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Somatic Embryogenesis and Root proliferation from Internode of *Anthocephalus cadamba In Vitro*

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Abstract : MS medium with 2.3 μM 6-furfuryl amino purine (KIN) plus 5.4 μM α-naphthaleneacetic acid (NAA) induced best proliferation of roots and medium with KIN (9.3 μM) plus NAA (5.4 μM) produced maximum number of somatic embryos in internodal explants of *Anthocephalus cadamba*. Embryoids differentiated directly from explant without going to callus phase. The best differentiation of roots was achieved from callus cultured on medium with KIN (4.6 μM), NAA (5.4 μM) and 15% coconut water (CW). Largest number of somatic embryos was produced in calluses cultured on the medium with KIN (23.2 μM), NAA (2.7 μM) and CW (15%).

Key words : Callus culture, Internode culture, Micropropagation, Rubiaceae.

Introduction

The genus *Anthocephalus* (Rubiaceae) comprises only three species distributed throughout the Indo-Malayan region out of which one species, the *Anthocephalus cadamba* is found in India (Santapan and Henry, 1973). Hindu mythological texts describe its beauty, shade and medicinal values (Bose and Chaudhary, 1991). It is a large tree that bears orange flowers with white stigmas on head inflorescences which appear like feathered balls. Its flower-receptacle is eaten and timber is used in packaging and paper industries. *A. cadamba* is used as medicine to make breast attractive & normal, enhance sperm count and increase milk production. It is aphrodisiac and used in mouth ulcer also. It contains cinchotannic acid which was used to control fever long before the knowledge of quinine to be effective against malaria (Bhatnagar *et al*. 1948; Visharad, 1985).

Such an important plant is propagated only through seeds and production of viable seeds is limited (Bose and Chaudhary, 1991). Other methods of propagation like micropropagation through tissue culture have not been exploited for this species. However, multiple shoot regeneration has been reported in its closely related species, the *A. indica* by Haque *et al*. (1991). The objective of the present work was to develop a protocol for micropropagation of *A. cadamba* to overcome its problem of propagation and conservation.

Materials and Methods

Explants taken from 2-4th internodes from shoot tip of branch of a mature tree were washed with 0.1% (v/v) Tween 80 for 30 min, surface sterilized with 0.2% (w/v) $HgCl_2$ for 5 min and finally rinsed with sterile distilled water 3-4 times. About 1-1.5 cm segments were taken out from these sterilized explants, dried on sterile filter paper and cultured on

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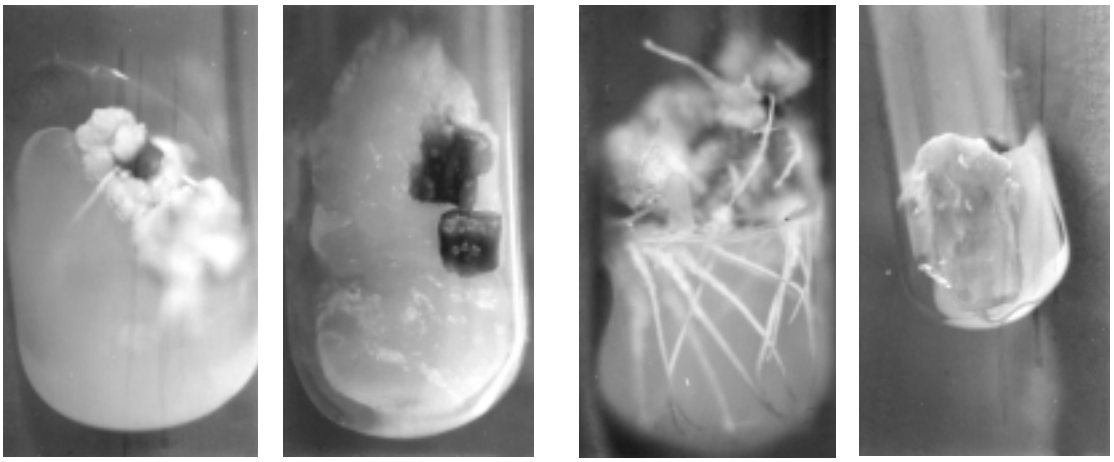
Murashige and Skoog (1962) (MS) medium with 87.6 mM sucrose, 8 g L^{-1} agar, KIN and NAA (Hi-media) at pH 5.6 (Table 1). The cultures were grown under 24-h photoperiod maintained by florescent light at 26 ± 2ºC. Calluses produced were subcultured on previously described media with/without coconut water (CW) from immature fruit under similar physical conditions. Ten replicates were maintained for each treatment. The cultures were observed weekly for development of callus, root, shoot and somatic embryos. After six weeks the cultures were evaluated for total number of roots, its length and somatic embryos.

Results and Discussion

NAA (2.7 – 5.4 μM) induced development of pale yellow/light green callus and proliferation of roots directly from the explant. The roots were white and unbranched with root-hair (Figure 1). NAA (5.4 μM) with KIN (2.3 μM) proved to be the best medium for rhizogenesis (Table 1). NAA was found suitable to induce rhizogenesis in *Ixora coccinea* (Rubiaceae) also (Lakshmanan *et al*., 1997). Increase in the concentration of KIN (Table 1) in the medium induced development of somatic embryos directly from the internode explant without going to callus phase. These embryoids were small and round with cotyledon like structures (Figure 2) that resembled somatic embryos of *Mussaenda* (Das *et al*. 1993). Calluses subcultured on KIN (2.3 μM) and NAA (5.4 μM) induced differentiation of roots. When CW was added to the medium, frequency of differentiation, number of roots per explant and length of roots increased (Table 1). Roots were un branched without root hair (Figure 3). Medium supplemented with KIN (4.6 μM), NAA (5.4

Table 1 : Somatic Embryogenesis and Rhizogenesis from Internode and Callus of *Anthocephalus cadamba* Cultured on MS Medium with KIN, NAA and CW

Treatment (μM)			Mean no. of roots per explant	Mean length (cm) of roots	Mean no. of somatic embryos per explant
Internode culture					
KIN	NAA				
0	0		--	--	--
0	2.7		2.8±0.6	1.2±0.3	--
0	5.4		6.2±0.2	1.0±0.2	--
2.3	5.4		7.2±2.1	1.8±0.2	--
9.3	5.4		--	--	18.2±3.2
13.9	5.4		--	--	12.2±3.4
Callus culture					
KIN	NAA	CW(%)			
0	0	0	--	--	--
2.3	5.4	0	8.8±2.4	1.9±0.2	--
2.3	5.4	15	10.2±2.8	2.8±0.4	--
4.6	5.4	15	15.8±2.4	3.2±0.5	--
18.6	2.7	15	--	--	4.5±1.1
23.2	2.7	15	--	--	5.1±1.2



(1) **(2)** **(3)** **(4)**

Figure 1-4 : Differentiation of roots and somatic embryos in Anthocephalus cadamba after six weeks of culture.

1. **Callus development and root proliferation from internode cultured on MS medium with KIN (2.3 μM) and NAA (5.4 μM).**
2. **Development of somatic embryos from internode cultured on MS medium with KIN (13.9 μM) and NAA (5.4 μM).**
3. **Root differentiation from callus subcultured on MS with KIN (4.6 μM), NAA (5.4 μM) and CW (15%).**
4. **Differentiation of somatic embryos from callus subcultured on MS with KIN (23.2 μM), NAA (2.7 μM) and CW (15%).**

μM) and CW (15%) was found best for rhizogenesis. When concentration of KIN was increased to 18.6 μm and that of NAA decreased to 2.7 μM, somatic embryos (Figure 4) were differentiated in the subcultured callus as was found by Santana *et al*. (1988) in coffee. Medium with KIN (23.2 μM), NAA (2.7 μM) and CW (15%) produced maximum number of somatic embryos. Increase in concentration of KIN in the medium suppressed root differentiation and increased callus compactness that ultimately resulted to development of somatic embryos.

In order to search out micropropagation protocol, we achieved somatic embryos and rhizogenesis that may make foundation for future experimentation.

References

Bhatnagar S.S., Chopra R.N., Prasad B., Ghosh J.C., Qureshi M., Saha M.N., Ram L.S. and Manjunath B.L. (eds.). (1948): *The wealth of India,* CSIR Publication, New Delhi, vol 1, p 83

Bose T.K. and Chaudhary B. (eds.). (1991): *Tropical garden plants in colour,* Horticulture and allied publishers, Kolkata, pp 13-14

Das P., Rout G.R. and Das A.B. (1993): Somatic embryogenesis in callus cultures of *Mussaenda erythrophylla* cvs Queen Sirikit and Rosea. *Plant Cell Tiss. Org. Cult.*, **35**, 199-201.

Haque M.I., Sarkar R.H., Haque M.M. and Islam A.S. (1991): *In vitro* multiple shoot regeneration in *Anthocephalus indica* L.. *Bangladesh J. Bot.*, **20**, 193-197.

Lakshmanan P., Lee C.L. and Goh C.J. (1997): An efficient in vitro method for mass propagation

of a woody ornamental *Ixora coccinea* L.. *Plant Cell Rep.*, **16**, 572-577.

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

Santana N., Martinez O. and Gonzalez M.C. (1988): Somatic embryogenesis in *Coffea arabica*. *Cultivos Tropicalis*, **10**, 36-43.

Santapan H. and Henry A.N. (1973): *A dictionary of Flowering Plants in India*, CSIR Publication, New Delhi.

Visharad C.B. (1985): *An Encyclopeadia of Indian Botanies and Herbs*, Chaukhambha Sanskrit Sansthan, Varanasi, vol 2, p 42.