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Full Length Research Paper

In vitro propagation of *Ceropegia juncea* Roxb

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The present study was conducted to establish a protocol for *in vitro* propagation of an endemic medicinal plant *Ceropegia juncea* (Asclepiadaceae) from one month old aseptic seedling nodal explants. The highest shoot multiplication rate of 20.65 ± 0.20 shoots/explant was achieved on Murashige and Skoog medium supplemented with BAP 8.87 µM + TDZ 4.54 µM. Excised shoots were rooted on half strength MS medium with IBA 4.90 µM + NAA 1.27 µM. 78% of the rooted shoots survived in the field.

Key words: *Ceropegia juncea*, in *vitro* propagation, endemic medicinal plant, nodal explants, multiple shoots.

INTRODUCTION

Ceropegia L. is old world tropical genus containing about 200 species which are distributed form South Africa around the perimeter of the Indian Ocean to Australia (Bruyns, 2003). Of the 48 *Ceropegia* species found in India, 28 species are endemic to the Peninsular India (Ahmedulla and Nayar, 1986). The existence of the *Ceropegia* species has become restricted to remote pockets in the Himalayas and the Western Ghats, two biodiversity hot spots. Regrettably, the *Ceropegia* genus has now been added to the list of Indian endangered plants. *Ceropegia juncea* Roxb. (Asclepiadaceae) is an important medicinal herb, which is used as a source of "Soma", a plant drug of the ayurvedic medicine with a wide variety of uses (Asolkar et al., 1992; Jagtap and Singh, 1999). The fleshy stem is used as a raw material for traditional and folk medicines for the treatments of stomach and gastric disorders (Jain and Defillips, 1991). The alkaloid ceropegin was isolated and identified as pyridone type alkaloid, which are relatively rare in nature (Adibatti et al., 1991). The total alkaloidal fraction exhibited promising hepatoprotective, antipyretic, analgesic, local anesthetic, anti-ulcer, mast-cell stabilizing, tranquilising and hypotensive activities and was devoid of side effects as noted out by the sub-acute toxicity studies (Adibatti et al., 1991). Although *C. juncea* is an important source of ayurvedic drug, but due to the lack of proper cultivation practice, low number of seed formation, destruction of plant habitats and its removal is leading to a progressive devastation of the species. Several workers have proposed micro-propagation protocols for *Ceropegia* spp. Multiple shoot regeneration and alkaloid ceropegin accumulation in callus culture of *C. juncea* Roxb. was reported by Nikam and Savant (2009). However our results were better than earlier report for multiple shoot regeneration of *C. juncea*.

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MATERIALS AND METHODS

The mature fruits of *C. juncea* were collected from Kalasamudram forest of Kadiri in Anantapur District Andhra Pradesh. Fruits were shade dried and seeds were collected for raising aseptic seedlings. The seeds of *C. juncea* were taken in 100 ml clean erlenmeyer flask then washed with two drops of liquid detergent (1% Tween-20) for 20 min with constant shaking followed by running tap water for half-an-hour, then repeated rinsing with millipore water. Further operations were carried under aseptic conditions inside laminar air flow cabinet. Seeds were first washed with sterilized millipore water and then subjected to 70% (v/v) ethyl alcohol treatment for 30 s and again washed with sterilized Millipore water followed by 20% hydrogen peroxide (H_2O_2) treatment for 6 min and later rinsed 5 times with sterilized Millipore water. The seeds were individually sown in culture tubes containing half strength MS medium (Murashige and Skoog, 1962) without hormones. Seedlings of 40 days incubation were used as explants source.

Asceptic seedling explants such as shoot tip, node and

cotyledonary node were excised aseptically and placed singly in test tubes containing 15 ml of agarified MS medium fortified with 3% (w/v) sucrose with various concentrations of plant growth regulators individually or in combination to obtain multiple shoot bud induction. Elongated shoots were excised from culture and rooted on half strength MS medium containing 2% sucrose and 0.6% phytoagar with different concentrations of NAA, IAA and IBA alone and in combination. The pH of media was adjusted to 5.8 before addition of gelling agent and sterilized by autoclave at 121°C for 15 min under 15lbs pressure. All the cultures were incubated at 25 ± 2°C under a 16 h photoperiod (60 $\mu/E^2/s$ irradiance) provided by cool white fluorescent tubes.

After 30 days plants with well developed roots were thoroughly washed to remove the adhering gel and planted in 5 cm plastic cups containing sterilized soil rite mix covered with polythene bag and incubated at 25 ± 2°C for 15 days. During this period liquid quarter strength MS basal nutrient medium devoid of sucrose was provided instead of water until the new leaves developed. Later small perforations were made on the polythene bag to reduce the relative humidity. Slowly the width of the holes was increased until the relative humidity inside the polythene bag and outside the chamber come to equal. After this conformity polythene bag was removed and the pots were directly exposed to the controlled temperature (25 ± 2°C). Slowly the pots were transferred to room temperature having diffuse light. And finally plants were shifted to pots containing organic manure, garden soil and forest humus (1: 1: 1). The pots were watered at a two-day interval and were maintained in greenhouse. The survival rate was recorded one month after transfer to pots.

All experiments were repeated thrice with 15 replicates each. The data was statistically analyzed using one-way analysis of variance (ANOVA), means were compared using the DMR test at the 0.05% level of significance.

RESULTS AND DISCUSSION

Shoot regeneration efficiency of different explants was analyzed by supplementing different concentrations of cytokinin. Among three explants used for shoot induction, nodal explants responded better when compared to shoot tip and cotyledonary node. Nodal explants have been selected for micropropagation of *Caralluma sarkariae* (Sreelatha et al., 2008), *Ceropegia intermedia* (Karuppusamy et al., 2009), *Ceropegia spiralis* (Murthy et al., 2010) and *Huernia hystrix* (Amoo et al., 2009). Among the various concentrations of BAP, Kn, 2-iP, TDZ and Zeatin, Nodal explants showed better organogenic response than cotyledonary node and shoot tip explants, best response was observed on BAP 8.87μM (Table 1). Maximum 6.37 ± 0.18 shoots/explant with 4.87 ± 0.12 cm of shoot length (Figure 1A). The effectiveness of BAP for bud multiplication has been reported in *Caralluma sarkariae* (Sreelatha et al., 2008), *Ceropegia intermedia* (Karuppusamy et al., 2009), *Ceropegia spiralis* (Murthy et al., 2010), *Hemidesmus indicus* (Raghuramulu, 2001) and *Sarcostemma intermedium* (Prasad, 2004). The effect of various cytokinins from nodal explants in *Ceropegia juncea* is in the order of BAP > TDZ >2-iP > Kn > Zeatin Culturing of nodal and shoot tip explants on Kn produced less number of shoots. But TDZ was found to be more effective (Table 1). Zeatin and 2-iP were inferior to TDZ in terms of percentage of response, shoot number and shoot length. Shoots along with basal callus formation was observed in all concentrations of TDZ containing medium. BAP was found to be more effective cytokinin when compared to other cytokinins in other members of Asclepiadaceae such as *Ceropegia* spp. (Patil, 1998).

After four weeks of incubation cultures were transferred to fresh medium. The shoot number further increased in subsequent subcultures like other Asclepiadaceae members such as *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Hemidesmus indicus* (Sree Kumar *et al*., 2000) and *Holostemma ada-kodien* (Martin, 2002).

In number of cases cytokinin alone was effective for multiple shoot multiplication (Garland and Stoltz, 1981), but for this species combination of different cytokinins could improve further multiplication rate of shoots, the effect of different concentrations and combinations were studied.

Combination of BAP 8.87μM + TDZ 4.54μM in *Ceropegia juncea* produced maximum number of 20.65 ± 0.20 shoots per explant and shoot length of 3.56 ± 0.03 cm with 76% response (Table 1 and Figure 1C).

For rooting shoots of 4 to 5 cm length were excised and transferred to half strength MS medium containing auxin for *in vitro* rooting. Half strength MS medium supplemented with auxins at different concentrations showed varied effect on *in vitro* rooting (Table 2). Of the three auxins tested, IBA 4.90μM was most effective for root induction. Combination of auxins were also tested. Thin delicate roots were noticed when the medium was supplemented with NAA and IAA. Half strength MS medium fortified with IBA 4.90μM + NAA 1.27μM was effective for better rooting in *Ceropegia juncea* and produced 8.23 ± 0.14 roots per explants and root length is 4.76 ± 0.14 cm (Figure 1D). The highest mean length of longest roots 5.17±0.11cm with the root number of 4.62±0.11 was recorded in IBA 4.90μM + NAA 0.54μM media composition. Similarly IBA was rooting hormone in many Asclepiads such as *Ceropegia bulbosa, Ceropegia bulbosa* var. *lushii, Ceropegia jainii* (Patil, 1998), *Ceropegia candelabrum* (Beena et al., 2003), *Decalepis hamiltonii* (Giridhar et al., 2004), *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Hemidesmus indicus* (Sreekumar et al., 2000) and *Holostemma ada-kodien* (Martin, 2002).

The plantlets from *in vitro* conditions were transferred to pots containing soil rite mixture and covered with polythene bags to maintain high rate of relative humidity and kept in the culture room conditions initially for two weeks. The hardened plants were transferred to earthen pots and kept under shade not exposing to directsunlight. Then acclimatized plants were finally transferred to soil. Nearly 78% plants of *C. juncea* were successfully acclimatized to field conditions (Figure 1E).

In conclusion, the outlined procedure offers a potential

Table 1. Effect of different concentrations of cytokinins alone and in combination on multiple shoot induction from nodal explants of *Ceropegia juncea* cultured on MS medium.

Cytokinins (µM)					Response± (%)	No. of shoots/explants (mean±SE)	Shoot length (cm) (mean±SE)	Shoots with basal callus
BAP	Kn	TDZ	2-iP	Zeatin				
Cytokinin free MS						No response	No response	
0.44					69	2.47±0.14^{ef}	3.29±0.06^{f}	-
4.44					62	5.12±0.11^{bc}	4.02±0.07^{d}	+
8.87					79	6.37±0.18^{a}	4.07±0.12^{d}	+
13.3					73	5.89±0.21^{b}	4.48±0.03^{c}	+
22.2					58	4.79±0.16^{c}	4.34±0.08^{b}	++
	0.46				40	1.69±0.09^{g}	3.29±0.09^{f}	-
	4.65				46	1.83±0.12^{g}	4.09±0.04^{d}	-
	6.07				53	2.07±0.06^{f}	3.42±0.05^{ef}	-
	9.29				63	2.94±0.13^{e}	3.54±0.07^{e}	+
	23.2				58	2.31±0.19^{ef}	2.14±0.03^{g}	+
		0.22			46	1.23±0.11^{f}	3.96±0.03^{bc}	-
		2.27			57	2.73±0.18^{cd}	3.79±0.06^{bc}	+
		4.54			69	4.32±0.16^{b}	5.21±0.13^{a}	+
		9.29			82	5.73±0.12^{a}	4.93±0.07^{b}	++
		22.7			53	3.27±0.14^{c}	4.69±0.09^{b}	+
			0.49		44	1.36±0.15^{f}	0.92±0.03^{f}	-
			4.90		68	2.83±0.14^{cd}	2.17±0.62^{d}	-
			9.80		76	3.53±0.19^{c}	2.32±0.29^{c}	-
			14.70		69	2.34±0.16^{e}	3.19±0.23^{d}	+
			24.60		54	1.47±0.17^{f}	1.47±0.03^{e}	+
				0.46	29	1.27±0.11^{f}	0.43±0.09^{g}	+
				4.56	42	2.09±0.15^{e}	1.32±0.04^{e}	+
				9.12	53	2.54±0.13^{cd}	2.57±0.03^{b}	+
				13.6	38	1.34±0.14^{f}	1.86±0.04^{d}	+
				22.8	34	1.17±0.11^{f}	1.24±0.03^{e}	+
8.87	0.46				49	5.42±0.17^{f}	3.04±0.07^{d}	+
8.87	2.32				52	7.39±0.15^{e}	3.17±0.12^{c}	+
8.87	4.62				69	9.97±0.12^{d}	3.18±0.03^{c}	++
8.87	9.32				57	4.69±0.09^{e}	2.61±0.06^{e}	+
8.87		0.23			69	8.24±0.23^{d}	2.97±0.06^{e}	+
8.87		2.27			64	18.32±0.16^{b}	4.74±0.04^{a}	++
8.87		4.54			76	20.65±0.20^{a}	3.56±0.03^{bc}	+++
8.87		9.05			58	12.25±0.18^{c}	4.32±0.04^{b}	++
8.87			0.49		69	2.32±0.13^{g}	3.05±0.04^{d}	-
8.87			2.46		63	6.82±0.19^{e}	3.68±0.03^{bc}	+
8.87			4.90		71	5.69±0.17^{e}	3.34±0.06^{c}	+
8.87			9.80		32	3.71±0.15^{f}	2.89±0.04^{e}	+
8.87				0.46	32	2.26±1.09^{g}	1.09±0.04^{f}	-
8.87				2.28	57	4.27±2.89^{f}	2.89±0.05^{e}	+
8.87				4.56	70	6.93±2.36^{e}	2.36±0.05^{e}	++
8.87				9.12	63	4.98±1.73^{f}	1.73±0.06^{f}	+

Values represent mean ± standard error of 15 replicates per treatment in three repeated experiments. Means followed by the same letter not significantly different by the Turkey test at 0.05% probability level; + Less, ++ Moderate, +++ Profuse, NR - No response.

system for conservation of *C. juncea* from various seedling explants. In the present investigation it was observed that BAP 8.87 µM +TDZ 4.54 µM on MS medium is more effective for shoot multiplication. Half

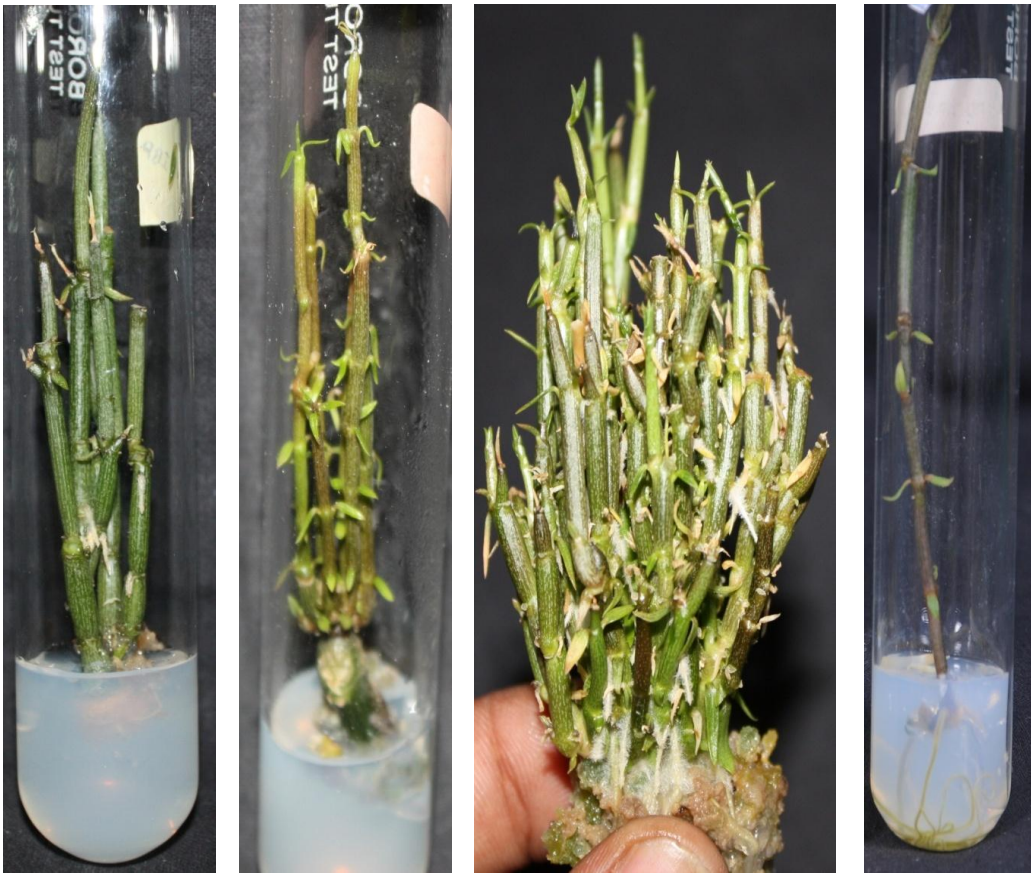

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Figure 1. *In vitro* propagation of *Ceropegia juncea* Roxb. (a)Multiple shoots from nodal explants cultured on MS medium supplemented with BAP 8.87 μM; (b) nodal explants cultured on MS medium supplemented with TDZ 4.54 μM; (c) multiple shoots form nodal explants cultured on BAP 8.87 μM + TDZ 4.54 μM; (d) *In vitro* rooting on half strength MS medium supplemented with IBA 4.90 μM + NAA 1.27μM; (e) acclimatized plant after 45 days.

Table 2. Effect of different auxin on rooting of *Ceropegia juncea* micro shoots cultured in ½MS medium after 30 days.

Auxins (µM)			Response (%)	No. of roots/explant (mean±SE)	Length (cm) of roots(mean±SE)	Basal callusing
IAA	IBA	NAA				
Auxin free ½ MS medium			-	No rooting	--	--
0.57			-	NR	NR	-
2.85			24	0.83±0.11^{e}	0.53±0.06^{e}	-
5.71			52	1.32±0.13^{d}	1.39±0.11^{d}	-
11.4			38	1.18±0.16^{d}	0.79±0.04^{e}	+
17.1			22	0.68±0.11^{e}	0.43±0.05^{e}	+
	0.49		78	3.27±0.18^{cd}	1.21±0.15^{d}	-
	2.46		76	5.17±0.09^{b}	3.62±0.12^{b}	-
	4.90		72	6.42±0.13^{a}	2.35±0.11^{c}	-
	9.80		54	5.93±0.16^{ab}	2.93±0.12^{bc}	
	14.7		42	4.36±0.11^{c}	1.43±0.06^{de}	+
		0.54	22	1.23±0.15^{d}	1.04±0.04^{e}	-
		2.69	35	1.39±0.17^{d}	3.27±0.05^{b}	-
		5.37	42	1.89±0.12^{cd}	4.42±0.03^{a}	-
		10.7	58	2.32±0.10^{d}	2.79±0.10^{bc}	+
		16.1	33	1.17±0.04^{de}	1.96±0.09^{c}	++
0.57	4.90		-	NR	NR	-
1.43	4.90		35	3.56±0.16^{d}	1.42±0.09^{c}	+
2.85	4.90		48	4.51±0.12^{c}	1.94±0.10^{a}	+
4.28	4.90		42	2.89±0.14^{d}	2.72±0.12^{d}	-
5.71	4.90		32	2.15±0.13^{e}	2.16±0.06^{d}	-
	4.90	0.54	78	4.62±0.11^{bc}	5.17±0.11^{c}	-
	4.90	1.27	63	8.23±0.12^{a}	4.76±0.14^{a}	-
	4.90	2.69	56	7.54±0.10^{b}	4.12±0.05^{b}	-
	4.90	3.96	32	2.73±0.09^{d}	3.12±0.05^{c}	+
	4.90	5.37	28	1.96±0.04^{f}	2.73±0.11^{cd}	+

Values represent mean ± standard error of 15 replicates per treatment in three repeated experiments. Means followed by the same letter not significantly different by the Turkey test at 0.05% probability level; + Less, ++ moderate, +++ profuse, NR - No response.

strength MS medium supplemented with 4.90 µM IBA + NAA 1.27 µM is best for root induction.

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