Taiwania, **49**(1): 57-65, 2004

Micropropagation of *Cryptolepis buchanani* Roem. & Schult.

P. J. N. Prasad[1], T. Chakradhar[1] and T. Pullaiah[1,2]

(Manuscript received 17 December, 2003; accepted 20 February, 2004)

ABSTRACT: *In vitro* regeneration protocol was developed for multiplication of *Cryptolepis buchanani* by using shoot tip, cotyledonary node and nodal explants derived from seedlings grown *in vitro*. The best response was achieved with nodal explants. Cultures were established placing the nodal explants on Murashige and Skoog's (MS; 1962) medium supplemented with various cytokinins singly or in combination with auxin and gibberellin. Of the various cytokinins used singly or in combination with auxins, 6-Benzyl aminopurine (BAP) was found to be most effective for shoot proliferation. The maximum number of shoots (12.5 to 13.0 shoots / explant with a shoot length of 4.5 to 5.0 cm) were produced on MS medium fortified with BAP 2mg/L, Kinetin (KN) 0.1mg/L, Napthalene acetic acid (NAA) 0.05 mg/L and gibberellic acid (GA_3) 0.05 mg/L with 60% response. Individual shoots (grown on shoot proliferation medium) were rooted on MS medium supplemented with various auxins—Indole acetic acid (IAA), Indole butyric acid (IBA), NAA—singly or in combination. Of these IBA 1 mg/L resulted in higher number of microshoots (80%) to form roots (about 6.5 to 7.0 roots / shoot and the root length of 4.0 to 4.5 cm). The *in vitro* raised plantlets were acclimatized successfully to pots containing a mixture of autoclaved peatmoss and compost in 1:1 ratio.

KEY WORDS: ***Cryptolepis buchanani*, Micropropagation, Acclimatization.**

INTRODUCTION

Cryptolepis buchanani Roem. & Schult., commonly called as "Adavipalatiga", belongs to Asclepiadaceae. It is a woody twiner found in Eastern Ghats and is widely used as demulcent, diaphoretic, diuretic, cure for paralysis (Datta *et al.*, 1978), rickets in children (Chopra *et al.*, 1956); combined with *Euphorbia microphylla* is given to women as galactogogue when milk supply is deficient or ills (Venkateswara *et al.*, 1989). The roots and leaves of the plant are reported to consist of cardiac glycosides and anticarcinogens (Venkateswara *et al.*, 1987). Sarverogenin and isosarverogenins of the plant (Purushothaman *et al.*, 1988) possess a potent cytotoxic activities against tumour cells and shows antibacterial and antiparasitic properties (Deepak *et al.*, 1999; Dassonneville *et al.*, 2001). The plant is categorized as weeds, over looking all its medicinal importance. Hence, the systematic propagation of this medicinally important species was undertaken for the first time through micropropagation method. An important step in the development of a transformation system for plant species is to establish a facile regeneration procedure. Several other species of Asclepiadaceae have been manipulated in culture using nodal segments on MS full-strength medium fortified with various plant growth regulator combination. These included, *Asclepias currassavica* (Pramanik and Datta, 1986), *Tylophora indica* (Chattopadhyay *et al.*, 1992), *Hemidesmus indicus* (Sharma and Yelne, 1995), *Gymnema elegans* (Komalavalli and Rao, 1997), *Holostemma annulare* (Sudha *et al.*, 1998), *Decalepis hamiltonii* (Bais *et al.*, 2000; Anitha and Pullaiah, 2002), *Cynanchum callialatum* (Raghu Ramulu *et al.*, 2002), and *Holostemma ada-kodien* (Martin, 2002). There have been no reports of a regeneration system for *Cryptolepis buchanani*. An efficient procedure for the induction of shoot proliferation from various seedling explants, and conditions required for root proliferation are described here.

1. Department of Botany, Sri Krishnadevaraya University, Anantapur-515003, Andhra Pradesh, India.
2. Corresponding author. Email: pullaiaht@yahoo.co.in

MATERIALS AND METHODS

Matured follicles of *Cryptolepis buchanani* were collected during April year from the natural stands of Bairluti in Nallamalai reserve forest, Eastern Ghats, Kurnool district, Andhra Pradesh, India. Seeds collected from shade dried follicles, taken in 100 ml clean Erlenmeyer flasks and washed with a detergent (1% Tween–20) for 30 minutes and then washed in running tap water for another 30 minutes. Further operations were carried under aseptic conditions inside laminar chamber. Seeds were first washed with sterilized distilled water and then subjected to 70% ethyl alcohol treatment for 60 seconds and again washed with sterilized distilled water. It is followed by 15% v/v hydrogen peroxide treatment for 3 minutes and later rinsed 4-5 times with sterilized distilled water. Seeds were soaked for 24 hour in sterilized double distilled water and then placed on quarter strength Murashige and Skoog's (MS) medium (Murashige and Skoog, 1962) without hormones and incubated at 25 ± 2^{o}C under 16 hr photoperiod with light intensity of 2000 lux. Seedlings of 30-day incubation are used as an explant source. Seedling explants like shoot tip, cotyledonary node and node were excised aseptically and placed singly in test tubes (150 x 25 mm Borosil, India) containing 20 ml of agarified MS medium fortified with 20 g/L sucrose and various plant growth regulators like 6-Benzyl aminopurine (BAP), Kinetin (KN), Thidiazuron (TDZ), Zeatin (Z), Indole acetic acid (IAA), 2,4-Dichloro phenoxyacetic acid (2,4-D), Napthalene acetic acid (NAA) and gibberellic acid (GA_3) at different concentrations individually or in combination for shoot proliferation. The regenerated microshoots were rooted on half strength MS medium supplemented with auxins like IAA, IBA, 2,4-D and NAA and different concentration singly or in combination. Rooted plantlets were washed thoroughly to remove all the traces of agar and transferred to pots containing sterilized peatmoss and compost (1:1). Pots were first placed in culture room at 25 ± 2^{o}C and then slowly acclimatized with normal room temperature and later to the field climate. All experiments were repeated twice with 15 replicates each time. Observations were recorded for every week after the initiation of the experiment.

RESULTS AND DISCUSSION

Amongst the various concentrations of BAP, KN, TDZ, and Z in single, explants responded best and produced increased number of shoots (4.50 ± 0.71) in MS medium supplemented with BAP (2 mg/L) (Fig. 1). In the present study it is evident that higher concentrations of cytokinins resulted in profuse callusing and reduction of shoot multiplication. Increasing of the concentration of BAP at more than 2 mg/L, TDZ and Z above 0.1 mg/L in the medium decreased the shoot number with retardation of shoot growth. Nodal segments cultured on medium fortified with either TDZ or Z of more than 0.05 mg/L developed callus having sectors of green patches from the base of shoots. However, shoot differentiation from the callus did not occur even after subculture or transferred to medium with BAP. Shoots developed on TDZ/Z containing medium showed stunted growth after 40-45 days of incubation. Leaves of plants cultured on TDZ turned dark green and thick, however, such effect was not found with Z treatments. The stunted shoot formation from nodal explants on TDZ supplemented medium has been reported by Preece and Imel (1991), Koroch *et al.* (1997) and Tavares (1996). One important observation noticed after the shoots raised on KN was the senescence of young shoots after an incubation period of 30 days. The best efficiency of BAP for maximum shoot induction was also observed in several other

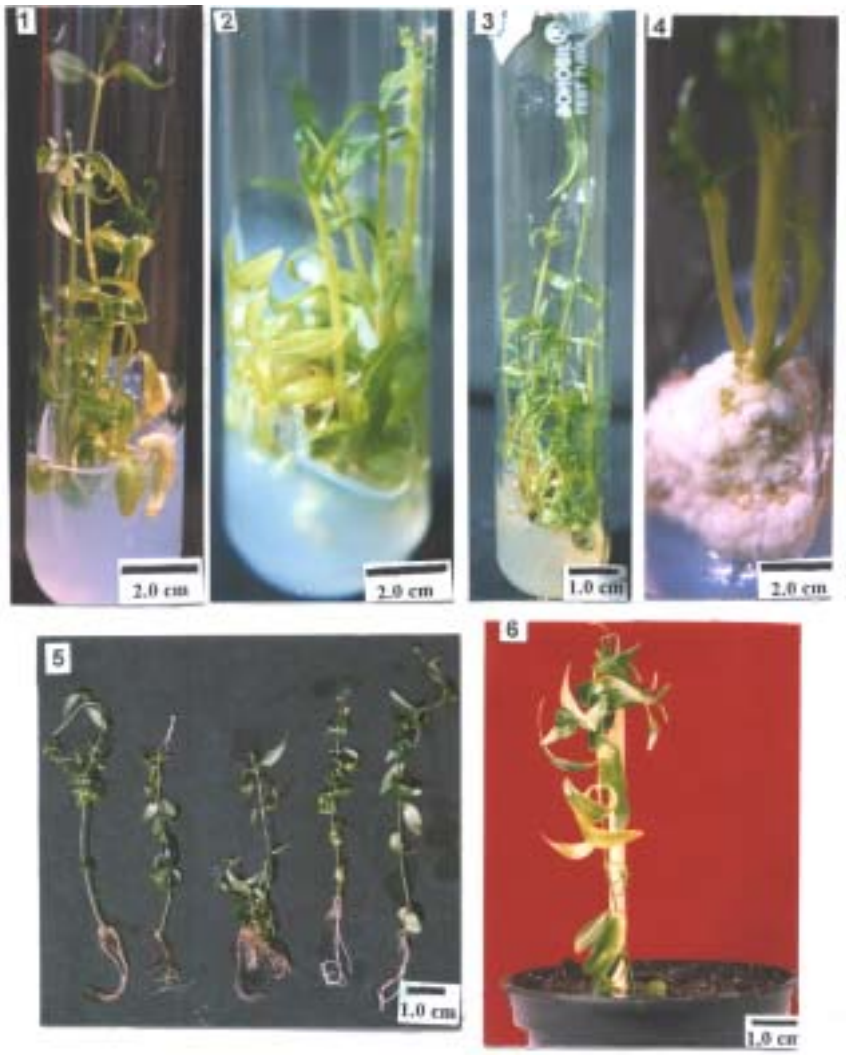

Fig. 1. Multiple shoots formed from nodal explants with BAP 2 mg/L Fig. 2. Effect of BAP 2 mg/L + KN 0.1 mg/L + NAA 0.05 mg/L on nodal explants in the induction of multiple shoots. Fig. 3. Multiple shoot induction from nodal explants with BAP 2 mg/L + KN 0.1 mg/L + NAA 0.05 mg/L + GA_3 0.05 mg/L. Fig. 4. Effect of BAP 2 mg/L + KN 0.10 mg/L + NAA 0.10 mg/L + GA_3 0.10 mg/L on shoot regeneration from nodal explants. Fig. 5. Rooting in regenerated shoots on MS medium containing ½ salts with various concentrations of plant growth regulators singly or in combinations. Fig. 6. *In vitro* plantlet potted in peatmoss and compost (1:1) after hardening. (Photographs of Fig. 1 to Fig. 5 had taken after 8 weeks of incubation from the date of inoculation).

Asclepiadaceae members such as *Ceropegia bulbosa* (Patil, 1998), *Hemidesmus indicus* (Raghu Ramulu *et al.*, 2002) and *Decalepis hamiltonii* (Anitha and Pullaiah, 2002).

Shoot regeneration efficiency of nodal explants was analyzed by supplementing different cytokinin combination and cytokinin + auxin combinations (Table 1). Among the treatments, medium fortified with BAP 2 mg/L + KN 0.1 mg/L + NAA 0.05 mg/L induced about 9.00 ± 0.90 shoots/explant (Fig. 2). This is better than single cytokinin or two cytokinin supplemented media. Improved shoot regeneration with cytokinin + auxin combination was proved in several other species such as *Pergularia pallida* (Bapat *et al.*, 1986), *Gymnema elegans* (Komalavalli and Rao, 1997), *Decalepis hamiltonii* (Bais *et al.*, 2000) and *Holostemma ada-kodien* (Martin, 2002). However, the addition of auxin to the cytokinin enriched medium enhanced the shoot number but caused growth of unnecessary basal callus.

Table 1. Effect of various plant growth regulators on shoot multiplication from nodal explants of *Cryptolepis buchanani* after 8 weeks of culture on MS medium.

Plant growth regulator (mg/L)							% of response	No. of shoots / Explant	Shoot length (cm)	Basal callusing
BAP	KN	TDZ	Z	IAA	2,4-D	NAA		Mean ± S.D.	Mean ± S.D.	
1	-	-	-	-	-	-	70	2.15 ± 0.60	2.15 ± 0.25	-
2	-	-	-	-	-	-	80	4.50 ± 0.71	3.00 ± 0.12	-
3	-	-	-	-	-	-	75	3.40 ± 0.45	3.10 ± 0.55	-
-	1	-	-	-	-	-	55	1.15 ± 0.10	2.15 ± 0.25	-
-	2	-	-	-	-	-	65	1.95 ± 0.31	3.10 ± 0.52	-
-	3	-	-	-	-	-	60	1.60 ± 0.22	2.95 ± 0.25	-
-		0.05	-	-	-	-	50	1.70 ± 0.21	2.00 ± 0.05	-
-		0.10	-	-	-	-	45	2.80 ± 0.82	1.85 ± 0.32	+
-		0.50	-	-	-	-	40	1.20 ± 0.60	0.85 ± 0.17	++
-		-	0.05	-	-	-	35	1.95 ± 0.25	2.15 ± 0.62	-
-		-	0.10	-	-	-	40	2.00 ± 0.31	1.95 ± 0.95	+
-		-	0.50	-	-	-	40	1.80 ± 0.52	1.25 ± 0.62	++
2	0.10	-	-	-	-	-	80	7.00 ± 0.92	3.95 ± 0.22	-
2	0.5.	-	-	-	-	-	75	7.10 ± 0.55	3.50 ± 0.50	-
2	-	0.05	-	-	-	-	50	6.00 ± 0.43	2.80 ± 0.35	-
2	-	-	0.05	-	-	-	40	3.00 ± 0.35	2.15 ± 0.11	++
2	0.10	-	-	-	-	0.05	60	9.00 ± 0.90	3.15 ± 0.71	+
2	0.10	-	-	-	-	0.10	55	7.75 ± 0.67	3.65 ± 0.48	+
2	0.10	-	-	0.05	-	-	40	4.10 ± 0.20	2.95 ± 0.19	+
2	0.10	-	-	0.10	-	-	35	3.80 ± 0.13	2.25 ± 0.71	+
2	0.10	-	-	-	0.05	-	35	3.25 ± 0.62	2.10 ± 0.34	+
2	0.10	-	-	-	0.10	-	25	2.15 ± 0.73	2.15 ± 0.34	++

+ scanty
++ medium

In many cases such as Pissaridi plum in Garland and Stoltz (1981), *Artocarpus heterophyllus* in Amin and Jaiswal (1993), it was noticed that cytokinin alone is enough for optimal shoot multiplication than in combination with auxins.

Shoot senescence that was observed during incubation in KN alone fortified medium could be controlled when NAA was supplemented to KN. Similar trial has also given a successful result to Pramanik *et al.* (1986) in *Hemidesmus indicus* and Maraffa *et al.* (1981) in *Hoya* spp. Apart from the above treatments a combination of auxin, cytokinin, GA_3 were also used to find the possible growth regulatory effect on different seedling explants. The requisite concentration of each type of regulant differs according to the kind of explant being cultured. Addition of GA_3 to NAA + BAP + KN was better than cytokinins alone and cytokinin + auxin treatments. When GA_3 concentration was increased from 0.05 mg/L to 0.1 mg/L shoot number was drastically decreased and enhanced the basal callus formation. Among the three explants tested, nodal explants showed better response when compared with other two (Table 2) on MS medium fortified with BAP 2 mg/L + KN 0.1 mg/L + NAA 0.05 mg/L + GA_3 0.05 mg/L (12.5 ± 0.33 shoots / explant) (Fig. 3). However, on the same hormonal combination maximum shoot length of 5.85 ± 0.35 cm was observed with shoot tip explants. Number of shoots induced from shoot tip explants (6.75 ± 0.20 shoots/explant) also is lesser than that of nodal explants. Further increase in the concentration of NAA or GA_3 reduced the number of shoots, enhanced the basal callus formation and also caused gradual decrease in shoot length (Table 2) (Fig. 4).

Table 2. Effect of BAP, KN, NAA and GA_3 at various concentrations for multiple shoot initiation from different seedling explants of *Cryptolepis buchanani* after 8 weeks of culture on MS medium.

Plant growth regulator (mg/L)				Cotyledonary node			Node			Shoot tip		
BAP	KN	NAA	GA_3	% of response	No. of shoots / explant Mean ± S.D.	Shoot length (cm) Mean ± S.D.	% of response	No. of shoots / explant Mean ± S.D.	Shoot length (cm) Mean ± S.D.	% of response	No. of shoots / explant Mean ± S.D.	Shoot length (cm) Mean ± S.D.
2	0.10	0.05	0.05	45	7.25 ± 0.55	5.00 ± 0.18	60	12.50 ± 0.33	4.73 ± 0.16	55	6.75 ± 0.20	5.85 ± 0.35
2	0.10	0.05	0.10	45	6.35 ± 0.65	4.25 ± 0.15	55	9.15 ± 0.75	4.60 ± 0.10	45	4.00 ± 0.11	4.85 ± 0.35
2	0.10	0.10	0.05	40	3.95 ± 0.10	3.80 ± 0.75	60	4.80 ± 0.27	4.50 ± 0.35	40	3.35 ± 0.15	3.85 ± 0.12
2	0.10	0.10	0.10	40	2.25 ± 0.33	2.95 ± 0.35	45	3.50 ± 0.35	3.70 ± 0.12	35	2.80 ± 0.85	3.25 ± 0.05

There are reports on the beneficial effects of GA_3 in the medium on enhanced frequency of bud break and growth in *Vitex negundo* (Sahoo and Chand, 1998), *Grevillia robusta* (Rajasekharan, 1994) and *Anacardium occidentale* (Bogetti *et al.,* 1999). *In vitro* cultures can generally be induced to grow and differentiate without gibberellins, although gibberellic acid may become an essential ingredient of media for culturing cells at low densities (Stuart and Street, 1971). When GA_3 is added to culture media, it often produces effects which are of a similar nature to those of auxins. In some cases it can also be used as a replacement for auxin in the induction of shoot formation (Sekioka and Tanaka, 1981), a precise gibberellin and cytokinin ratio (instead of auxin and cytokinin) may be required. But in the present study the combination of GA_3 and cytokinin is generally less satisfactory for the induction of shoots than that of auxin + cytokinin + GA_3. However, GA_3 was a preventive for directing shoot regeneration in sugar beet floral axillary buds (Coumans-Gilles *et al.,* 1981).

The rooting assays were performed on full and half strength MS media supplemented with 0.1, 1.0, 2.0 mg/L IAA or IBA or NAA singly or in combination (Fig. 5). In the absence of growth regulators both in half and full strength media no roots developed. When growth regulators were added to the medium, excised shoots cultured on half strength medium induced roots better than the shoots cultured on full strength medium. Elongated shoots of 2.0 – 2.5 cm length were used to test the effect of various auxin treatments on root induction. Growth regulator concentration significantly influenced rooting percentage, root number and root length as compared to control. As shown in Table 3, IBA was more effective than IAA or NAA. About 80% of shoots produced an average of 6.50 ± 0.57 white thick roots, each averaging 4.00 ± 0.52 cm length within four weeks of transfer to MS medium incorporated with IBA 1 mg/L. Superiority of IBA in root induction was also proved in *Ceropegia bulbosa* (Patil, 1998), *Ceropegia bulbosa* var. *lushi* (Patil,1998), *Ceropegia jainii* (Patil, 1998), *Exoecaria agallocha* (Rao *et al.,* 1998) and *Hemidesmus indicus* (Raghu Ramulu *et al.,* 2002). Higher concentration of auxin lowered the rooting percentage as well as root number. In general root formation is much better with one auxin than in combinations. But in the present investigation on *Cryptolepis buchanani* a combination of IBA 1.0 mg/L + NAA 0.05 mg/L triggered the root number to 8.75 ± 0.31 and IBA 1.0 mg/L + NAA 0.10 mg/L to 6.65 ± 0.82. When combination of IAA 1 mg/L + NAA 0.03 mg/L is used, the root number was less (1.00

Table 3. Effect of various auxins on rooting response from *in vitro* regenerated shoots of *Cryptolepis buchanani* Cultured on MS half strength medium.

Auxin concentration (mg/L)			% of response	No. of roots formed after 4 weeks of culture	Length of roots (cm) after 4 weeks of culture	Days to rooting
IAA	IBA	NAA		Mean ± S.D.	Mean ± S.D.	
0.10	-	-	35	1.00 ± 0.00	3.05 ± 0.82	12
1.00	-	-	40	1.00 ± 0.50	2.95 ± 0.15	14
2.00	-	-	30	1.00 ± 0.00	3.00 ± 0.00	15
-	0.10	-	65	1.50 ± 0.67	2.95 ± 0.31	18
-	1.00	-	80	6.50 ± 0.57	4.00 ± 0.52	7
-	2.00	-	40	4.20 ± 0.22	3.25 ± 0.72	9
-	-	0.10	60	1.20 ± 0.67	5.25 ± 0.11	11
-	-	1.00	65	2.90 ± 0.41	3.30 ± 0.27	18
-	-	2.00	55	1.35 ± 0.32	2.10 ± 0.15	18
1.00	-	0.05	65	1.00 ± 0.10	4.30 ± 0.05	20
1.00	-	1.00	50	1.80 ± 0.27	5.10 ± 0.61	18
-	1.00	0.05	70	8.75 ± 0.31	3.10 ± 0.21	12
-	1.00	0.10	65	6.65 ± 0.82	2.85 ± 0.18	14

± 0.10 root shoots), but root length was good with this combination when compared to IBA + NAA combination (3.10 ± 0.21 cm with IBA 1 mg/L + NAA 0.05 mg/L). When the concentration of auxins was increased in the combination, a cream coloured profuse callus developed from the base of excised shoots within three weeks of culture.

Plantlets with fully expanded leaves and well developed roots were transferred to pots containing a mixture of autoclaved peatmoss and compost (1:1). Within four weeks of transfer into potting mixture, 72% plantlets formed new leaves (Fig. 6).

ACKNOWLEDGEMENTS

This work was supported by a grant from Council of Scientific and Industrial Research.

LITERATURE CITED

Amin, M. N. and V. S. Jaiswal. 1993. *In vitro* response of apical bud explants from immature trees of jack fruit (*Artocarpus heterophyllus*). Plant Cell Tiss. Org. Cult. **33**: 59-65.

Anitha, S. and T. Pullaiah. 2002. *In vitro* propagation of *Decalepis hamiltonii*. J. Trop. Med. Plants. **3**: 227-232.

Bais, H. P., J. George and G.A. Ravishankar. 2000. *In vitro* propagation of *Decalepis hamiltonii* Wight & Arn. an endangered shrub, through axillary bud culture. Curr. Sci. **79**: 408-410.

Bapat, V. A., M. Mahtre and P. S. Rao. 1986. Regeneration of plantlets from protoplast cultures of *Pergularia pallida.* J. Plant Physiol. **124**: 413-417.

Bogetti, B., J. Jasik and S. Mantell. 1999. *In vitro* multiplication of cashew (*Anacardium occidentale* L.) using shoot node oxplants of glasshouse raised plants. Plant Cell Rep. **18**: 456-461.

Chattopadhyay, S., S. K. Datta and M. Ray. 1992. *In vitro* effect of NH_4NO_3 on growth and alkaloid content of *Tylophora indica* Merr. Phytomorphology **42**: 134-144.

Chopra, R. N., S. L. Nayar and L. C. Chopra. 1956. Glossary of Indian Medicinal plants. CSIR, NewDelhi: 82.

Coumans-Gilles, M. S., C. Kevers, M. Coumans, E. Ceulemans and T. H. Gastar. 1981. Vegetative multiplication of sugar beet through *In vitro* culture of inflorescence pieces. Plant Cell Tiss. Org. Cult. **1**: 93-101.

Datta, S. K., B. N. Sharma and P. V. Sharma. 1978. Buchanine, a novel pyridine alkaloid from *Cryptolepis buchanani.* Phytochemistry **17**: 2047-2048.

Deepak, D., S. Srivastava, A. Sethi and A. Khare. 1999. Mass spectral studies of pregnane glycosides. ISMAS Bulletin. **12**.

Dassonneville, L., A. Lansiaux, A. Wattel et, N. Wattez, C. Mahien, S. Van Miert, L. Pieters and C. Bailly. 2001. Cytotoxicity and cell cycle effects of the plant alkaloids Cryptolepine and neocryptolepin. Relation to drug – induced apoptosis. European. J. Pharm. **409**: 9-18

Garland, P. and L. P. Stoltz. 1981. Micropropagation of Pissardi plum. Ann. Bot., **48**: 387-389.

Komalavalli, N. and M. V. Rao. 1997. *In vitro* micropropagation of *Gymnema elegans* W. & A. – A rare medicinal plant. J. Exp. Biol. **35**: 1088-1092.

Koroch, A. R., H. R. Juliani, Jr. H. R. Juliani and V. S. Trippi. 1997. Micropropagation and acclimatization of *Hedeoma multiflorum*. Plant Cell Org. Cult. **48**: 213-217.

Maraffa, S. B., W. R. Sharp, H. K. Tayama and T. A. Fretz. 1981. Apparent asexual embryogenesis in cultured leaf sections of *Hoya carnosa.* Z. Pflanzen Physiol. **102**: 45-56.

Martin, K. P. 2002. Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. Plant Cell Rep. **21**: 112-117.

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

Patil, V. M. 1998. Micropropagation of *Ceropegia* spp. In vitro Cell Dev. Biol. Plant. **34**: 240-243.

Pramanik, T. K. and S. K. Datta. 1986. Plant regeneration and ploidy variation in culture derived plants of *Asclepias curassavica* L. Plant Cell Rep. **5**: 219-222.

Pramanik, T. K., S. De, M. Dasgupta. and S. K. Datta. 1986. Organ induced cardenolides biosynthesis in cultured tissues of *Asclepias curassavica*, *Calotropis gigantea* and *Thevetia peruviana*. Intl. Congr. Plant Tiss. Cell Cult. 6 meet 115.

Preece, J. E. and M. R. Imel. 1991. Plant regeneration from leaf explants of *Rhododendron* 'P. J. H. Hybrids'. Scientia Hort. **48**: 159-170.

Purushothaman, K. K., V. Sarada, J. D. Connolly and D. S. Rycroft. 1988. New sarverogenin and isosarverogenin glycosides from *Cryptolepis buchanani* (Asclepiadaceae). Rev. Latinoam. Quim. **19**: 28-31.

Raghu Ramulu, D., K. Sri Rama Murthy and T. Pullaiah. 2002. *In vitro* propagation of *Cynanchum callialatum*. J. Trop. Med. Plants. **3**: 233-238.

Rajasekaran, P. 1994. Production of clonal plantlets of *Grevillia robusta* in *in vitro* culture via axillary bud activation. Plant Cell Tiss. Org. Cult. **39**: 277-279.

Rao, C. S., P. Eganathan, A. Anand, P. Balakrishna and T. P. Reddy. 1998. Protocol for *in vitro* propagation of *Excoecaria agallocha* L., a medicinally important mangrove species. Plant Cell Rep., **17**: 861-865.

Sahoo, Y. and P. K. Chand. 1998. Micropropagation of *Vitex negundo* a woody aromatic

medicinal shrub, through high frequency axillary proliferation. Plant Cell Rep. **18**: 301-307.

Sekioka, T. J. and J. S. Tanaka. 1981. Differentiation in callus cultures of cucumber (*Cucumis sativus* L.) Hort. Sci. **16**: 451.

Sharma, P. C. and M. B. Yelne. 1995. Observation on *in vitro* propagation of sarivas *Hemidesmus Indicus* R. Br. Bull. Medico Ethnobotanical Res. **16**: 129-132.

Stuart, R. and H. E. Street. 1971. Studies on the growth in culture of plant cells. J. Exp. Bot. **22**: 96-106.

Sudha, C. G., P. N. Krishnan and P. Pushpangadan. 1998. *In vitro* propagation of *Holostemma annulare* (Roxb.) K. Schum, a rare medicinal plant. In vitro cell. Dev. Biol. Plant. **33**: 57-63.

Tavares, A. C., M. C. Pinenta and M. T. Goncalves. 1996. Micropropagation of *Melissa officinalis* L. through proliferation of axillary shoots. Plant Cell Rep. **15**: 441-444.

Venkateswara, R., K. Sankara Rao and C. S. Vaidyanathan. 1987. Cryptosin—a new cardenolide in tissue culture and intact plants of *Cryptolepis buchanani* Roem. & Schult. Plant Cell. Rep. **6**: 291-293.

Venkateswara, R., N. Narendra, M. A. Viswamitra and C. S. Vaidyanathan. 1989. Cryptosin, a cardenolide from the leaves of *Cryptolepis buchanani*. Phytochemistry **28**: 1203-1205.

Cryptolepis buchanani 微繁殖法的研究

P. J. N. Prasad[(1)], T. Chakradhar[(1)] and T. Pullaiah[(1,2)]

(收稿日期：2003 年 12 月 17 日；接受日期：2004 年 2 月 20 日)

摘　　要

建立 *Cryptolepis buchanani* 在試管中的再生步驟繁殖法，是利用在試管中生長的幼苗莖頂、子葉的節以及節的外植體；其中有最好表現的是節的外植體。栽培的方法是將節的外植體放到只含有各種細胞分裂素或者還包含生長素及吉貝素 (gibberillic acid, GA_3) 的 Murashige and Skoog (MS) 培養基上。單獨使用不同的細胞分裂素或者結合使用生長素 BAP，對於莖的增生有最好的效果。放在添加 2 mg/L BAP、0.1 mg/L Kinetin (KN)、0.05 mg/L Napthalene acetic acid (NAA) 以及 0.05 mg/L 吉貝素的 MS 培養基中，有 60% 的反應會產生最多的莖 (12.5~13.0 個/4.5 到 5.0 公分長)。單獨將莖放到含有一種或多種生長素，例如 IAA、IBA 以及 NAA 的 MS 培養基中進行生根。在 IBA 濃度為 1 mg/L 時，會得到最多的微小莖來形成根 (大約 6.5 到 7.0 個根/莖，其根的長度為 4.0 到 4.5 公分)。此方法可成功地馴化植物幼苗至盆中含有等比例消毒滅菌過的泥煤苔及堆肥中生長。

關鍵詞：*Cryptolepis buchanani*、微繁殖法、馴化。

1. Department of Botany, Sri Krishnadevaraya University, Anantapur-515003, Andhra Pradesh, India.
2.通信作者。Email: pullaiaht@yahoo.co.in