IAA. Plants were hardened and introduced in soil for in vivo trails. In vitro regenerated plants had shown 65 % viability in vivo. During growth of the cultures formation of tubers was recorded. The tubers are hard light green in color and if sub cultured give rise to new plantlets. Similar results were recorded for callus and shoot multiplication using various explants in different plants *like Tylophora indica (*Gupta *et al*. 2010, Shah and Kapoor, 1976*)*. Proliferating shoot cultures was established by repeatedly sub culturing the mother explants on the hormone free medium. Repeated sub-culturing was said to be one of the methods of maintaining juvenility (Choudhary and Jha. 2004). In the present work highest number of shoot percentage was recorded in third sub culturing. Somatic embryos were developed into plantlets and subsequently grown to maturity (Kirtikar and Basu, 2001). These results indicate that nodal explants have high competence for somatic embryogenesis in *Plumbago indica* (Das and Rout, 2002). In the present study nodal explants have shown direct multiple shoot formation.

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REFERENES

1. Choudhary KN, Ghosh B, jha S. (2004). May The root: a potential new source of competent cells for high-frequency regeneration in *Tylophora indica*, Plant cell report; 22^{10}:731-40
2. Das G and Rout GR (2002) Plant regeneration through somatic embryogenesis in leaf derived callus of *Plumbago indica.* Biologia Plantarum. 45^{2}: 299-302.
3. Gupta Mayank, Singh Mhaveer, Mukhatr Hayat M., Ahmad Sayeed.(2010). pharmacology journal (Vol2,Issue 11, Page 381-385
4. Kirtikar KR, Basu BD (2001). Indian Medicinal Plants, Vol. 12nd ed. Dehradun publisher Ltd, India, 1994; 1: 830-832.
5. Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassay for tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.
6. Nadkarni, K. M. (1976). Indian materia medica, vol. I. Bombay: Popular Prakashan; 303–304.
7. Shah N.C., Kapoor L. D., (1976). Botany of *Tylophora indica* (Burm.f.) Merr, Vol. 14, No. 1, Pages 27-34
8. Thomas D, Philip B (2005). Thidiazuron-induced high-frequency shoots organogenesis from leaf-derived callus of a medicinal climber, *Tylophora indica*. In Vitro Cellular and Dev. Biol. Plant, 41:124-128.

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