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Micropropagation of *Crataeva magna* (Lour.) DC.—A medicinal plant

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Rapid multiplication of *C. magna*, a medicinal plant, was achieved by culturing nodal segments on Murashige and Skoog's (MS) medium supplemented with sucrose (3%) and different concentrations of benzyl amino purine (BAP). Nodal segments cultured on MS medium supplemented with 8.8 µ*M* of BAP produced multiple shoots (4.4+0.09) with maximum length (63.2+0.92 mm). Rooting of the excised shoots cultured on 9.84 µ*M* of IBA in combination with 0.54 µ*M* of NAA in half strength MS medium was found suitable. Rooted plants established with 68% success rate in pots after hardening.

Keywords: *Crataeva magna*, *in vitro* multiplication, nodal segments

Crataeva magna (Lour.) DC. belonging to the family *Capparidaceae* is an important medicinal tree. Its bark and leaves are astringent, bitter, acrid, diuretic, lithotriptic stimulant, detergent, expectorant, demulcent depurative, astiperiodic and tonic, and are useful in vitiated conditions of *vata* and *kapha* (Ayurveda), dyspepsia, colic, flatulence, helminthiasis, strangury, renal and vesical calculi, cough, asthma, bronchitis pruritus, skin diseases, intermittent fevers, visceromegaly, scrofula, inflammations and hepatopathy. The leaf paste is applied externally on piles and the juice is drunk to get relief from bleeding piles[1].

Conventionally, the plant can be propagated through seeds. The seeds, however, remain viable for a short period of only 3 months or less and also germinate poorly thus producing few plants. Also, the vegetative propagation through cuttings is not efficient under varied climatic conditions and the rooting behaviour is erratic. Because of its medicinal importance and potential, there is a need to mass propagate this species. Plant tissue culture offers an effective alternative method for rapid multiplication to overcome the natural impediments in propagation of this species[2]. A method for rapid multiplication of *C. magna* through nodal cultures is described. There is no earlier report on *in vitro* micropropagation of this useful tree.

Plants of *C. magna*, collected from herbal garden of medicinal plants survey unit, Government Siddha College, Palayamkottai, were established in the greenhouse of the department. Young shoots were used as the source of explants. The shoots were excised, defoliated and cut into 5-7 cm long pieces having three to four nodes. After surface sterilization with 0.1 % (w/v) mercuric chloride for 2 min they were thoroughly rinsed thrice in sterile distilled water. The nodal segments (1 cm long) were prepared and cultured on MS (Murashige & Skoog, 1962) medium supplemented with 3% sucrose, 0.5 % (w/v) agar (Himedia, Mumbai) and different concentrations of BAP (Table 1)[3]. Half strength MS medium having 3% sucrose and supplemented with auxins, IBA, IAA and NAA were used for rooting *in vitro* grown shoots (Table 2). The *p*H of the medium was adjusted to 5.8 before adding agar and autoclaved at 121°C under 1.0 kg/cm^2 pressure for 15 min. The cultures were incubated at 25°±2°C under cool white fluorescent light (2000 lux 12 hr photoperiod). Ten replicates were used for the studies on shoot initiation and 20 replicates for rooting. Subculturing was carried out at periodic intervals of 20-30 days using MS medium supplemented with IBA (0.84 µ*M*) and NAA (0.54 µ*M*).

Cultures were free from exudation and browning and showed shoot initiation and elongation of axillary buds within 6 days (Fig. 1A). Shoot initiation from the nodal segments was mainly a cytokinin effect because the explants in cytokinin free medium did not respond. The role of BAP in bud breaking has been recorded in many medicinal plants, such as *Wadelia calendulaceae*, *Solanum anguivi*, *Baliospermum montanum* and *Plectranthus vettiveroides*[4-6]. The number of multiple shoots developed after 4 weeks on MS + 8.8 µ*M* BAP was registered as 4.44 ± 0.09 and 63.2 ± 0.92, respectively (Table 1; Fig 1B.). After 30 days, MS medium supplemented with 4.44 µ*M* was used for routine subculture. As compared to the primary cultures there was no significant improvement in the rate of multiplication during subsequent subcultures.

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Table 1—Effect of BAP on shoot production from the nodal segments of *C. magna*

BAP Concentration (μ*M*)	% of shooting response	No. of shootlets/ node ±S.D after 6 weeks of culture	Mean length of shootlets (mm)
0	0	0	0
0.44	0	0	0
0.88	60.27±1.34	1.1 ± 0.07	19.8±0.83
2.22	65.10±0.737	1.4 ± 0.07	31.8±1.03
4.44	70.09±0.090	2.7± 0.09	43.6±1.11
8.88	74.02±0.632	4.4 ± 0.09	63.2±0.92
2.20	65.01±0.737	1.7 ± 0.08	16.3±0.82

Table 2—Effect of auxins on rooting of *in vitro* shoots of *C. magna*

Auxin concentration (μ*M*)			% of rooting response	Mean no. of roots/shootlets ± S.D	Mean length of rootlets (mm)
IAA	IBA	NAA			
0	0	0	0	0	0
0	0.49	0	65	2.2 ±0.5	11±0.12*
5.71	0	2.69	0	0	0
0	4.92	5.37	0	0	0
	9.84	0.54	70	3.0 ± 0.48	1.36±0.08*
0.57	0	5.37	0	0	0

*Rhoot base showed callusing

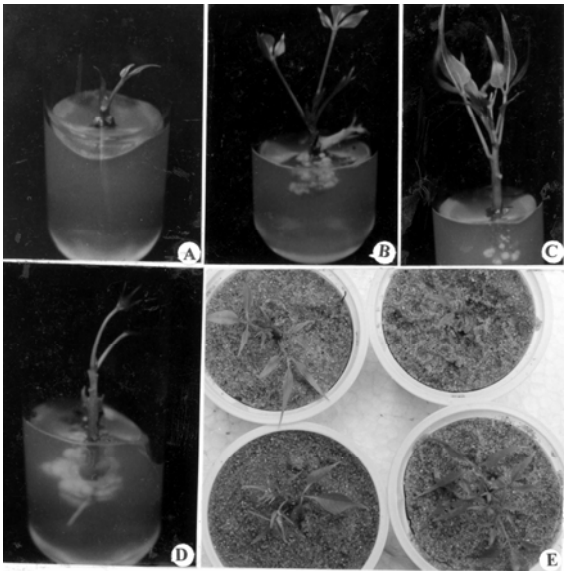

Fig.1—Micropropagation stages of *C. magna:* A, Shoot initiation stage after 6 days of culture; B, Multiple shoots induction after 6 weeks of culture; C, Multiple shoots induction after 8 weeks; D, Root induction on *in vitro* raised shootlets; & E, Hardened plantlets

The *in vitro* raised multiple shoots were excised and transferred individually to half strength MS medium supplemented with various concentrations and combinations of growth hormones (Table 2). *In vitro* raised shoots (31.8-43.6 mm) were transferred to half strength MS medium supplemented with IBA alone and also in combination with NAA for rooting. Maximum rooting percentage (70%) was observed in 9.84 μ*M*/IBA with 0.54 μ*M*/NAA (Table 2). It was observed that the roots were thin like a thread in 9.84 μ*M*/IBA with 0.54 μ*M*/NAA. In the present study, initially the rooting percentage was very low. These results are in accordance with those on *Kaempferia rotunda* and Indian ginseng by others[7,8]. When IAA was tested for rooting, there was not only decrease in the rooting response but also enhanced basal callusing from the shoots[6].

Rooted shoots after attaining a height of 2.0-2.5 cm were hardened *in vitro* by placing them in liquid ¼ strength MS medium having 2 % sucrose for 7-8 days and thereafter they were transferred to the earthen pots containing the mixture of soil, sand and manure (1:1:1) and covered with perforated polythene bags. These bags were removed periodically, and finally when emergence of new pair of leaves started. After transfer of plantlets to soil in pots, 68% success in survival was observed (Fig.1D). The tissue culture raised plants after attaining a height of 50 cm, were successfully planted in the field.

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