In vitro propagation of *Justicia gendarussa* Burm. f.–A medicinal plant

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An efficient protocol for *in vitro* propagation of *Justicia gendarussa* Burm. f. has been developed. MS medium supplemented with NAA induced prolific callus in both leaf and nodal explants. Organogenic and chlorophyllous calli were produced at lower concentrations of NAA (1.0 mg L\(^{-1}\)) and BAP (0.1 mg L\(^{-1}\)). Thick and long roots with numerous root hairs were produced with NAA (1.0 mg L\(^{-1}\)) and BAP (0.1 mg L\(^{-1}\)). Long shoots were also formed. Of the *in vitro* grown 120 plantlets transferred to the field 94% survived after 2 months of transplantation to natural environment.

**Keywords:** *in vitro* propagation, organogenesis, *Justicia gendarussa*

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*Justicia gendarussa* Burm. f. belonging to the family Acanthaceae, is well-known for many of its medicinal properties. It has anodyne, diaphoretic, diuretic, antiphlogistic, antispasmodic, carminative, emetic, febrifuge and laxative properties. The roots and leaves are acrid, bitter, therogenic, emmenagogue, antiperiodic and insecticidal. They are useful in the treatment of chronic rheumatism, cephalalgia, hemiplegia, facial paralysis, ostalgia, hemicrania, cough, bronchitis, arthritis, dysmenorrhoea, amenorrhoea, internal haemorrhages, intermittent fevers, ascites and debility\(^1\). Fresh leaves are used topically to treat oedema of beriberi and rheumatism\(^2\). Dried leaves are used as insecticide.

*J. gendarussa* grows along the beds of streams in Himalayas. It is an erect, branched, smooth, undershrub with long leaves having acute tips; small flowers on long terminal pinkish spikes with purple spots. The reported chemical constituents of *Justicia* spp. are 2-(2’-amino benzylamine)-benzyl alcohol-o-methyl ether, 2-amino benzyl alcohol, 2-amino benzyl alcohol o-methyl ether and β-sitosterol. Some spp. exhibited varied responses like antimicrobial, anti-inflammatory and antiplatelet aggregation effects\(^3\). Moreover, cytotoxic and anticancer activities of lignans isolated from *Justicia* spp. have also been reported\(^4\).

The plant is propagated by seeds, whose viability is only for a very short period and hence, it is important to look for alternative methods to conserve it. Moreover, owing to increasing exploitation of natural population for its wide uses in traditional medicine, the plant is also getting depleted. Hence an attempt has been made for *in vitro* propagation of this medicinal plant and this protocol offers an alternative method for rapid multiplication of desirable clones.

Mature plants of *Justicia gendarussa* were collected at a relatively warm (25 to 38°C) and humid (70 to 95%) climate with about 20 cm rainfall during the monsoon months (September-November) and were grown in the garden. Tender leaves and nodal regions of stem were used as explants. The explants were soaked in 10% tween-20 for ten min. Then they were washed thrice with sterile distilled water followed by treatment with 0.03% bavistin, (a fungicide) for 20 min and again washed thrice in sterile distilled water. Explants were further surface sterilized with 0.1% mercuric chloride for 4 min, washed 5-7 times with sterile distilled water in a complete aseptic ambience. Explants were trimmed and placed on full strength MS medium\(^5\) supplemented with 8 g L\(^{-1}\) agar, 30 g L\(^{-1}\) sucrose and appropriate concentrations of phytohormones. Naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP) were used in a 4 by 4 latin square with concentrations 0, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 2.5 mg L\(^{-1}\). In all the cases, \(pH\) was maintained between 5.6 and 5.8. Each treatment consisted of fifteen replicates and the experiments were repeated three times. A complete randomized block design was used in all experiments and analysis of variance and mean separation were carried out using Duncan’s Multiple Range Test (DMRT) and significance was assessed at 5% level\(^6\). Explants were grown in a culture environment of either 25±2°C or

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20°C in continuous darkness, or a 16 h light/8 h dark cycle with a light intensity of 27 μE m⁻² s⁻¹ or a continuous low light regime of 0.5 μE m⁻² s⁻¹.

When the medium was supplemented with NAA, prolific callus induction occurred in leaf and nodal explants (Fig. 1; Table 1). To maximize callus proliferation, a solid medium supplemented with 2 mg L⁻¹ NAA was used. In this medium the callus doubled in size every 15 d with routine subcultures. Callus was found to be big, friable and white-pale green in colour. When nodal explants were grown in medium supplemented with low concentrations of NAA (1.0 mg L⁻¹) and BAP (0.1 mg L⁻¹), they produced compact chlorophyllous calli with shoots (Fig. 2). When leaf explants were grown in medium supplemented with BAP (2.0 mg L⁻¹), hard callus was formed after longer incubation time. This may be attributed to differential physiological status and endogenous concentration of growth regulators as revealed by the studies on Quercus spp 

The roots, developed from the callus after 15-20 d of culturing in rooting medium, exhibited varied morphological features. Lower concentrations of NAA (0.1 to 1.0 mg L⁻¹) without BAP gave maximum number of tender roots (8/nodal explant) also recorded earlier in Hybanthus ennaespermus and Datura metal. Thick and long (25 cm) roots were produced in large numbers in medium supplemented with NAA (1 mg L⁻¹) and BAP (0.1 mg L⁻¹) (Fig. 3). Long chlorophyllous roots were formed in medium supplemented with NAA (0.2-2.0 mg L⁻¹). Very minute, dense root hairs were produced from the thick roots.

The inclusion of cytokinins and auxins to the culture medium stimulated in vitro multiplication and growth of shoots in several plant species. In the present study, shoots were produced from 10th day onwards and final observations were made on 20th day. All combinations of NAA and BAP produced long shoots (7.6±0.7 cm) with two to three shoots per callus. However, 3 to 4 medium sized shoots appeared in medium containing 0.5 mg L⁻¹ NAA and 0.1 mg L⁻¹ BAP (Fig. 4). Only short shoots appeared at higher concentrations of NAA and BAP. Nodal explants produced calli with long shoots (10.71 cm) and profuse rooting occurred at 0.1 mg L⁻¹ NAA and 1.0 mg L⁻¹ BAP. Maximum number (4.33) of shoots was obtained on MS medium supplemented with 3 mg L⁻¹ NAA.

Figs 1-5—Plant regeneration in J. gendarussa Burm. f.: 1, Callus induction in nodal explant; 2, Shoot induction in callus using nodal explant with single axillary bud; 3, Induction of shoot and root from nodal explant; 4, Multiple shoot induction from in vitro nodal explant; & 5, Transfer of plantlet for hardening.

### Table 1—Effect of NAA and BAP on callus induction and organogenesis from nodal explants of J. gendarussa

<table>
<thead>
<tr>
<th>Medium</th>
<th>Optimal hormonal concentrations (mg L⁻¹)</th>
<th>Percentage of response</th>
<th>Number of shoots</th>
<th>Length of shoots</th>
<th>Nature of Callus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Callus induction</td>
<td>Shoot formation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>—</td>
<td>—</td>
<td>50.34 (2.71)*</td>
<td>1.00 #</td>
<td>4.8(0.52)* Compact, very slow growth</td>
</tr>
<tr>
<td>MS + NAA</td>
<td>2.0</td>
<td>0.2</td>
<td>96.66 (2.50)*</td>
<td>2.11(0.70)*</td>
<td>5.4(0.61)* Friable, bulky, organogenic</td>
</tr>
<tr>
<td>MS + BAP</td>
<td>0.2</td>
<td>3.0</td>
<td>75.34 (2.01)*</td>
<td>4.33(1.03)*</td>
<td>6.3 (0.68)* Compact, slow growth with thick roots</td>
</tr>
<tr>
<td>MS + NAA + BAP</td>
<td>1.0</td>
<td>0.5</td>
<td>80.41 (1.60)*</td>
<td>3.20(1.24)*</td>
<td>7.6 (0.70)* Friable, bulky, organogenic with lengthy shoot and roots</td>
</tr>
</tbody>
</table>

(n = 15; values in parenthesis are SD)

LSD at 5% level (* = 0.90) ( # = 0.66)
of BAP. Similar findings have been reported in *Dictyospermum ovalifolium*\(^{11}\). 120 rooted plantlets were transferred directly to sand supplemented with Hoagland solution (Fig. 5), and after acclimatization, they were transferred to the field. 94% survival rate was recorded after 2 months.

In the present study, both BAP and NAA induced organogenesis and it appears that BAP is very essential to induce organogenesis. Similar results have been reported for legumes\(^ {12}\) and Foxtail millet\(^ {13}\). Higher concentrations (>1.5 mg L\(^{-1}\)) of NAA and BAP induced thick and compact callus with no shoot formation. These results presumably indicate a threshold level of endogenous hormones in the explants\(^ {14}\). In conclusion, the present work demonstrates a simple procedure for callus induction, shoot and root formation of *J. gendarussa* using leaf and nodal explants.

### References