7. BUTEA—ROOTING AND TRANSPLANTATION

7.1 Introduction

Axillary shoots induced in-vitro from cotyledonary and mature tree nodes need to be separated and rooted in order to establish them in the soil. Shoots elongated to a minimum length of 2 cm were isolated periodically and transferred to various rooting media.

7.2 Effect of auxins on root initiation.

Shoots which had elongated to a minimum length of 2 cm were harvested and transferred for rooting to MS media incorporated with various concentrations of IBA, NAA and filter sterilized IAA. IBA and NAA were added to the medium before autoclaving, IAA was added after autoclaving just before the medium started gelling. The cultures were kept under total darkness and under a 16 hour photoperiod.

7.2.1 Effect of IAA.

Roots were not produced with IAA under dark and light conditions from the excised shoots. (Table 7.1). Only callus was produced at the cut end of the shoots. The amount of callus increased with increase in concentration of IAA. The rate of response in dark was lower compared to cultures incubated under a 16 hour photoperiod.
Table 7.1: Response of excised microshoots of *Butea* at the end of 30 days on MS medium incorporated with 20 g/l sucrose and varying concentration of IAA under a 16 hour photoperiod and in total darkness.

<table>
<thead>
<tr>
<th>IAA (mg/l)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Callus</td>
</tr>
<tr>
<td>0.1</td>
<td>21.32</td>
<td>-a</td>
</tr>
<tr>
<td>0.5</td>
<td>19.42</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>20.31</td>
<td>-</td>
</tr>
<tr>
<td>1.5</td>
<td>21.93</td>
<td>-</td>
</tr>
<tr>
<td>2.0</td>
<td>20.46</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>20.93</td>
<td>-</td>
</tr>
</tbody>
</table>

+ : Presence, - : Absence of roots/callus

Table 7.2: Response of excised microshoots to IBA at the end of 30 days on MS medium incorporated with 20 g/l sucrose, various concentrations of IBA and incubated under a 16 hour photoperiod and in total darkness.

<table>
<thead>
<tr>
<th>IBA (mg/l)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Callus</td>
</tr>
<tr>
<td>0.0</td>
<td>10.37</td>
<td>-a</td>
</tr>
<tr>
<td>0.5</td>
<td>10.93</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>11.01</td>
<td>-</td>
</tr>
<tr>
<td>1.5</td>
<td>10.74</td>
<td>-</td>
</tr>
<tr>
<td>2.0</td>
<td>10.25</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>10.81</td>
<td>-</td>
</tr>
</tbody>
</table>

+ : Presence, - : Absence of roots/callus

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7.2.2 Effect of IBA.

IBA both under light and in dark failed to induce roots on the excised shoots (Table 7.2). Only callus was produced at the cut surface of the microshoots. On further subculturing the microshoots become necrotic.

7.2.3 Effect of NAA.

No roots were produced from the excised shoots with NAA (Table 7.3). Only callus was formed at the cut end of the shoots.

Table 7.3: Response of excised microshoots to NAA at the end of 30 days on MS medium incorporated with 20 g/l sucrose, varying concentrations of NAA and incubated under a 16 hour photoperiod and in total darkness.

<table>
<thead>
<tr>
<th>NAA (mg/l)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response of explants (%)</td>
<td>Roots</td>
</tr>
<tr>
<td>0.1</td>
<td>14.93 - a</td>
<td>15.32 - a</td>
</tr>
<tr>
<td>0.5</td>
<td>16.87 -</td>
<td>16.24 -</td>
</tr>
<tr>
<td>1.0</td>
<td>15.09 -</td>
<td>16.64 -</td>
</tr>
<tr>
<td>1.5</td>
<td>14.12 -</td>
<td>17.71 -</td>
</tr>
<tr>
<td>2.0</td>
<td>12.47 -</td>
<td>15.46 -</td>
</tr>
<tr>
<td>2.5</td>
<td>12.01 -</td>
<td>14.32 -</td>
</tr>
</tbody>
</table>

+ : Presence, - : Absence of roots/callus

75
7.3 Effect of 2iP and Zeatin singly and in combination.

As zeatin and 2iP induced roots on leaf discs of *Butea* they were tested for rooting of microshoots. Excised microshoots were inoculated to cotton plugged test tubes containing 10 ml of MS medium with varying concentrations of 2iP and zeatin under light and dark conditions at the end of 30 days.

Table 7.4: Response of excised microshoots of *Butea* on MS medium with varying concentrations of 2iP and zeatin under light and dark conditions at the end of 30 days.

<table>
<thead>
<tr>
<th>Zeatin (mg/l)</th>
<th>2iP (mg/l)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Roots</td>
<td>% Callus</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>21.32</td>
<td>-a</td>
</tr>
<tr>
<td>0.1</td>
<td>-</td>
<td>24.46</td>
<td>+</td>
</tr>
<tr>
<td>0.5</td>
<td>-</td>
<td>23.32</td>
<td>+</td>
</tr>
<tr>
<td>1.0</td>
<td>-</td>
<td>24.39</td>
<td>+</td>
</tr>
<tr>
<td>1.5</td>
<td>-</td>
<td>23.74</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>-</td>
<td>22.36</td>
<td>+</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>23.10</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>11.32</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>0.1</td>
<td>14.36</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>0.5</td>
<td>12.39</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>1.0</td>
<td>11.36</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>1.5</td>
<td>11.91</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>2.0</td>
<td>12.28</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>2.5</td>
<td>12.74</td>
<td>+</td>
</tr>
<tr>
<td>0.1</td>
<td>0.5</td>
<td>9.46</td>
<td>+</td>
</tr>
<tr>
<td>0.1</td>
<td>1.0</td>
<td>9.91</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>1.5</td>
<td>18.49</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>1.0</td>
<td>18.36</td>
<td>+</td>
</tr>
<tr>
<td>2.5</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a

+: Presence, -: Absence of roots/callus

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medium incorporated with 20 g/l sucrose, varying concentration of 2ip and zeatin both singly and in combination in the range of 0 - 2.5 mg/l. The cultures were incubated both in light and total darkness. The response at the end of 30 days was noted (Table 7.4).

Zeatin and 2ip singly or in combination did not induce roots on excised microshoots. Only callus was induced which failed to produce roots on further subculturing.

7.4 Effect of Catechol.

7.4.1 Catechol incorporated in the medium.

Microshoots more than 2 cm in length were isolated, transferred to MS medium incorporated with varying concentrations of catechol in the range of 0-5 mg/l and were incubated both under a 16 hour photoperiod and in complete darkness (Table 7.5).

Table 7.5: Response of microshoots on MS medium incorporated with 0-5 mg/l catechol and cultured under a 16 hour photoperiod and in complete darkness at the end of 30 days.

<table>
<thead>
<tr>
<th>Catechol</th>
<th>Length of root (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3 - 5</td>
</tr>
<tr>
<td>5</td>
<td>3 - 5</td>
</tr>
</tbody>
</table>
When catechol was incorporated into the medium only a single root was produced. The root was fibrous, strong and brown in colour and had no root hairs. Increase in the concentration of catechol increased the length of the root but the shoot size remained the same (Fig 7.1). The rooted microshoots when transplanted to soil did not survive.

7.4.2 Excised microshoots dipped in catechol solution.

The cut ends of the excised microshoots were dipped in catechol solutions of varying concentration for 15 minutes. The shoots were then planted in conical flasks containing sterile sand moistened with sterile distilled water. Sterile water was added every alternate day to retain the moisture of the sand. The cultures were kept in total darkness and under a 16 hour photoperiod in the culture room. Observations were made at the end of 30 days (Table 7.6).

Table 7.6: Response of microshoots dipped in catechol, planted in sterile sand and incubated in total darkness and under a 16 hour photoperiod.

<table>
<thead>
<tr>
<th>Catechol (mg/l)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Callus</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>a</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5.0</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

a

+ : Presence, - : Absence of roots/callus

With catechol only callus was produced in light. Roots were induced only in total darkness. The roots were very delicate and broke off easily during transplantation to soil (Fig 7.2).
7.5 Transplantation to soil.

Soil mixtures of the following composition (v:v) were prepared.

a) Sand:Soil (1:1)
b) Sand:Soil:Soilrite mix (2:2:2)
c) Sand:Coconut husk (1:1)

The soil mixtures were oven sterilized at 200°C for 3 hours, allowed to cool to the room temperature and transferred to perforated polythene bags of 20 X 12 cm size.

Excised microshoots were dipped in 5 mg/l catechol for half an hour and planted in the plastic pots containing various soil mixtures. The plants were kept in a glass house (Fig 7.3) where they received diffused day light filtered through tinted fibre glass sheets and were protected with polythene covers to maintain humidity. The number of plants which survived at the end of 60 days of transplantation was noted (Table 7.7).

Table 7.7: Survival of microshoots dipped in catechol and planted in different soil mixtures.

<table>
<thead>
<tr>
<th>Composition of soil mixtures</th>
<th>Survival of plants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand:Soil (1:1)</td>
<td>12.04</td>
</tr>
<tr>
<td>Sand:Soil:Soilrite mixture$^a$ (2:2:2)</td>
<td>-</td>
</tr>
<tr>
<td>Sand:Coconut husk (1:1)</td>
<td>14.36</td>
</tr>
</tbody>
</table>

$^a$ Soilrite is a commercial product (mixture of expanded vermiculite, exfoliated perlite and peat moss) of Karnataka Explosives, Ltd., Bangalore.

Only a few microshoots transplanted to the soil survived on sand:soil and sand:coconut husk mixture. Most of them had only 1 or 2 roots which were weak and thin.
The rooted plants were carefully removed from the soil mixture and transferred to pots containing unsterilized soil. The pots were kept in partial shade and medium humidity for hardening. The plants grew slowly and were weak (Fig 7.4).

7.6 Conclusion.

Microshoots of *Butea* could not be rooted with any of the root inducing auxins.

A single fibrous root without root hairs was formed when catechol was added to the medium. Plantlets with such roots transferred to the soil did not survive. Dipping the microshoots in catechol and planting them in the soil results in root formation.

Plants transferred to soil in pots survive but the stems remain weak and the plants appear like climbers.