Micropropagation of *Crataeva religiosa* Hook. f. & Thoms

M. Jothi Basu1*, R. Ramanathan, N. Yogananth1 and S. Baburaj

1 J.J. College of Arts and Science, Pudukkottai-622 422, Tamilnadu, India.
Thiagarajar College (Autonomous), Madurai-625 009, Tamilnadu, India.
*Present Address: Alagappa University, Karaikudi-630 003, Tamilnadu, India
*For Correspondence - jothibasu77@gmail.com

**Abstract**

A protocol was developed for *in vitro* propagation by multiple shoot induction of *Crataeva religiosa* Hook. f. & Thoms, a medicinal tree having high medicinal values belonging to the family Capparidaceae. High frequencies of multiple shoot regeneration were achieved from apical bud on MS medium fortified with 8 mg/L BAP alone. Five to seven shoots per explant were obtained. The elongated shoots were subcultured for rooting on half strength MS supplemented with various concentrations of IBA and IAA. The *in vitro* raised plantlets were acclimatized in green house and successfully transplanted to natural condition with 72% survival.

**Key words:** *Crataeva religiosa*, Medicinal plant, Micropropagation, Acclimatization.

**Abbreviations:** MS-Murashige Skoog medium, NAA-Napthalene Acetic Acid, BAP-Benzyl Amino Purine, IAA-Indole Acetic Acid, IBA-Indole Butric Acid.

**Introduction**

Tissue culture techniques are being increasingly exploited for clonal multiplication and *in vitro* conservation of valuable indigenous germ-plasm threatened with extinction. Greater demand for these plants especially for the purpose of food and medicines which is one of the causes of their rapid depletion from primary habitats. Micro-propagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation (1). *Crataeva religiosa* Hook. f. & Thoms., non Frost. F. belongs to the family Capparidaceae is a moderate-sized deciduous tree (Tamil: Mavilankai, Telugu: Magalingam, Malayalam: Nirmatalam, Hindi: Barun). *C. religiosa* is characterized with astringent, bitter, acrid, diuretic, anthelmintic, carminative, laxative and stomachic. Root and bark promote appetite, and increase biliary secretion. Leaves are stomachic and tonic. Juice of leaves is given internally to cure rheumatism. Bark is demulcent, alterative, tonic, stomachic, laxative, diuretic, antipyretic, and useful in calculus affections and for disorders of urinary organs. Powdered bark is useful in urinary and renal troubles, gastro-intestinal and uterine infections (2). There has been progress in tissue culture studies in many Capparidaceae members such as *C. nurvala* (3), *C. magna* (4) and *Capparis decidua* (5) to propagate them. But no such *in vitro* culture studies have been carried out in this valuable medicinal tree, *C. religiosa*. The present investigation elucidates *in vitro* multiple shoot regeneration through apical bud segments of *C. religiosa* for better exploitation and also preservation of this valuable germ-plasm which has already undergone a severe biotic pressure.
Materials and Methods

Apical buds of *C. religiosa* were collected from the campus of Thiagarajar College, Madurai, washed thoroughly in running tap water and treated with detergent solution. The apical buds were initially disinfected by rinsing it in 90% ethanol for 15 seconds followed by surface sterilization in an 0.1% (w/v) HgCl₂ aqueous solution of 0.1% (w/v) HgCl₂ for 2-3 minutes. The explants were once again rinsed thrice with sterile distilled water. The sterilized explants were inoculated in MS medium containing 3% sucrose and 0.8% agar with supplemented with BAP alone (0.5 — 10.0 mg/L) and different concentrations (1.0 — 5.0 mg/L) of BAP along with NAA (0.25 mg/L). The pH of the media was adjusted to 5.8 and autoclaved at 1.06 kg/cm² pressure and 121°C temperature for 15 min. For root induction, the well developed shoots were transferred to half strength MS medium with different concentrations of IAA (3.0 mg/L) and IBA (3.0 mg/L). All subsequent subculturing were performed at four week of interval to fresh medium. The cultures were incubated in a culture room maintained at 25 ± 2°C under 16 hours photoperiod with light intensity of 3000 lux. For each treatment 14 replicate cultures were maintained and all the experiments were replicated thrice. The data were statistically analyzed using one way analysis of variance and means were compared using the Duncan’s Multiple Rank Test at the 0.05% level of significance.

Results and Discussion

The use of pre-existing buds for propagation reduces the possibility of variation among the progeny and therefore can be safely applied for rapid propagation of *Crataeva religiosa*. We optimized shoot multiplication conditions and novel rooting techniques for mass clonal propagation without interference of callus. This method is quite common for the propagation of *Fragaria indica* (6), and *Acacia mearnsii* (7) and *Sandalum album* (8). The apical bud explants showed slight swelling prior to the emergence of shoot buds developing from the pre-existing material 20 days after inoculation. Initially two to four shoot buds per explant emerged 30 days after inoculation and gradually the number of shoot buds per explant increased up to 5 – 7 (Table 1; Plate 1a) on MS media fortified with 8 mg/L BAP. But low number of buds developed in the concentration of 0.5 mg/L BAP. Superiority of BAP over other cytokinin has been reported and discussed in relation to shoot proliferation in cultures of trees (9, 10) and the regeneration of shoots from nodal explants has also been encountered in Capparidaceae plants like *C. nurvala* (3), *C. magna* (4) *C. adansonii* (11) and *Capparis decidua* (5). Sometimes callus formation from the basal cut ends of the apical bud explant was also observed (Plate 1b).

The *in vitro* developed shoots from the apical bud cultures were harvested and transferred to half strength MS medium added with various concentrations of IBA and IAA. A maximum number of 4 - 5 roots were observed when the medium was supplemented with 3.0 mg/L IBA or IAA after 5 weeks, irrespective of the type of auxins used (Fig. 1). Similarly, pulse treatments of IBA were given for root induction in shoots produced in cultures from nodal explants of adult plants of *Camellia sinensis* (12), *Maytenus emayginata* (13) and *Prosopis cineraria* (14) and *Sandalum album* (8). IBA is the most commonly used auxin for root formation from shoots of woody trees (15). The *in vitro* regenerated rooted plantlets were washed with sterile water and transferred to small plastic pots containing sand, garden soil and digested coir pith (1:1:1) mixture (Plate 1c). Then the plants were maintained under shade and partial shade for one more week. The regenerated plants were transferred to the soil with 72% survival.

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Table 1: Effect of NAA and BAP on \textit{in vitro} shoot formation from apical shoot bud explants of \textit{Crataeva religiosa} after 30 days of culture

<table>
<thead>
<tr>
<th>Growth Regulators mg/L</th>
<th>Mean number of Shoots/explant</th>
<th>Morphogenic Response</th>
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<tbody>
<tr>
<td>NAA</td>
<td>BAP</td>
<td></td>
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<tr>
<td>0</td>
<td>0.5</td>
<td>2.571 ± 0.327&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>2.0</td>
<td>3.570 ± 0.589&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>0</td>
<td>4.0</td>
<td>4.420 ± 1.496&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>0</td>
<td>6.0</td>
<td>5.710 ± 0.822&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>8.0</td>
<td>6.850 ± 0.839&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>10.0</td>
<td>5.710 ± 0.822&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>0.25</td>
<td>0.5</td>
<td>3.714 ± 0.826&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>0.25</td>
<td>1.0</td>
<td>4.290 ± 0.629&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>4.290 ± 0.509&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>0.25</td>
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<td>0.25</td>
<td>4.0</td>
<td>5.420 ± 0.639&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>0.25</td>
<td>5.0</td>
<td>4.857 ± 0.841&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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± - Standard Error. Means followed by the same letter not significantly different by the Duncan’s Multiple Rank Test at P< 0.05 level of significance.

Where CS – Callus + Shoot, S - Shoot

Fig. 1: Effect of various auxins on rooting response from \textit{in vitro} regenerated shoots of \textit{Crataeva religiosa} cultured on half strength MS medium after 5 weeks of culture.

Fig. 2: Plate one

Vertical line on the bar indicates Standard Error. Means followed by the same letter not significantly different by the Duncan’s Multiple Rank Test at P< 0.05 level of significance.

a) Shoot proliferation from apical bud on MS media fortified with 8 mg/L BAP
b) Direct shoot formation from callus on MS media supplemented with 0.25 mg/L NAA and 1.0 mg/L BAP
c) Successful establishment of rooted plants in plastic cup.
The present investigation has demonstrated a potentially efficient technique for the large scale micropropagation of *C. religiosa* from apical bud explant. The data indicated that BAP at 8.0 mg/L in MS medium is more effective for shoot multiplication from the shoot apical bud. Half strength MS medium supplemented with 3.0 mg/L IBA or IAA is best for root induction.

The protocol described is an efficient and could be used as a means of propagation and multiplication of *Crataeva religiosa* — a potential medicinal plant for commercial exploitation while previously published protocols are having some complications like incubation in dark for 6 days (5).

**References**


