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Research note

In vitro micropropagation from nodal segments of *Cleistanthus collinus*

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Abstract

Cleistanthus collinus Benth. was micropropagated using nodal explants on MS medium supplemented with 2.2 μM benzyladenine (BA). April to June was the best time for initiating shoot cultures. Shoot proliferation was enhanced when the BA concentration was lowered to 1.1 μM. Rooting was achieved on half-strenth MS medium with 22.8 μM indole-3-acetic acid for 7 days and continuous darkness for the first 72 h of the 7 days.

Abbreviations: MS – Murashige & Skoog's medium; WPM – Woody Plant Medium; BA – 6-benzyladenine; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; NAA – 1-naphthalenacetic acid

Cleistanthus collinus Benth. (Euphorbiaceae) is a small deciduous fuelwood tree. Its leaves contain the poisonous glucoside oduvin (Chopra *et al.*, 1965). Leaf extracts inhibit neuromuscular function in the isolated phrenic nerve-diaphragms of mice (Nandakumar *et al.*, 1989). This species has great potential for afforestation of hills exposed to browsing because of its natural resitance to these predators (Haines, 1925). The wood is durable and used for making posts and poles, moreover it is not attacked by white ants. The tree sprouts readily from newly cut stumps and can be used for biomass production in short rotation plantations. Vegetative propagation by cuttings etc. has not been reported for this species. The objectives of this study were to determine a suitable medium and season for *in vitro* plantlet production from juvenile nodal explants of *C. collinus.*

Seeds of *C. collinus* were collected from native trees in Bastar, India, and sown in polyethylene bags containing 1 fine-grained black soil: 1 coarse sand: 1 cow-dung manure (by volume). Shoot tips with 4–5 nodes were obtained from 6 to 10-month-old seedlings. After removing the leaves, the shoots were thoroughly washed in running water: disinfested in 0.15% (w/v) aqueous mercuric chloride for 10 min and rinsed 4–5 times in sterile distilled water. Each nodal segment was cut to 1.5 cm long and placed *in vitro* on MS (Murashige & Skoog, 1962) supplemented with 2.2 μM BA during January–March, April–June, July–September and October–December for 2 consecutive years (1993, 1994) to study seasonal variation in axillary shoot elongation. Similary, the effect of different media and BA levels on establishment of shoot cultures was investigated by placing the nodal segments on MS, WPM (Lloyd & McCown, 1980) or White's medium (White, 1963) supplemented with 0, 2.2, 8.9 or 17.8 μM BA during April 1993. The number and length of shoots (> 0.5 cm long) were recorded after 4 weeks *in vitro.*

After 4 weeks, microshoots that grew from nodal explants on medium with 2.2 μM BA were excised and their nodes were placed on MS with 0, 0.56, 1.1 or 2.2 μM BA for further axillary shoot multiplication. Data on shoot number, length, and number of nodes were taken after 8 and 12 weeks on the proliferation media.

For rooting of microshoots, a medium with half-strength MS macronutrients was used. Three to 4 cm long microshoots were cut and placed vertically on media supplemented wih 2.8, 5.7 or 22.8 μM IAA, 2.4, 4.9 or 19.6 μM IBA or 2.7, 5.4 or 21.5 μM NAA, individually or in combination. In another set of experiments, microshoots were given a joint pulse treatment;

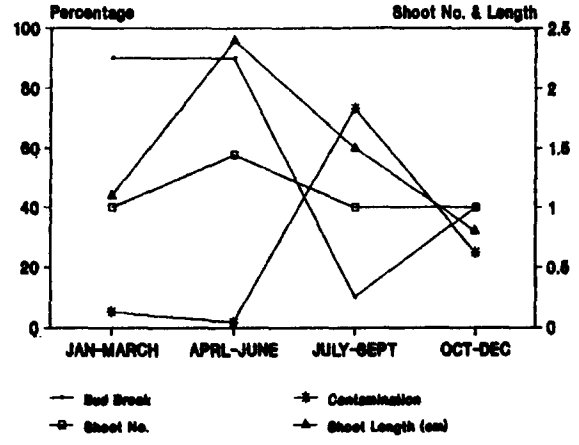

Fig. 1. Effect of seasonal variation on axillary shoot outgrowth. Each treatment consisted of 30 replicates and each experiment was repeated twice. Regression: $Y_{Shoots} = 1.299 - 0.069X$; $p<0.0001$; $R^2=0.146$. $Y_{Length} = 2.148 - 0.129X$; $p<0.0001$; $R^2=0.165$

in which they were placed on media containing 2.8, 5.7 or 22.8 µM IAA, and incubated for 72 h in continuous darkness and then for 4 days in a 16-h photoperiod. Thereafter, the microshoots were transferred to plant growth regulator free basal medium. Rooted microshoots were removed from the culture vessels, washed thoroughly to remove the nutrient medium and transplanted in pots containing 1 fine-grained black soil: 1 sand (by volvme) mixture. These pots were placed at 30 ± 2 °C and 16-h photoperiod; and initially covered by polyethylene bags, for 3 weeks.

In all the above experiments, each treatment consisted of 10 replicates and each experiment was conducted three times. Regression analysis was employed to analyze trend and relationship between variables.

The time of the year that explants were collected from seedling stock plants had an influence on axillary shoot outgrowth. This may be related to the differences in the physiological condition of the stock plants grown under natural environmental conditions. The best period for shoot culture initiation was April to June (Fig. 1). At this time explants produced significantly more shoots and the shoots were longer than during other times of the year. Seasonal variation in axillary shoot proliferation has been reported for *Artocarpus heterophyllus* (Amin & Jaiswal, 1993) and *Eucalyptus tereticornis* (Das & Mitra, 1990).

Contamination of the cultures also appeared to be dependent on the season. Maximum contamination occurred during July to September and minimum contamination occurred during January to June.

Table 1. Effect of different media and BA levels on axillary shoot growh from nodal explants of *C. collinus*.

Medium	BA µM	Bud break (%)	Shoot No. Mean ± SE	Shoot length (in cm) Mean ± SE
MS	0	100	1.0 ± 0	2.2 ± 0.2
	2.2	100	1.8 ± 0.2	3.8 ± 0.4
	8.9	100	1.4 ± 0.1	1.0 ± 0.3
	17.8	100	1.0 ± 0	1.1 ± 0.2
WPM	0	100	1.0 ± 0	2.5 ± 0.4
	2.2	100	1.8 ± 0.2	2.5 ± 0.4
	8.9	100	1.0 ± 0	0.9 ± 0.04
	17.8	100	1.0 ± 0	0.8 ± 0.1
White's	0	60	1.0 ± 0	0.9 ± 0.1
	2.2	80	1.0 ± 0	0.8 ± 0.04
	8.9	80	1.0 ± 0	0.8 ± 0.04
	17.8	80	1.0 ± 0	0.7 ± 0.04

Each treatment consisted of 10 replicates and the experiment was repeated three times.
Regression- MS: ^{y} Shoots = 1.426 - 0.077 ×; p =0.0655; R^2=0.028
^{y}Length = 2.868 - 0.516 ×; p<0.0001; R^2=0.355
WPM: ^{y}Shoots=1.702 - 0.186 ×; p<0.0001; R^2=0.155
^{y}Length = 2.467 - 0.471 ×; p<0.0001; R^2=0.366
White's: ^{y}Length = 0.886 - 0.044 ×; p=0.0017; R^2 =0.080

Axillary shoots elongated from explants placed on all three basal media, regardless of the presence of BA (Table 1). However, the explants on MS supplemented with 2.2 µM BA had the longest shoots.

The nodes of microshoots that had elongated from the original explants, were used for further shoot multiplication. There was a significant increase in shoot multiplication when the level of BA in MS was lowered (Table 2). On the basis of the number of nodes produced by each node of explant, MS with 1.1 µM was found to be the best medium for shoot multiplication. A similar trend was found by De Fossard *et al.*, (1978) in *Eucalyptus ficifolia.* After 8 weeks, the microshoots on MS with 1.1 µM BA started swelling and a mean of 11.9 ± 1.0 axillary shoots grew from each explant, within 12 weeks.

Shoot multiplication is an important factor for suitability of tissue culture method for mass propagation of tree species. On the basis of the results of this study, it is estimated that a single juvenile nodal explant can produce more than 22 shoots within three months (Fig. 2a).

Table 2. Effect of BA on shoot multiplicaton in micro-nodes of microshoots obtained from nodal explants of *C. collinus* placed on MS with 2.2 μM BA.

BA (μM)	Shoot No. Mean ± SE	Length of longest shoot (cm) Mean ± SE	Nodes per explant Mean ± SE
0	1.0 ± 0	1.1 ± 0.3	2.6 ± 0.2
0.56	2.3 ± 0.2	3.3 ± 0.4	8.3 ± 0.8
1.1	2.5 ± 0.7	4.1 ± 0.5	11.2 ± 0.7
2.2	1.8 ± 0.4	2.7 ± 0.1	6.1 ± 0.4

Each treatment consisted of 10 replicates and the experiment was repeated three times.
Regression: $Y_{Shoots} = 1.584 + 0.362X$; p=0.0131; R^2 =0.051
Y_{Length} =1.753 + 0.534X; p=0.0009; R^2=0.090
$Y_{Nodes} = 5.975 + 1.140X$; p=0.0038; R^2=0.068

Table 3. Rooting respons of microshoots of *C. collinus* to auxin(s) in half-MS, after 4 weeks.

Treatment (μM)	Rooted shoots (%)	Root number per shoot Mean ± SE	Root length (cm) Mean ± SE	Callus between root and shoot
Effect of auxin(s)				
00	-	-	-	–
2.8 IAA	-	-	-	–
5.7 IAA	20	1.0 ± 0	1.5 ± 0.1	–
22.8 IAA	20	1.0 ± 0	0.4 ± 0.1	–
2.4 IBA	30	9.3 ± 3.7	0.9 ± 0.1	+
4.9 IBA	20	1.0 ± 0	0.8 ± 0.2	+
19.6 IBA	20	6.0 ± 1.0	0.4± 0.1	+
2.6 NAA	20	2.0 ± 1.0	0.6 ± 0.1	+
5.4 NAA	40	4.0 ± 1.0	0.7 ± 0.1	+
21.5 NAA	-	-	-	+
0.7 IAA + 0.6 IBA + 0.6 NAA	20	2.0 ± 1.0	0.5 ± 0.2	+
1.4 IAA + 1.2 IBA + 1.3 NAA	20	2.0 ± 1.0	0.5 ± 0.1	+
2.8 IAA + 2.4 IBA + 2.6 NAA	-	-	-	+
Effect of IAA (7 days) + darkness (first 72h)				
2.8 IAA	-	-	-	–
5.7 IAA	60	1.3 ± 0.3	1.1 ± 0.4	–
22.8 IAA	80	2.1 ± 0.2	2.3 ± 0.2	–

Each treatment consisted of 10 replicates and the experiment was repeated three times.
Callus: +, present; –, absent.

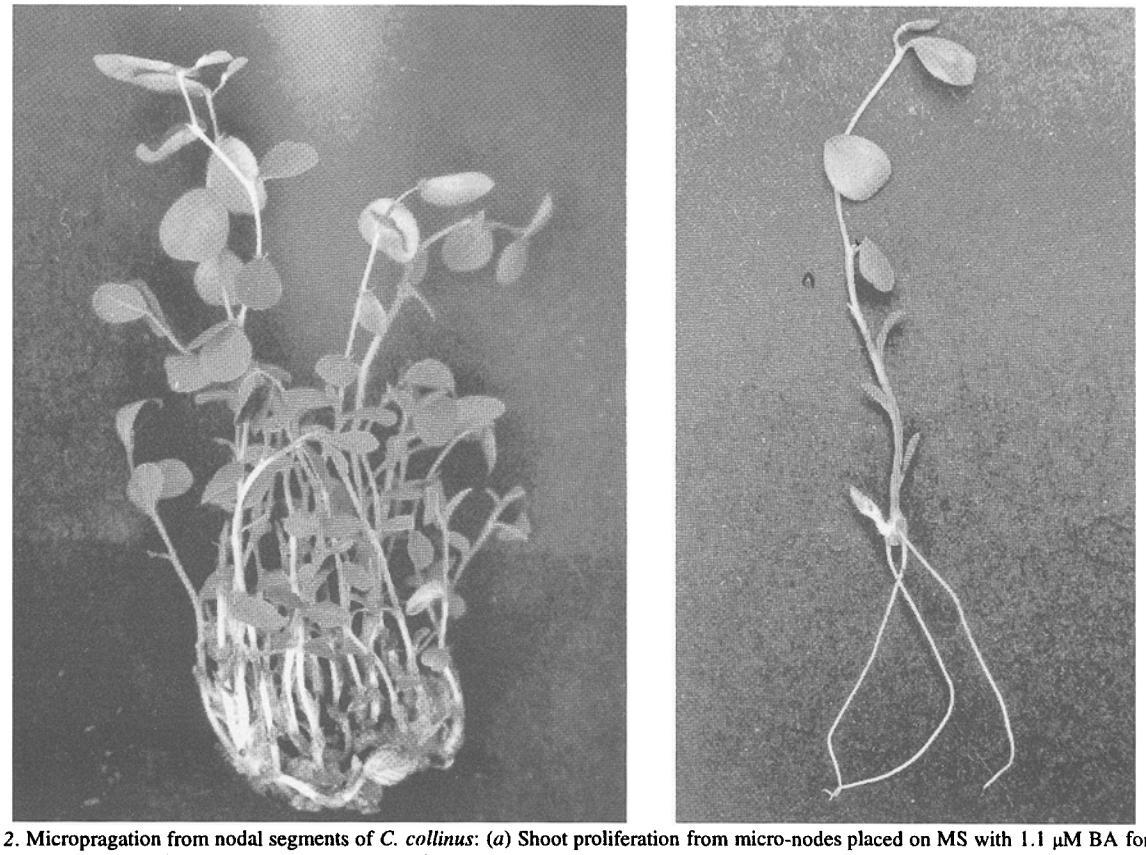

Fig. 2. Micropragation from nodal segments of *C. collinus*: (*a*) Shoot proliferation from micro-nodes placed on MS with 1.1 μM BA for 12 weeks (*b*) Microshoot showing roots without intervening callus after joint pulse treatment.

Rooting of microshoots of *C. collinus* is difficult. Rooting did not occur in microshoots placed on half-MS medium without growth regulators (Table 3). In presence of NAA and IBA, singly or in combination with IAA, 20 to 40% shoots formed roots with intervening callus. Continuous culture on medium with 5.7 μM IAA resulted in rooting of 20% of the microshoots; this increased to 80% when a pulse treatment of 22.8 μM IAA in 72 h darkness was given. These roots were without intervening callus (Fig. 2b). Use of simultaneous auxin and dark pulses for improvement in rooting has been reported in *Mitragyna parvifolia* (Roy *et al.*, 1988) and *Terminalia bellerica* (Roy *et al.*, 1987). Perhaps, the initial dark period prevents degradation of IAA, which is unstable in light. The rooted plants were transferred to pots containing soil: sand mixture, where only 30% plants survived for 5 weeks. Efforts are being made to achieve completely acclimatized plants

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References

Amin MN & Jaiswal VS (1993) *In vitro* response of apical bud explants from mature trees of jackfruit (*Artocarpus heterophyllus*). Plant Cell Tiss. Org. Cult. 33: 59–65

Chopra RN, Badwar RL & Ghosh S (1965) Poisonous Plants of India. No II (pp 774–775). Indian Council of Agricultural Research, New Delhi

Das T & Mitra GC (1990) Micropropagation of *Eucalyptus tereticornis*. Plant Cell Tiss. Org. Cult. 22: 95–103

De Fossard RA, Bennett MT, Gorst JR & Bourne RA (1978) Tissue culture propagation of *Eucalyptus ficifolia*. F Muell Comb. Int. Proc. Plant Prop. Soc. 28: 427–435

Haines HH (1925) Botany of Bihar and Orissa,Part 1 (p 122). Adlard and Son and West Newman, LTD., London

Lloyd G & McCown B (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Proc. Int. Comb. Plant Prop. Soc. 30: 421–427

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

Nandakumar NV, Pagala MKD, Venkatachari Sriman AT, Namba T & Grob D (1989) Effect of *Cleistanthus collinus* leaf extract on neuromuscular function of the isolated mouse phrenic nerve-diaphragm Toxicon 27: 1219–1228

Roy SK, Pal PK & Das AK (1987) Propagation of a timber tree, *Terminalia bellerica* Roxb. by tissue culture. Bangladesh J. Bot. 16: 125–130

Roy SK, Lutfar Rahman Sk & Datta PC (1988) *In vitro* propagation of *Mitragyna parvifolia* Korth. Plant Cell Tiss. Org. Cult. 12: 75–80

White PR (1963) A Handbook of Plant Tissue Culture. Jacques Cottell Press, Lancaster, Pennsylvania