African Journal of Biotechnology Vol. 11(61), pp. 12443-12449, 31 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.4302
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

In vitro conservation of *Ceropegia elegans*, an endemic plant of South India

P. V. Krishnareddy and T. Pullaiah*

Department of Botany, Sri Krishnadevaraya University, Anantapur-515 003, Andhra Pradesh, India.

Accepted 22 March, 2012

The purpose of this study was to develop *in vitro* techniques for conserving endemic species, *Ceropegia elegans* by axillary shoot multiplication. Murashige and Skoog (MS) medium with kinetin (Kn) 23.20 µM + indole-3-acetic acid (IAA) 5.71 µM was the best for axillary bud proliferation inducing a mean of 7.11 ± 0.07 shoots per node. Shoots developed were rooted best on half strength MS medium with 4.90 µM indole-3-butyric acid (IBA). Plantlets established in pots exhibited 72% survival rate.

Key words: *Ceropegia elegans*, endemic species, micropropagation.

INTRODUCTION

The genus *Ceropegia* L. belongs to the sub family Asclepiadoidae under the family Apocynaceae in APG III Classification. *Ceropegia* is the largest genus in the tribe Ceropegieae with more than 200 species distributed in the old World ranging from the Spanish Canary Islands (Anonymous, 1992) in the west, through central, southern and northern Africa, Madagascar, Arabia, India, southeast Asia to northern Australia in the east (Bruyns, 2003). In India, there are about 50 species and four varieties that have been described so far, of which 33 are endemic to the subcontinent, which are distributed mainly in Western Ghats and most of them are enlisted under endangered category (Nayar and Sastry, 1983, 1988). *Ceropegia* shows a variety of growth habits, such as non-succulent twiners, leafless succulent twiners, erect herbs and rarely subshrubs. The tuberous roots of many *Ceropegia* species are edible (Mabberley, 1987) and many others are of medicinal value (Jain and Defilips, 1991). The root tubers contain starch, sugar, gum, albuminoids, fats and crude fiber and are valuable constituents in many traditional medicinal systems in India (Kirtikar and Basu, 1935). Active principle of tuberous roots contains an alkaloid ceropegine which is active against diarrhoea and dysentery (Nadkarni, 1976).

Ceropegia elegans is endemic to India (Karnataka, Kerala and Tamilnadu) and Srilanka. Scanty population of this species is distributed in edges of moist deciduous forests in Tamil Nadu (Jagtap and Singh, 1999). Propagation through seed is hampered due to less viability rate, poor germination of seeds, and scanty and delayed rooting of seedlings. Seed-derived progenies are not true-to-type due to cross-pollination. Vegetative propagation by root tubers and stem cuttings is very arduous. The rapid deforestation in the Western Ghats has depleted the wild population of *C. elegans* and hampered its natural regeneration potential. The natural regeneration as well as conventional propagation of this plant has also been attributed to several other factors (Nayar and Sastry, 1988). Conventional propagation of *C. elegans* is through seeds and stem cuttings. Lack of proper cultivation practice, low number of seed formation, destruction of plant's habitat and its removal is leading to a progressive devastation of the species. Consequently, it has been enlisted as a rare species. Large scale propagation is a prerequisite for effective conservation of this endangered species. Axillary bud multiplication is an effective alternative for clonal propagation. Until now, no *in vitro* studies have been reported on this endangered species.

*Corresponding author. E-mail: pullaiah.thammineni@gmail.com.

Abbreviations: IAA, Indole-3-acetic acid; **IBA,** indole-3-butyric acid; **Kn,** kinetin; **MS,** Murashige and Skoog (1962) medium; **NAA,** naphthalene acetic acid; **TDZ,** thidiazuron; **2-iP,** 2-isopentyladenine; **2,4-D,** 2,4-dichlorophenoxy acetic acid.

MATERIALS AND METHODS

Plant material and surface sterilization

Plants of *C. elegans* were collected from Sirumalai hills of Eastern Ghats of Tamil Nadu (Figure 1A) and grown in earthen pots under green house condition in Botanical Garden at Sri Krishnadevaraya University, Anantapur. The young shoots with six internodes were collected from the garden grown plants and washed with running tap water for 15 min. The nodes were cut (4 cm) separately and they were washed with Tween 20 (Merck, India) detergent solution (5% v/v) for 15 min. The surface sterilization of explants was followed by rinsing with sterile distilled water 3 to 4 times to remove trace of detergent, rinsed in 80% ethanol for 30 s and finally treated with mercuric chloride (0.1% w/v) ($HgCl_2$) for 6 min. To remove every trace of the sterilant, the shoot material was then washed with sterile distilled water at least 4 to 5 times. The shoot segments containing nodes (1 to1.5 cm) were prepared from the surface sterilized shoots and used as explants. The whole process was carried out under the laminar air flow chamber.

Culture media

For culture media, MS medium (Murashige and Skoog, 1962) was used for shoot bud proliferation from nodal explants of *C. elegans*. Axillary bud induction and multiplication of shoots were examined using MS medium variously supplemented with benzylamino purine (BAP), Kn, thidiazuron (TDZ), 2-isopentyladenine (2-iP) and zeatin alone and in combination with various auxins. For rooting, half strength MS medium fortified with various concentrations of auxins like IAA, IBA and naphthalene acetic acid (NAA) were examined.

Culture conditions

MS medium was fortified with 30 g/L sucrose (Qualigens, India) and gelled with 0.8% (w/v) agar (Sd-fine chemicals, India), 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 for 20 min. All the cultures were incubated under 50 µMol M^{-2} S^{-1} light provided by cool white fluorescent tubes for a photoperiod of 16 h at 25 ± 2°C. Fifteen cultures were raised for each treatment and all the experiments were performed three times.

Effect of cytokinins on shoot proliferation

MS basal media containing different cytokinins (BAP, Kn, TDZ, 2-iP and zeatin) with different concentrations (0.5, 1, 2, 3 and 5 mg/L) either alone and combination with auxins like IBA 2,4-D, IAA and NAA were investigated for their effects on shoot multiplication from nodal explants.

Shoots 5 cm in height were separated individually and transferred to rooting half strength MS medium containing different concentrations of IAA, IBA and NAA. The cultures were incubated under 16 h photoperiod for 30 days until the microshoots developed roots. Then, the rooting frequency was measured.

Acclimatization and transplantation of plantlets

The rooted plantlets were removed from the culture tubes and washed with tap water to remove traces of agar. Then, the plantlets were planted into plastic cups containing autoclaved soilrite mixture (Keltch energies Ltd, Bangalore). The plastic cups were covered with transparent polythene cover to maintain humidity until the development of new leaves for 10 days. Later on, the percent of humidity was decreased by pricking the polythene cover with the needle. After 20 days, the plastic cups were transferred to green house and polythene covers were removed. Quarter strength MS salt solution free of sucrose was poured with 2 days intervals up to 20 days of hardening and followed by pouring of tap water. Hardened plants were transferred to pots containing mixture of garden soil and forest humus (1:1 ratio). The pots were watered with two days interval under green house condition. After 60 days, the frequency of survival was calculated.

Statistical analyses

Data were measured after 30 and 40 days for shoot multiplication and rooting, respectively. Mean values with the same superscript were not significantly different (p = 0.05%) according to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of cytokinins on shoot multiplication

The morphogenic response of nodal explants cultured on MS basal medium showed that axillary nodes remained green and fresh but failed to sprout. However, the multiplication rate and shoot number were high in media fortified with various concentrations of cytokinins (Table 1). The percentage of response varied with the type of growth regulator and its concentration. All the concentrations of BAP (2.22, 4.44, 8.87, 13.37 and 22.19 µM), Kn (2.32, 4.65, 9.29. 13.94 and 23.2 µM), TDZ (0.22, 2.27, 4.54, 9.27 and 22.7 µM), 2-iP (2.46, 4.92, 9.84, 14.76 and 24.61 µM) and zeatin (2.28, 4.56, 9.12, 13.68 and 22.81 µM) alone facilitated shoot bud differentiation. Swelling of the dormant axillary bud took place within ten days and then, differentiation into multiple shoots occurred after four weeks.

Kn was the most efficient cytokinin for axillary bud initiation and subsequent proliferation of shoots (Table 1). Among the various concentration of Kn used for shoot induction, better response (85%) was observed with 23.2 µM with 4.96 ± 0.15 shoots/explant and 6.42 ± 0.03 cm of shoot length (Table 1 and Figure 1C). As Kn concentration increased, shoot number decreased significantly. Better results were also obtained with BAP 13.37 µM alone with maximum shoot sprouting frequency of 80% and 3.49 ± 0.21 shoots/explant and attained a length of 3.55 ± 0.03 cm (Table 1 and Figure 1B). TDZ 0.5 mg/l induced a mean 1.62 ± 0.11 shoots/explant and shoot length of 4.72 ± 0.04 cm in 2.27 µM. 2-iP resulted to maximum 66% of shoot sprouting frequency and 2.32 ± 0.09 shoots/explant and 5.10 ± 0.04 cm shoot length at 14.70 µM. Better response (52%) was observed with zeatin 13.6 µM that induced 1.96 ± 0.14 shoots/explant with 3.58 ± 0.15 cm shoot length. All cultures containing different concentrations of zeatin induced a mean of single vitrified shoot invariably (Table 1). A callus was occasionally formed at the base of the explants retarding axillary bud formation and the subsequent growth of

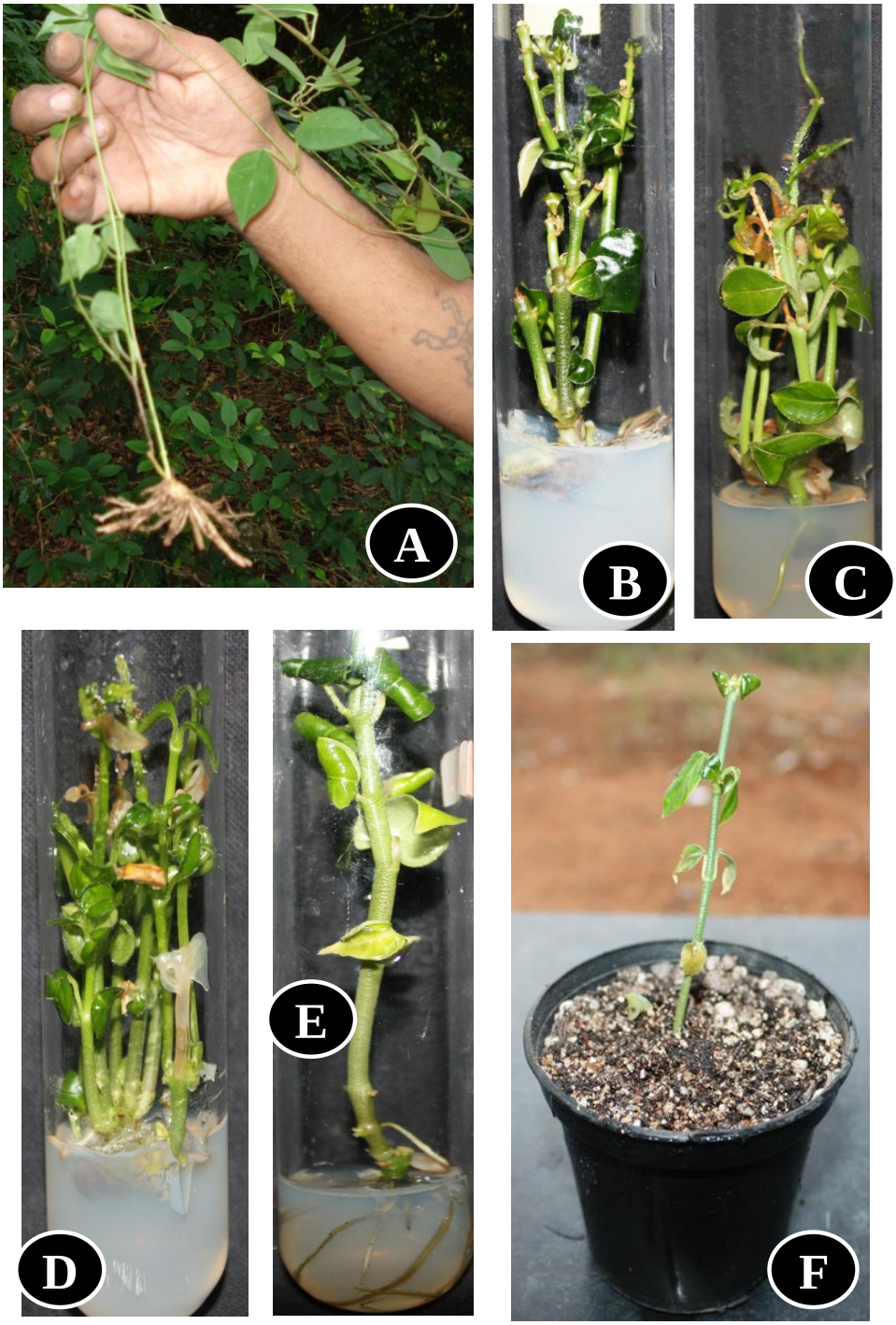

Figure 1. *In vitro* propagation of *C. elegans*. (A) Natural habit; (B) multiple shoot induction with BAP 13.37 µM; (C) multiple shoot induction with Kn 23.20 µM; (D) multiple shoot induction on MS medium supplemented with Kn 23.20 µM + IAA 5.71 µM; (E) *in vitro* rooting on ½ MS + IBA 4.90 µm after 30 days; (F) acclimatized plant growing in plastic cup 45 days after transfer to soil.

shoots. Therefore, precautions were taken to remove such callus growth while sub culturing.

Nodal segments containing axillary buds have quiescent or active meristems depending upon the physiological stage of the plant. These buds have the potential to develop into complete plantlets. The conventional method used for the vegetative propagation of stem cuttings relies on the axillary bud taking over the

Table 1. Effect of different concentrations of cytokinins on multiple shoot induction from nodal explants of *C. elegans* cultured on MS medium with 3% sucrose.

Cytokinin (µM)					Response	Number of shoots/explants	Shoot length (cm)	Shoots with
BAP	Kn	TDZ	2-iP	Zeatin	(%)	(Mean±SE)	(Mean±SE)	basal callus
Cytokinin free MS						No response	No response	
0.44					42	1.47±0.13^{f}	2.82±0.06^{g}	-
4.44					64	2.32±0.10^{e}	3.61±0.03^{ef}	-
8.87					76	2.87±0.15^{d}	3.72±0.05^{ef}	-
13.3					80	3.49±0.21^{b}	3.55±0.03^{f}	+
22.2					63	1.97±0.16^{e}	4.14±0.05^{e}	++
	0.46				28	1.26±0.07^{f}	5.34±0.06^{d}	-
	4.65				32	1.34±0.06^{f}	6.03±0.04^{b}	-
	6.07				42	2.46±0.13^{e}	5.69±0.04^{cd}	-
	9.29				72	3.27±0.16^{c}	5.08±0.05^{d}	+
	23.2				85	4.96±0.15^{a}	6.42±0.03^{a}	++
		0.22			28	1.00±0.07^{f}	5.07±0.06^{b}	-
		2.27			36	1.28±0.12^{d}	4.69±0.03^{c}	-
		4.54			57	1.62±0.11^{d}	4.72±0.04^{c}	++
		9.29			48	1.36±0.12^{de}	5.12±0.05^{ab}	+
		22.7			38	1.28±0.14^{cd}	5.43±0.03^{a}	+
			0.49		40	1.12±0.12^{f}	3.89±0.09^{f}	+
			4.90		45	1.00±0.07^{f}	3.95±0.10^{ef}	-
			9.80		54	1.69±0.11^{d}	4.15±0.05^{e}	-
			14.70		66	2.32±0.09^{a}	5.10±0.04^{ab}	+
			24.60		60	2.12±0.04^{b}	4.95±0.08^{b}	++
				0.46	30	1.00±0.03^{f}	2.55±0.08^{g}	-
				4.56	35	1.11±0.06^{f}	2.95±0.04^{g}	-
				9.12	38	1.89±0.09^{d}	3.25±0.06^{f}	-
				13.6	52	1.96±0.14^{c}	3.58±0.15^{ef}	+
				22.8	47	1.79±0.10^{d}	3.49±0.18^{ef}	++

Values represent mean ± standard error of 15 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the Tukey test at 0.05% probability level; + less, ++ moderate, +++ profuse, NR- no response. BAP, Benzylamino purine; Kn, kinetin; TDZ, thidiazuron; 2-iP, 2-isopentyl adenine.

function of the main shoot in the absence of a terminal bud. In nature, these buds remain dormant for a specific period depending on the growth pattern of the plant. However, using tissue culture, the rate of shoot multiplication can be greatly enhanced by performing axillary bud culture in a nutrient medium containing suitable cytokinin or cytokinin and auxin combinations. Due to continuous availability of cytokinin, shoots formed by the bud already present in the explant (nodal segment) develop into axillary buds, which then grow directly into shoots. Multiple shoot formation following the *in vitro* culture of nodal segments has proved to be an effective method of mass multiplication.

Kn was effective than other cytokinins in bud multiplication in *Cryptolepis buchanani* (Prasad et al., 2004), *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Hoya wightii* spp. *palniensis* (Lakshmi et al., 2010) and *Wattakaka volubilis* (Chakradhar and Pullaiah, 2006).

Effect of cytokinin with auxin on shoot multiplication

The efficiency of the optimal concentration of BAP and Kn with various auxins (NAA, IAA, 2,4-D and IBA) was also valuated for multiple shoot induction (Table 2). The highest percentage of cultures that regenerated shoots was 86% on MS medium supplemented with Kn 23.20 µM + IAA 5.71 µM (Table 2 and Figure 1D) with highest mean number of shoots per explant (7.11 ± 0.07). The highest mean length of 2.79 ± 0.01 cm with 5.42 ± 0.04 shoots/explant was recorded at BAP 13.3 + IAA 5.71 µM.

Among the various combinations of Kn and IBA used, the highest shoot regeneration frequency (72%) and number of shoots per explant (2.41 ± 0.10) together withthe maximum shoot length (4.58 ± 0.11 cm) were recorded on MS medium supplemented with Kn 23.20 µM + IBA 4.90 µM after six weeks of inoculation (Table 2). The elevated concentration of IBA, 4.90 µM resulted in

Table 2. Effect of different combinations of BAP and Kn with NAA, IAA and IBA on shoot regeneration of mature nodal explants of *C. elegans.*

Cytokinin (µM)					Response (%)	Number of shoots/explants (Mean±SE)	Shoot length (cm) (Mean±SE)	Shoots with basal callus
BAP	IBA	2,4-D	IAA	NAA				
13.3	2.46				47	2.26±0.12^{cd}	5.15±0.05^{b}	+
13.3	4.90				68	2.37±0.13^{c}	4.86±0.06^{d}	++
13.3		2.26			61	1.40±0.13^{e}	4.87±0.72^{d}	+
13.3		4.52			74	2.40±0.09^{b}	5.06±0.04^{c}	++
13.3			2.85		64	2.33±0.23^{c}	5.26±0.08^{b}	-
13.3			5.71		73	2.79±0.06^{a}	5.42±0.04^{a}	+
13.3				2.69	61	2.15±0.26^{cd}	4.54±0.07^{d}	+
13.3				5.37	54	1.30±0.18^{ef}	4.48±0.05^{e}	+
Kn	**IBA**	**2,4-D**	**IAA**	**NAA**				
23.20	2.46				59	2.30±0.09^{e}	5.06±0.09^{a}	+
23.20	4.90				72	2.41±0.10^{e}	4.58±0.11^{b}	++
23.20		2.26			54	2.59±0.19^{e}	4.07±0.74^{d}	-
23.20		4.52			69	3.61±0.09^{d}	3.90±0.11^{d}	+
23.20			2.85		74	5.57±0.13^{c}	3.29±0.03^{f}	-
23.20			5.71		86	7.11±0.07^{a}	4.21±0.09^{e}	-
23.20				2.69	64	6.72±0.21^{b}	4.28±0.06^{c}	-
23.20				5.37	53	3.54±0.10^{d}	3.36±0.05^{e}	+

Values represent mean ± standard error of 15 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the Tukey test at 0.05% probability level; + less, ++ moderate, +++ profuse, NR- no response. BAP, Benzylamino purine; IBA, indole-3-butyric acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indole-3-acetic acid; NAA, naphthalene acetic acid; Kn, kinetin.

little callusing at the cut end thus, reducing the percent shoot regeneration and the number of shoots per explant.

Kn 23.20 µM + NAA 2.69µM produced a maximum of 6.72 ± 0.21 shoots/explant with 4.28 ± 0.06 cm shoot length with 64% shoot sprouting frequency (Table 2). Among the BAP and NAA combinations, the maximum frequency (70%) of shoot bud formation and the highest number of shoots (2.0 ± 0.28) per explant were obtained on MS medium containing BAP 13.3 µM with 2,4-D 4.9 µM. Combination of 2,4-D with cytokinins resulted to maximum shoots at Kn 23.20 µM + 2,4-D 4.52 µM and produced 3.61 ± 0.09 shoots/explant with shoot length of 3.90 ± 0.11 cm (Table 2).

A low concentration of auxin together with a high concentration of cytokinin was most promising for the induction and multiplication of shoots in *C. elegans* and MS medium supplemented with Kn 23.20 µM in combination with IAA 5.71 µM proved most effective for direct shoot regeneration. The synergistic effect of Kn in combination with an auxin has been demonstrated in many medicinal plants such as *Decalepis hamiltonii* (Giridhar et al., 2004), *Hedychium spicatum* (Koul et al., 2005), *Ocimum gratissimum* (Gopi et al., 2006) and *Salvadora persica* (Mathur et al., 2002). In accordance with these reports, the present study also exemplifies the positive modification of shoot induction efficacy obtained by employing a low concentration of auxin in combination with a cytokinin.

Rooting

In vitro shoots of nodal explants of *C. elegans* cultured on MS medium supplemented with auxins such as IAA, IBA and NAA, 0.1 to 3 mg/l induced rooting (Table 3). Maximum number of 5.23 ± 0.11 roots/shoot with 3.70 ± 0.11 root length was achieved with IBA 4.90 µM (Figure 1E). IAA 5.71 µM showed maximum of 56% response with 1.37 ± 0.06 roots/shoot with 2.11 ± 0.14 cm root length. NAA 10.7 µM showed maximum response of 63% with 2.13 ± 0.09 roots/shoot and 4.10 ± 0.10cm in length (Table 3). Increase in concentration of NAA decreases the root number and length, higher concentrations of NAA resulted in callusing.

Number of roots formed, root length with IBA treatments was far better than that of NAA and IAA (Table 3). Rooting capacity of these hormones is IBA > NAA > IAA. The reduced survival in higher concentrations of auxin treatments may be due to poor vascular connection of the root with the stem because of the intervention of callus. The positive response of rooting in the present study is similar to observation in other Asclepiads and *Caralluma*

Table 3. Effect of different auxins on rooting of *C. elegans* microshoots cultured on ½ MS medium after 40 days.

Auxin	Concentration (µM)	Number of roots/shoot (Mean±SE)	Length (cm) of roots (Mean±SE)	Basal callusing
	Free medium	No rooting		
IAA	0.57	0.92± 0.19^{f}	1.43± 0.09^{f}	-
	2.85	1.00±0.11^{f}	1.68±0.05^{e}	-
	5.71	1.37±0.06^{e}	2.11±0.14^{e}	-
	11.4	1.18±0.11^{e}	1.64±0.05^{e}	+
	17.1	1.20±0.12^{e}	1.39±0.04^{e}	++
IBA	0.49	2.37±0.14^{cd}	3.21±0.11^{d}	-
	2.46	3.10±0.07^{c}	5.31±0.09^{a}	-
	4.90	5.23±0.11^{a}	3.70±0.11^{c}	-
	9.80	4.25±0.13^{b}	4.15±0.10^{bc}	+
	14.7	3.14±0.10^{c}	3.29±0.07^{d}	++
NAA	0.54	1.11±0.13^{f}	1.84±0.05^{f}	-
	2.69	1.62±0.14^{de}	3.27±0.06^{d}	+
	5.37	1.41±0.12^{e}	4.35±0.07^{b}	++
	10.7	2.13±0.09^{d}	4.10±0.10^{bc}	++
	16.1	1.34±0.08^{e}	2.07±0.13^{e}	++

Values represent mean ± standard error of 15 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the Tukey test at 0.05% probability level; + less, ++ moderate, +++ profuse, NR- no response. IAA, Indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, naphthalene acetic acid.

spp. (Aruna et al., 2009), *Caralluma edulis* (Rathore et al., 2008), *Ceropegia candelabrum* (Beena and Martin, 2003), *Ceropegia intermedia* (Karuppusamy et al., 2009), *Cryptolepis buchanani* (Prasad et al., 2004), *Decalapis arayalpatra* (Gangaprasad et al., 2005; Sudha et al., 2005), *Hoya wightii* ssp. *palniensis* (Lakshmi et al., 2010), *Leptadenia reticulata* (Rathore et al., 2010) and *Pergularia daemia* (Kiranmai et al., 2008).

Acclimatization

During hardening, the gradual exposure of plants to conditions outside the polythene covering helped to conserve and develop a proper balance of relative humidity and thereby increased the rate of survival.

The well developed plantlets were transferred to the cups containing autoclaved soilrite mixture for hardening at diffuse light (16/8 h photoperiod). The potted plantlets were covered with polythene membrane to ensure high humidity and watered every 2 days with quarter strength MS salt solution free of sucrose. Later on, the percent of humidity was decreased by pricking the polythene cover with the needle. On the 20th day, the plants were transferred to the pots. The survival rate of plantlets was 72% (Figure 1F). The plantlets were successfully adapted to the natural environment and exhibited similarity with mother plants.

Conclusion

We have established a protocol for direct *in vitro* regeneration system for conservation and micro-propagation of *C. elegans* from nodal explants. MS medium fortified with Kn 23.20 µM + IAA 5.71 µM is the best for shoot proliferation, half strength MS basal medium supplemented with IBA (4.90 µM) is the best for root induction.

ACKNOWLEDGEMENT

Authors extend their gratitude to the University Grants Commission, New Delhi for financial assistance in the form of Major Research Project to T. Pullaiah.

REFERENCES

Anonymous (1992). *Ceropegia* Linn. (Asclepiadaceae) In: The Wealth of India 3. New Delhi, CSIR. pp. 448-449.

Aruna V, Kiranmai C, Karuppusamy S, Pullaiah T (2009). Micropropagation of three varieties of *Caralluma adscendens* via nodal explants. J. Plant Biochem. Biotech. 18:121-123.

Beena MR, Martin KP (2003). *In vitro* propagation of the rare medicinal plant *Ceropegia candelabrum* L. through somatic embryogenesis. *In vitro* Cell Dev. Biol. Plant 39:510-513.

Bruyns PV (2003). Three new succulent species of Apocynaceae (Asclepiadaceae) form southern Africa. Kew Bull. 58:427-435.

Chakradhar T, Pullaiah T (2006). Effect of explant source on axillary shoot multiplication during micropropagation of a rare medicinal plant– *Wattakaka volubilis* (L.f.) Stapf. J. Plant Biochem. Biotech. 15:43-45.

Duncan DB (1955). Multiple range and multiple *F*-test. Biometrics 11:1-42.

Gangaprasad A, Decruse SW, Seeni S, Nair GM (2005). Micropropagation and ecorestoration of *Decalepis arayalpathra* (Joseph & Chandra.) Venter – An endemic and endangered ethnomecicinal plant of Western Ghats. Indian J. Biotech. 4:265-270.

Giridhar P, Vinodkumar D, Obul Reddy B, Rajasekharan T (2004). Somatic embryogenesis, organogenesis and regeneration from leaf callus culture of *Decalepis hamiltonii* Wight & Arn., an endangered shrub. *In vitro* cell. Dev. Biol. Plant. 40:567-571.

Gopi C, Sekhar YN, Ponmurugan P (2006). *In vitro* multiplication of *Ocimum gratissimum* L. through direct regeneration. Afr. J. Biotechnol. 5:723-726.

Jagtap AP, Singh N (1999). Asclepiadaceae and Periplocaceae. fascicles of flora of India, botanical survey of India, Kolkata. Fascicle 24:211- 241.

Jain SK, Defillips RA (1991). Asclepiadaceae. In: Algonae, M. I. (ed.).Medicinal plants of India. Vol. 1. Reference Publications Inc., Michigan, USA. pp. 144-152.

Karuppusamy S, Kiranmai C, Aruna V, Pullaiah T (2009). *In vitro* conservation of *Ceropegia intermedia* - an endemic plant of South Inida. Afr. J. Biotechnol. 8(17):4052-4057.

Kiranmai C, Aruna V, Karuppusamy S, Pullaiah T (2008). Callus culture and plant regeneration from seedling explants of *Pergularia daemia* (Forsk.) Chiov. J. Plant Bochem. Biotechnol. 7(1):99-101.

Kirtikar KR, Basu BD (1935). Indian medicinal plants Vol. 3. Bishen Singh Mahendrapal Singh, New Delhi, India. p. 1638.

Komalavalli, N, Rao, MV (2000). *In vitro* micropropagation of *Gymnema sylvestre* – A multipurpose medicinal plant. Plant Cell Tissue Org. Cult. 61:97-105.

Koul S, Raina V, Sharma SK (2005). Conservation and propagation of high altitude medicinal and aromatic plant: *Hedychium spicatum.* J. Plant Biochem. Biotechnol.14:57-59.

Lakshmi SR, Benjamin JHF, Kumar ST, Murthy GVS, Rao MV (2010), *In vitro* propagation of *Hoya wightii* sp. *palniensis* Mathew KT, a highly vulnerable and endemic species of Western Ghats of Tamil Nadu, India. Afr. J. Biotechnol. 9(5):620-627.

Mabberley DJ (1987). The plant book. Cambridge University Press, Cambridge, p. 114.

Mathur S, Shekhawat GS, Batra A (2002). Micropropagation of *Salvadora persica* Linn. via cotyledonary node. Indian J. Biotechnol. 1:197-200.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant 15:473-497.

Nadkarni KM (1976). Indian Materia Medica Vol. 1. Popular Prakashan, Bombay, India, pp. 303-304.

Nayar MP, Sastry ARK (1983). Red data book of Indian plants, Vol. 1. botanical survey of India. Calcutta, p. 58.

Nayar MP, Sastry ARK (1988). Red data book of Indian plants, Vol. 2. botanical survey of India, Howrah, p. 23.

Prasad PJN, Chakradhar T, Pullaiah T (2004). Micropropagation of *Cryptolepis buchanani* Roem. & Schult. Taiwania, 49:57-69.

Rathore MS, Dagla HR, Singh M, Shekhawat NS (2008). Rational development of *in vitro* methods for conservation, propagation and characterization of *Caralluma edulis*. World J. Agric. Sci. 4(1):121-124.

Rathore MS, Rathore MS, Shekhawat NS (2010). *Ex vivo* implications of phytohormones on various *in vitro* responses in *Leptadenia reticulata* (Retz.) Wight & Arn. an endangered plant. J. Environ. Exp. Bot. doi: 10.1016

Sudha CG, Krishnan PN, Pushpangadan P, Seeni S (2005). *In vitro* propagation of *Decalepis arayalpathra,* a critically ethnomedicinal plant. *In vitro* Cell Dev. Biol. Plant. 41:648-654.