Research Article

"*In Vitro* Callus Induction of *Ceropegia bulbosa and Ceropegia attenuata*" – An Endangered Tuberous Plants of Rajasthan

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ABSTRACT

The genus *ceropegia* belongs to the Asclepiadoideae (milkweed) sub-family within the family Apocynaceae. *Ceropegia species* is a medicinal herb, this is useful in curing many diseases like kidney stone and deafness which in present time cured surgically but this plant product remove it naturally so this plant has great medical uses. Ethno botanical study showed that the plant has good medicinal value for tribe of Rajasthan. The effect of various phytohormones on *in-vitro* callus induction and shoot regeneration in *Ceropegia species* from leaf explant was evaluated. The sterilized explants were inoculated on ms medium supplemented with different concentrations of auxin alone or in combination with cytokinins. Best result was observed on MS medium fortified with NAA (1.5 mg/l). Callus generation capacity, length and morphology were observed. I*n vitro* generated callus was used further for large scale production of plant and biochemical study.

Keywords: Callus cultures, *Ceropegia*, MS medium, Plant hormones.

INTRODUCTION

Ceropegia L. (commonly known as Lantern Flowers) species are members of the family Asclepiadaceae (Apocynaceae), an old world tropical genus, which are distributed from south-east Asia, India, Madagascar, Tropical Arabia, Canary Islands, Africa except Mediterranean region, New Guinea and Northern Australia.[1-4] In India, about 55 species are present and most of them are endemic to Western Ghats, which is one of the centres of diversity of *Ceropegia* [5,6]; 38 species distributed mainly in Western Ghats[7-9]; and most of them are enlisted under endangered category.[10]

In India, several medical systems have evolved and prominent among these systems are Ayurveda, Siddha and the Unani Systems of Medicine. In different civilizations the contribution of floral biodiversity to health care has been well documented.[12] According to Schippmann *et al.*, more than 50,000 species are used for medicinal purposes worldwide, of which almost 13% are flowering plants.[13] Over 8000 plant species are used in traditional and modern medicine in India (Planning Commission 2000), and 90-95% collection of medicinal plants is from the wild, of which more than 70% collection involves destructive and unscientific extraction. Over-exploitation of trade species, destructive way of collection, and vulnerability due to anthropogenic pressure are some of the major threats to medicinal plants.[14] Active principle of tuberous roots contains an alkaloid cero-pegine which is active against diarrhea and dysentery.[15] Of the 44 species of *Ceropegia* found in India, 27 species are endemic to the Peninsular India,[16] which is distributed mainly in Western Ghats and most of them are enlisted under endangered category.[17] *Ceropegia intermedi* are also endemic and endangered species of south India *Ceropegia bulbosa* and *Ceropegia attenauta*, are rare medicinal plant belonging to the family Asclepiadoideae (milkweed) sub-family within the family Apocynaceae. It is an herbal climber and has great medicinal value. *Ceropegia bulbosa* and *Ceropegia attenauta* has in recent years suffered over-exploitation and has therefore been listed as one of the threatened species of India by BS. Various secondary metabolites present in the plant are responsible for its medicinal value. In recent years there has been a tremendous increase in the demand and consumption of herbal medicinal drugs as they have fewer side effects. There is an immense pressure on natural resources due to urbanization and industrialization, this coupled with harvesting of plants as source of drug has threatened their survival thus there is a great necessity for large scale multiplication of the plant which is simple rapid, genetically stable. Plant tissue culture offers a method for large scale multiplication of various medicinal herbs such as *Ceropegia* species and also secondary metabolite production. The technique of direct production of plant from nodal part made it possible several million individuals per year in number of species.[18] The present study was undertaken to study callus induction from various explants of *Ceropegia* species using various plant growth regulators.

MATERIALS AND METHODS

Plant material and explants preparation

Ceropegia Bulbosa and *Ceropegia attenuata* were collected from Ajmer and Amber hills area, Jaipur. The leaves and nodal parts were used for callus induction. The explants were thoroughly washed for 10-15 minutes under running tap water and subsequently they were rinsed with 0.2% tween-20 (mild detergent). Then they were rinsed several times using sterilized distilled water.

Further sterilization was done under aseptic conditions in laminar air flow cabinet. The explants were treated with 70% ethanol for 30 s and washed thrice in sterile distilled water. For surface sterilization, the explants were dipped in 0.1% aqueous solution (w/v) of $HgCl_2$ for 3 minutes then they were washed in sterilized distilled water for 3-4 times, till the sterilants were removed completely.

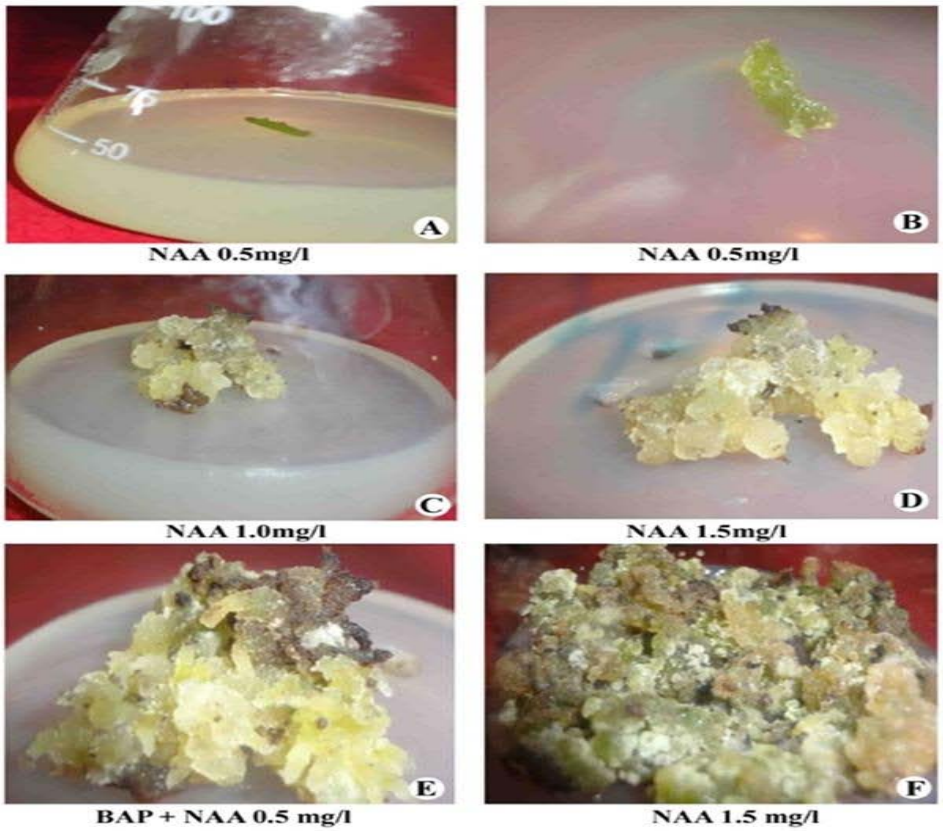

Fig. A: Callus formation from internode segment explants of *Ceropegia bulbosa* on MS medium supplemented with various auxins

Callus induction

For callus induction, The sterilized leaf, nodal segment and shoot tip explants were placed on full strength Murashige and Skoog's, (1962) basal medium (MS medium) fortified with sucrose and supplemented with different concentrations of auxins, 2,4-D/ NAA/IAA (0-2.5 mg/l) alone or in combination with cytokinins, BAP/ Kn (0-2.5 mg/l). Callus obtained from explants was transferred to MS medium supplemented with combinations of BAP (0-3.0 mg/l) and NAA (0.5 mg/l) for further subculture. After the inoculation, all the cultures were maintained in growth chamber which with regulated temperature (26±2°C), relative humidity (55±5%) and light conditions (16/8 hours photoperiod). 3000 lux intensity of constant light was provided in culture shelves by cool-white fluorescent tubes. Data was recorded after 4-6 weeks.

RESULTS AND DISCUSSION

Callus induction and *in vitro* plantlet regeneration system for *Ceropegia bulbosa* and *Ceropegia attenauta* were optimized by studying the influence of explants type (leaf, nodal segment and shoot tip) and different concentrations of plant growth regulators. Callus formation and shoot differentiation was initiated on Murashige and Skoog's (MS) medium containing different concentrations of auxin and cytokinin. The best result was obtained using nodal explants and callus production was maximum at 1.5 mg/L NAA (α-naphthalene acetic acid). NAA (1 mg/l) was found optimal for the induction of whitish green friable callus which started after 7th day of inoculation. On this medium, 70% of the cultures showed callus emergence from the basal cut end of the nodal cuttings. So, this protocol can be used for the rapid regeneration of *Ceropegia bulbosa* and *Ceropegia attenauta* through indirect organogenesis using a wide range of explants Among the various auxins tried, NAA was the most suitable auxin at 1.5 mg/l concentration and among various explants (leaf / internodes / nodal segment) tried, nodal explants proved to be the best for callus induction. The results showed in photo plate 1 and 2. Detail explanation given in table no. 1 and 2. BAP with GA & NAA showed the frequency of formation of shoot (85%) in for in vitro propagation of *Ceropegia bulbosa*.[19]

Table 1: Effect of auxins on callus formation in different explants of *Ceropegia bulbosa*

Auxins (mg/l)	% of explants responding for callus formation			Type of callus
	Leaf explants	Internode explants	Nodal explants	
NAA				
0.0	0.0-	0.0-	0.0-	nil
0.5	15*	25*	42*	Whitish friable
1.0	50**	50**	60*	Greenish white, friable
1.5	70***	43*	80***	Greenish friable
2.0	8*	15*	50*	Greenish friable
2.5	4*	5*	20*	Greenish friable
2,4-D				
0.0	0.0-	0.0-	0.0-	nil
0.5	12*	12*	30*	White friable
0.1	17***	20**	50**	Green, friable
1.5	40**	52**	55*	Greenish White friable
2.0	10***	20***	25***	Green friable
2.5	30*	20*	40*	Greenish friable
IAA				
0.0	0.0-	0.0-	0.0-	nil
0.25	8*	10*	15*	Whitish brown
0.5	10*	12*	20*	Dark brown
1.0	0.0	0.0	0.0	nil
2.0	0.0	0.0	0.0	nil
2.5	0.0	0.0	0.0	nil

Table 2: Effect of auxins on callus formation in different explants of *Ceropegia attenuta*

Auxins (mg/l)	% of explants responding for callus formation			Type of callus
	Leaf explants	Internode explants	Nodal explants	
NAA				
0.0	0.0-	0.0-	0.0-	nil
0.5	10*	15*	32*	Whitish friable
1.0	50**	50**	60*	Greenish white, friable
1.5	60***	53*	85***	Greenish friable
2.0	18*	25*	50*	whitish friable
2.5	4*	5*	20*	whitish friable
2,4-D				
0.0	0.0-	0.0-	0.0-	nil
0.5	22*	18*	23*	White friable
0.1	27***	20**	50**	Green, friable
1.5	40**	40**	85*	Greenish White friable
2.0	10***	22***	45***	whitish friable
2.5	30*	20*	30*	whitish friable
IAA				
0.0	0.0-	0.0-	0.0-	nil
0.25	12*	40*	15*	Whitish brown
0.5	10*	12*	30*	Dark brown
1.0	0.0	0.0	0.0	nil
2.0	0.0	0.0	0.0	nil
2.5	0.0	0.0	0.0	nil

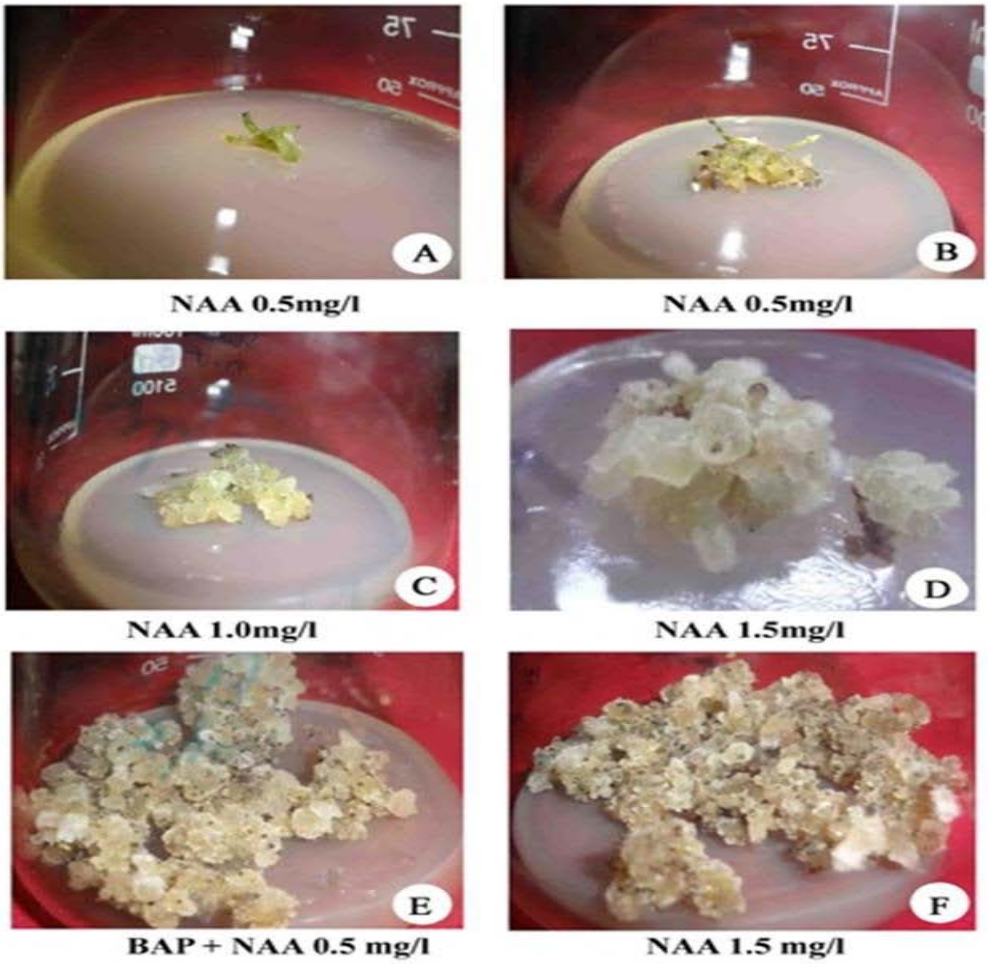

Fig.B: Callus formation from internode segment explants of *C. attenuata* on MS medium supplemented with various auxins

CONCLUSION

This is the first report describing callus induction protocol for *Ceropegia Bulbosa* and *Ceropegia attenuate* species found Rajasthan. This study reports an efficient and easy to handle protocol for callus induction for endangered plant of semi- arid region of Rajasthan, *Ceropegia Bulbosa* and *Ceropegia attenuate* medium supplemented with 1.5 mg/l NAA is the most effective medium for callus induction and among various explants (leaf / internodes / nodal segment) tried, nodal explants proved to be the best for callus induction. This protocol could be utilized for conservation of this medicinally important endangered plant.

REFERENCES

1. Meve U, Liede-Schumann S, Ceropegia (Apocynaceae, Ceropegieae, Stapeliinae): Paraphyletic, but still taxonomically sound, Ann Missouri Bot Garden, 94(2), 2007, 392-406.
2. Bruyns PV, Three new succulent species of Apocynaceae (Asclepiadoideae) from southern Africa, Kew Bull, 58, 2003, 427.
3. Mabberly DJ, The Plant-Book, Cambridge University Press, (first published 1987, repr. with corrections 1998, 2000), 1997.
4. Dyer RA, *Ceropegia*, *Brachystelma* and *Riocreuxia* in Southern Africa, Balkema, Rotterdam, 1983.
5. Malpure NV, Kamble MY, Yadav SR, A new species of Ceropegia L. (Asclepiadaceae) from the Western Ghats of India with a note on series Attenuatae Huber, Curr Sci, 91, 2006, 1140-1142.
6. Yadav SR, Flytrap flowers of Western Ghats, Hornbill, 1, 1996, 1–7.
7. Yadav SR, Mayur YK, Threatened *Ceropegias* of the Western Ghats and Strategies for Their Conservation, In: Rawat G.S. (Ed). Special Habitats and Threatened plants of India. ENVIS Bulletin: Wildlife and protected Areas, Wildlife Institute of India, Deharadun, India, Vol.11, 2008, 123-134.
8. Surveswaran S, Kamble MY, Yadav SR, Sun M, Molecular phylogeny of *Ceropegia* (Asclepiadoideae, Apocynaceae) from Indian Western Ghats, Plant Syst Evol, 281, 2009, 51-63.
9. Jagtap A, Singh NP, Fascicles of Flora of India, BSI, Calcutta, India, 1999, 211-241.

10. Nayar MP, Sastry ARK, Red data book of Indian plants, Botanical Survey of India, Kolkata, 1-2, 1987, 58.

11. Posey DA, Cultural and Spiritual Values of Biodiversity - A Complementary Contribution to the Global Biodiversity Assessment, UNEP, 1999.

12. Schippmann U, Leaman DJ, Cunningha AB, Impact of cultivation and gathering of medicinal plants on biodiversity, Global trends and issue, In Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries, Food and Agriculture Organization (FAO), Rome, 2002.

13. Adhikari BS, Babu MM, Saklani PL, Rawat GS, Medicinal Plants Diversity and their Conservation Status in Wildlife Institute of India (WII) Campus, Dehradun, *Ethnobotanical Leaflets,* 14, 2010, 46-83.

14. Nandkarni AK, India Materia Medica, Bombay, India, Popular Prakashan Pvt. ltd., 1976, 1308-1215.

15. Ahmedullah M, Nayar MP, Endemic Plants of Indian region Botanical Survey of India, Calcutta, India, Vol 1, 1986, 119.

16. Nayar MP, Meaning of Indian flowering plant names, *Bishen Singh, Mahendrapal singh*, Dehra Dun, India, 1985, 78.

17. Murashige T, Plant regeneration through tissue culture, Ann. Rev., Plant Physiol, 25, 1974, 135-166.

18. Goyal D, Bhadauria S, *In Vitro* Propagation of C. bulbosa using nodal segments, Indian Journal of Biotechnology, Vol 5, 2006, 565-567.

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