



Research Article

Micropropagation of traditional medicinal plant *Ceropegia juncea*

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Abstract: *Ceropegia* species which possess wide medicinal properties are being used in different traditional medicinal systems that are used by tribal people for curing different ailments. *Ceropegia juncea* was reported to be the source of 'Soma', a plant drug of the Ayurvedic system of medicine. The plant extract is used for the treatment of anti-inflammatory, analgesic, antiulcer activities, liver disorders, hypotension, ulcerative condition and fever. It is also used as typical anesthetic agent. The present study was conducted to establish a protocol for in-vitro propagation of an endemic medicinal plant *Ceropegia juncea* maximum shoot proliferation better shoots with a sprouting frequency of 86% and with an average of 8.28 ± 1.11 shoots /explants and attained a length of 5.37 ± 0.74 cm was achieved on Murashige and Skoog's, 1962 (MS) medium supplemented with 6-benzylaminopurine (BAP) 1.5 mg/L + NAA 1.0mg/L and highest rooting of *in vitro* derived shoots was achieved on half MS with IBA 0.75mg/L.

Keywords: *In vitro*; *Ceropegia juncea*

Introduction

The genus *Ceropegia* L. belongs to the sub-family Asclepiadoideae under the family Apocynaceae in APG III Classification. *Ceropegia* species are being used traditionally, due to their immense therapeutic potential to treat as well as to cure various diseases. In the fields of Ayurvedic drug preparation *Ceropegia* tubers are used as nutritive tonic and blood purifier (Jagtap and Singh, 1999). "Cerpegin" is a key compound of the genus *Ceropegia*. Sukumar *et al.*, (1995) revealed that the edible species of *Ceropegia juncea* contains steroids, polyphenols, sugars and potassium. This extract is used to cure kidney stone and urinary tract disorders (Paras *et al.*, 2011). Pavan Kumar *et al.*, (2012) focused on the phytochemical profile of the plant *Ceropegia juncea* constituents of alkaloids, tannins, sterols, glycosides, saponins, proteins, lipids and carbohydrates. The hydro-alcoholic extract of *Ceropegia juncea* whole plant possesses hepatoprotective activity against paracetamol induced hepatic damage and found it to have significant antioxidant activity in rats. *Ceropegia* species has enormous medicinal value, but the genus *Ceropegia* has now been added to the list of Indian endangered plants. Propagation through seed hampered due to its low viability rate, poor germination and scanty rooting of seedlings. Vegetative propagation by root tubers and stem cuttings is very arduous. The rapid deforestation hampers its natural regeneration potential in a big way therefore; large scale propagation has become a prerequisite for effective conservation of this endangered species.

Materials and Methods**Micropropagation studies**

Ceropegia juncea was collected from Marthualmalai hills, a part of the Southern Western Ghats, during rainy season. The collected plant was identified by using *Flora of Eastern Ghats* (Pullaiah *et al.*, 2011). The collected plant parts such as node, internode and shoot tips were used for micropropagation. Murashige and Skoog's (1962) medium was found to be the most commonly used medium for the propagation of *Ceropegia* as per the existing literature. Therefore, all trials were later on carried out on MS medium. However, use of full strength salt formulations with media supplements yielded poor roots results to the reduced salt strength i.e. half salt strength.

The plantlets were transferred to seven-centimeter diameter plastic cups containing different types of hardening materials. One of the hardening materials like equal ratio of sterilized soil, coir waste and *Azolla* (1:1:1). Others filled with potting mixture of cow dung, autoclaved river sand and garden soil (1:1:1). The individual plantlets were covered with polythene membrane to maintain humidity; small holes were made, and watered every two days with half strength MS solution free of sucrose. After twenty days, the polythene membrane was removed and plants were allowed to adjust to ambient conditions. Then the hardened plants were transferred to earthen pots and kept under shade not exposing to direct sunlight. The acclimatized plants were finally transferred to soil. Each experiment consisted of five to seven replicate per plant growth regulator treatment. The differences in

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root and shoot length among the micropropagated *Ceropegia* species with different concentration of hormones were calculated with Tukeys test.

Results

Effect of BAP on shoot apices of *Ceropegia juncea*

Nodal explants and shoot tip portion obtained from the mature plants were cultured on MS medium. The nodes remained green and fresh but failed to sprout on MS media without cytokinins. BAP was found to be the most efficient cytokinin for the axillary bud initiation and subsequent proliferation. MS medium supplemented with different concentrations of BAP were tested for shoot proliferation. Microshoots were proliferated on MS medium containing the best concentration of BAP (1.5mg/L). Sub-culture on the MS medium containing different concentrations of BAP, microshoots were observed and they measured twice. The sub-cultured microshoots and node, produced adventitious buds on the third day in MS medium containing BAP 1.5 mg/L were found to have grown faster. New shoots developed adjacent to the axillary shoots and had grown well within fifteen days. Shoot multiplication rate was evaluated after two weeks BAP 1.5 mg/L and proved to be most effective, as in this medium produced better shoots with a sprouting frequency of 86% and with an average of 8.28 ± 1.11 shoots /explants and attained a length of 5.37 ± 0.74 cm.



Plate 1. Combined effect of BAP and NAA in shoot regeneration of *C. juncea*



Plate 2. *Ceropegia juncea* - Callus formation and multiplication



Plate 3. *In vitro* roots of *C. juncea*



Plate 4. *C. juncea* -Hardened plants

Shoot multiplication was observed on all the combinations of BAP with KN, NAA, IAA and IBA, highest number of shoots was observed in third sub-culture on MS medium supplemented with BAP 1.5mg/L + NAA 1.0mg/L (9.71 ± 0.75) shoots/explants with 76% response. The results are reported in table 1. The developed shoots were healthy, strong and elongated. Creation of roots was inhibited by the content of this concentration. On the other hand, auxin had a stimulating influence on the creation of roots (Plate 1).

Callus induction from nodal explants of *Ceropegia juncea*

Pale green friable callus was observed the internodes of *Ceropegia juncea* on MS medium augmented with NAA within nine days of the third sub-culture. Higher percentage (80%) of node callus formation was observed on the medium augmented with a hormonal combination of BAP (1.5mg/L) + NAA (2mg/L) (Plate 2: A & B). These calli were relatively friable and yellowish green in color.

Table 1. Effect of different combination of plant growth regulators in shoot regeneration from nodal explant of *Ceropegia juncea* cultured on MS medium with 3% sucrose.

BAP	NAA	IAA	IBA	Shoot induction (%)	Number of multiple shoots/explant \pm SD	Average length of shoots/explant \pm SD (cm)	Shoots with basal callus
1.5				70%	7.85 \pm 1.06 ^{fg}	7.01 \pm 0.64 ^{cd}	+
1.5	0.5			76%	9.71 \pm 0.75 ^{gh}	5.31 \pm 0.55 ^c	+
1.5	1.0			50%	5.14 \pm 0.69 ^{cde}	5.01 \pm 0.12 ^b	++
1.5	1.5			80%	CP	CP	+++
1.5	2.0			70%	CP	CP	++
1.5	2.5			70%	1.57 \pm 0.78 ^{ab}	1.82 \pm 0.73 ^a	+
1.5		0.5		40%	1.14 \pm 0.37 ^a	1.25 \pm 0.35 ^a	++
1.5		1.0		60%	1.85 \pm 1.21 ^{ab}	3.84 \pm 0.36 ^{ab}	-
1.5			0.5	80%	1.28 \pm 0.48 ^a	3.42 \pm 0.40 ^b	-

Values are the mean \pm SD of 7 replicates. Different superscripts in the same column indicate significant differences within treatments (Tukey's HSD test, $P < 0.005$); (-) sign indicates no callusing, number of (+) sign represents the intensity of callusing; CP - callus production.

Table 2. Effect of different auxins on root response of *Ceropegia juncea* microshoots cultured on half MS medium after third sub-culture.

Auxins	Concentration (mg/L)	Days for root induction	Average number of root/explant \pm SD	Average length of roots/explant \pm SD (cm)
IAA	0.25	10 -14	3.20 \pm 0.44 ^b	4.42 \pm 0.20 ^{ab}
	0.5	10 -14	3.60 \pm 0.54 ^{ab}	5.06 \pm 0.52 ^{bc}
	0.75	10 -14	5.20 \pm 0.83 ^{bc}	5.44 \pm 0.35 ^{bc}
	1.0	10 -14	5.20 \pm 0.44 ^{bc}	5.94 \pm 0.15 ^{bcd}
	1.25	10 -14	3.80 \pm 0.44 ^{ab}	4.42 \pm 0.17 ^{ab}
IBA	0.25	5 -14	4.60 \pm 0.89 ^{bc}	5.52 \pm 0.26 ^{bc}
	0.5	5 -14	5.60 \pm 0.54 ^{cd}	5.82 \pm 0.44 ^{bc}
	0.75	5 -14	6.20 \pm 0.44 ^{cd}	5.96 \pm 0.59 ^c
	1.0	5 -14	6.60 \pm 0.54 ^d	4.16 \pm 0.11 ^b
	1.25	5 -14	4.40 \pm 0.54 ^b	3.42 \pm 0.22 ^a
NAA	0.25	9 -14	2.60 \pm 0.89 ^a	6.12 \pm 0.19 ^{bd}
	0.5	9 -14	3.60 \pm 0.54 ^{ab}	5.88 \pm 0.41 ^{bc}
	0.75	9 -14	2.40 \pm 0.54 ^a	5.28 \pm 0.25 ^{abc}
	1.0	9 -14	NR	NR

Each values represent mean value \pm SD of experiment carried out with five replicate. Different superscripts in the same column indicate significant differences within treatments (Tukey's HSD test, $P < 0.005$); NR- No response.

Root induction from microshoots of *Ceropegia juncea*

Root initiation was noticed within two week of culture and the data was recorded. After fifth day of incubation, rooting was observed and measured. Shoot inoculation on half strength MS medium supplemented with IAA (0.25-1.25 mg/L), improved the percentage of rooting up to 1.0 mg/L, along with maximum number of micro roots/shoots (4-5). Auxin promotes growth of roots only at extremely low concentrations. At higher concentrations, it always inhibits growth of root. Shoots kept on MS medium fortified with IBA (0.75mg/L) showed quick responses (5-14 days) 6 thick, long roots were observed (Plate 3).

Acclimatization and transplantation of plantlets

The regenerated plantlets were removed from the culture medium and washed with tap water. Rooted shoots were removed from the medium and they were transplanted in plastic cups with a mixture of different manures. Among the *in vitro* plantlets, 50% plants were hardened with a bio-fertilizer *Azolla* along with sterilized soil and coir waste (1:1:1) 50% plantlets with potting mixture of cow dung, autoclaved river sand and garden soil (1:1:1). The comparison was made between the plants transferred to different mixtures on the basis of their survival percentage and plant development. Plantlets were kept for 10-15 days in high humidity

and low light intensities. The humidity is gradually decreased to the ambient level over a period of 15-30 days and the light intensity is also gradually increased. Plants were irrigated initially with ¼ strength MS solution and afterwards whenever required with the tap water. The plantlets were acclimatized in hardening chamber for one month and subsequently transferred to the earthen pots and maintained under shade for one more week. Then, plants were exposed to sunlight for a few hours and then for a week. After two weeks of hardening, their response was recorded. Among the two different manures cow dung, autoclaved river sand and garden soil were tested to find out the best hardening medium. Afterwards, plants were transferred to soil and watered with tap water. The hardened plants produced well shoots, simultaneously produced flowers and follicles are representing in Plate 4. Tissue culture technique used for propagation may prove useful tool for the multiplication of *Ceropegia juncea* facilitate the bulking up of material for experimental purpose mentioned above without over harvesting the species from natural habitat from the point of view of its conservation also.

Discussion and Conclusion

In vitro techniques used for conserving wild and endemic species of *Ceropegia* by mass multiplication for subsequent reintroduction in their natural habitat (Patil, 1998). Axillary bud proliferation is considered the most simple and reliable method for the establishment of elite clones the potential to develop into complete plantlets (Gangaprasad *et al.*, 2005; Ugraiah *et al.*, 2011). Superiority of BAP for shoot multiplication in Apocynaceae has been reported in many studies (Martin, 2002; Ramulu *et al.*, 2002; Britto *et al.*, 2003; Karuppusamy *et al.*, 2009). BAP in combination with auxins NAA was required in optimal quantity for shoot multiplication in *Ceropegia juncea*. These results were in corroborative with the findings on *Gynmema sylvestre*, where BAP (5mg/L) in combination with NAA (0.2mg/L) is reported to induce maximum number of shoots along with callus production at the base of the proliferated shoots Reddy *et al.*, (1998). The same combination which was also found suitable for *in vitro* raising of several valuable medicinal plants (Kim *et al.*, 2003; Khosravi *et al.*, 2007; Catapan *et al.*, 2009; Pillai *et al.*, 2009; Tyagi *et al.*, 2010; Kumar *et al.*, 2012). The present study higher frequency of rooting was reported when MS medium with IBA (1.0 mg/L) on *Ceropegia juncea* and IBA (1.5mg/L). Similarly IBA was rooting hormone in many medicinal plants such as *Dalbergia sissoo* (Pradhan *et al.*, 1998), *Ceropegia bulbosa* and *Ceropegia bulbosa* var. *lushii* (Patil, 1998), *Decalepis arayalpathra* (Gangaprasad *et al.*, 2005), *Caralluma edulis* (Rathore *et al.*, 2008), *Ceropegia intermedia* (Karuppusamy *et al.*, 2009) and *Ceropegia fantastica* (Chandore *et al.*, 2010). The plantlets were successfully established in soil with 80% survival.

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