Corresponding Author: G. Ganapathy Selvam, Plant Tissue Culture Laboratory, PG and Research Department of Botany and Microbiology, AVVM Sri Pushpam College (Autonomous) Poondi - 613 503, Thanjavur District, Tamil Nadu, India.
Medium and Culture Conditions: The explants were cultured on Murashige and Skoog (MS) medium [7] containing 3% (W/V) sucrose was used in all the experiments. The pH of the medium was adjusted to 5.8 prior to the addition of 0.8% (w/v) agar (HiMedia Laboratories Private Limited Mumbai, India). Molten medium was dispensed in 15 ml aliquots into culture tubes (25x150mm) and closed with non-absorbent cotton plugs. The medium was autoclaved at 1.1 kg/cm$^2$ pressure and 121°C temperature for about 15 min. All the cultures were incubated in a culture room maintained at 25±2°C and 55 to 65% RH fewer than 12 h photoperiod of 50-60 µmol m$^{-2}$ s$^{-1}$ light intensity provided by cool white fluorescent tubes.

Culture Establishment: The nodal explants were then singly placed in test tubes (25x150mm) with basal MS [2] medium supplemented with 6-benzyl amino purine (1.0mg L$^{-1}$) and adenine sulphate (20mg L$^{-1}$), sucrose (3%) and solidified with 0.6% agar supplied by Duchefa Bio-chemie (NL) to induce bud support and to select sterile shoots. Activated charcoal (Sigma, USA), 3g L$^{-1}$, was added to the medium to reduce browning. Medium pH was adjusted to 5.8 before autoclaving. After 30 days, the aseptic auxiliary shoots were transferred to a fresh medium of the same composition for 3 subcultures to produce a large number of shoots.

Shoot Multiplication: MS basal medium supplemented with various concentrations (0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of plant growth regulators such as benzylaminopurine (BAP) and Kinetin (KN) were added with adenine sulphate individually. All the cultures were transferred to fresh medium after 2-3 weeks duration. The mean number of shoots and their length and basal callus fresh weight were calculated after 6 weeks of inoculation.

RESULTS AND DISCUSSION

The results of callus induction and shoot propagation were tabulated in Table 1 and Figure 1 and 2. In all the BAP treatments, the explants were produced the bud formation, but 1.0 mg ml$^{-1}$ concentration of BAP treatment did not show any bud formation. Similarly, several authors have reported the bud formation in teak plants with different concentrations of growth viz., Devi et al. [12] reported the auxiliary shoot formation in 10 day incubation. Tiwari et al. [13] also observed the auxiliary shoot formation after 6 weeks. Similarly Goswami et al. [14] reported the maximum shoot length 4.7 cm in 8 weeks incubation. But in the present study, the maximum (7.5 cm) shoot average was identified within 30 days (Table 1). In all the BAP treatments, the maximum (7.5 cm) shoot length was identified with the 3.0 mg/L concentration. Increased shoot length formation was identified with increased concentration of BAP treatments, but no callus formation was observed in 1.0 mg/L concentration of BAP treatment. In the KN treatments, the maximum (0.7 cm) shoot length was identified with 2.0 mg/L concentration, but all the other treatments were not showed any shoot induction. Likewise, Gangopadhyay et al. [5] also reported the stunning growth properties with KN treatments and enhanced growth properties with BAP treatments. According to the literature, BAP and KN is the most commonly used cytokinins for micropropagation of teak plants, but high concentration of KN did not show any

Table 1: Effect of BAP and Kn on shoot formation from the nodal explants of Tectona grandis on MS medium

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Growth regulators concentrations (mg/L)</th>
<th>Presence of callus (C) and shoot (S) formation</th>
<th>Days to callus and shoot formation</th>
<th>Height of shoots (cm)</th>
<th>Morphology and colour of callus</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 1</td>
<td>0.5</td>
<td>S</td>
<td>7</td>
<td>Live</td>
<td>Shoot only</td>
</tr>
<tr>
<td>T 2</td>
<td>1.0</td>
<td>S + C</td>
<td>14</td>
<td>Live</td>
<td>Shoot only</td>
</tr>
<tr>
<td>T 3</td>
<td>1.5</td>
<td>S + C</td>
<td>21</td>
<td>1.3</td>
<td>Yellow Green + S</td>
</tr>
<tr>
<td>T 4</td>
<td>2.0</td>
<td>S + C</td>
<td>28</td>
<td>3.3</td>
<td>Brown Yellow + S</td>
</tr>
<tr>
<td>T 5</td>
<td>2.5</td>
<td>S + C</td>
<td>35</td>
<td>4.8</td>
<td>Light Yellow + S</td>
</tr>
<tr>
<td>T 6</td>
<td>3.0</td>
<td>S + C</td>
<td>42</td>
<td>7.5</td>
<td>White, Light Greenish + S</td>
</tr>
<tr>
<td>T 7</td>
<td>0.5</td>
<td>C</td>
<td>7</td>
<td>Live</td>
<td>Brown</td>
</tr>
<tr>
<td>T 8</td>
<td>1.0</td>
<td>C</td>
<td>14</td>
<td>Live</td>
<td>White light Yellow</td>
</tr>
<tr>
<td>T 9</td>
<td>1.5</td>
<td>C</td>
<td>21</td>
<td>Live</td>
<td>Light Greenish</td>
</tr>
<tr>
<td>T 10</td>
<td>2.0</td>
<td>C + S</td>
<td>28</td>
<td>0.7</td>
<td>Greenish white + S</td>
</tr>
<tr>
<td>T 11</td>
<td>2.5</td>
<td>C + S</td>
<td>35</td>
<td>0.5</td>
<td>Yellow + S</td>
</tr>
<tr>
<td>T 12</td>
<td>3.0</td>
<td>C</td>
<td>42</td>
<td>Live</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

Notes - = No growth; S = Shoot; C = Callus; T = Treatment; BAP = Benzylaminopurine; KN = Kinetin
Fig. 1: Picture showing the shoot formation
1. Initiation of Burst from the nodal explants on MS medium fortified with 0.5 mg/L BAP with KN after 1 weeks; 2. Initiation of shoot in explants MS medium supplemented 1.0 mg/L BAP after 2 weeks; 3. Induction of basal callus from the micro shoots was observed on MS medium supplemented with 1.5mg/L KN after 3 weeks; 4. Formation of shoots from nodal plants on MS medium supplemented 2.0mg/L BAP after 4 weeks; 5. Formation of quality of shoot from Explants on supplemented 2.5mg/L KN after 5 weeks; 6. Developing of shoot from nodal plants on supplemented 3.0mg/L BAP with KN after 6 weeks.

Fig. 2: Picture showing the formation of root nodels from explants
7. Initiation of callus from the leaf on MS medium fortified with 0.5 mg/L KN after 2 weeks. 8. Formation of callus in leaf MS medium supplemented 1.0mg/L BAP after 3 weeks. 9. Induction of basal callus from the micro shoots was observed on MS medium supplemented with 1.5mg/L KN after 3 weeks. 10. Formation of callus from leaf on MS medium supplemented 2.0mg/L BAP after 4 weeks. 11. Formation of quality of callus with micro shoots from Explants on supplemented 2.5mg/L KN after 5 weeks. 12. Maturity of callus from leaf on supplemented 3.0mg/L BAP, KN with cytokinin after 6 weeks.

formation of the callus indication and this might be due to the inhibitory properties of auxiliary bud sprouts [15]. Phenolic compounds cause necrosis and death in in vitro tissues. It is, hence, a prerequisite to remove these compounds from explants before culturing to avoid medium darkening that is usually a result of phenolic exudation [16]. In some instances the blackening of explants were observed in the beginning stage of budding formation and this might be due to the higher exudation of phenolic [17], further the phenolic content was reduced by the addition of the charcoals and this might be due to the absorption properties of the charcoal [18]. The present study provides an efficient in vitro propagation method which could be commercially feasible for teak, by providing a protocol for producing genetically similar plants from selected genotypes. This work showed the highest number of micropropagated shoots reported for teak, up to now, in the available literature and in a relative short period of time, producing about quality of shoot within 6 weeks. Some steps were important for improving quality of shoots, aimed at improving shoot length percentage, by MS medium composition in salt balance.

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REFERENCES


