Micropropagation of white palash tree 
(*Butea monosperma* [Lam.] Taub. Var. *lutea* [Witt.])

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**ABSTRACT**

An efficient and reproducible protocol is established for rapid *in vitro* multiplication of an endangered, valuable, medicinal plant, *Butea monosperma* (Lam.) Taub. Var. *lutea*, through cotyledonal nodes of mature seeds. Among various cytokinins tested, high frequency of direct shoot regeneration was induced on Murashige and Skoog (MS) medium supplemented with benzylaminopurine, which found to be more effective and showed an optimal response at 2 mg/L with a maximum number of 8.35 ± 0.32 multiple shoots per explant. Proliferation of shoots was established by repeated subculturing onto same regeneration medium with 2-3 weeks of the time interval. Rooting of regenerated shoots was achieved after 3 weeks of culture on MS medium containing 1 mg/L indole-3-butyric acid. *In vitro* raised plantlets were transferred to pots containing sterilized soil and vermiculate mixture in 1:1 ratio and then shifted to greenhouse. Well-established plantlets exhibited 75% survival rate.

**KEY WORDS:** Benzylaminopurine, *Butea monosperma* var. *lutea*, cotyledonal node, thidiazuron

**INTRODUCTION**

*Butea monosperma* (Lam.) Taub. Var. *lutea*, belonging to the family Fabaceae, is an endangered, valuable, woody plant. This plant is popularly known as white palash having ivory-white flower buds (Jadhav et al., 2001). This plant species is in much demand in folk medicine and possesses unique phytochemicals such as flavonoids, chalcones, linoleic acid, and unsaturated fatty acids (Thirupataiah, 2007). Chemical screening of different parts of this plant species has shown multipurpose values. The presence of effective compounds such as cyclitols which are known to heal asthma and chronic bronchitis. The extract from stem bark is used to cure leukorrhea, jaundice, and several skin diseases (Reddy et al., 2001). The distribution of this species has been limited in area that needs immediate conservation. This tree species is reported around Sabarkantha of Gujarat (Hitesh and Patel, 2015), Aurangabad in Maharashtra, and in few regions of Telangana such as Siricilla forests of Karimnagar, Peddagutta of Nizamabad, and Mallakpally of Warangal district (Reddy et al., 2008). Conventional propagation of this species through seeds has limited scope for large scale propagation because of its poor seed germination percentage, and however, enhanced *in vitro* seed germination protocol for its conservation has been developed with the highest percentage (65%) (Mahender et al., 2014). Clonal propagation of most forest trees was generally carried out from seedling derived juvenile explants as adult tissues are resistant in nature (The Wealth of India, 1988). However, compared to conventional propagation methods, *in vitro* clonal propagation is a common alternative means, which has been extensively applied for large scale multiplication of many other important forest tree species (Ahuja, 1993; Bonga and von Aderkas, 1992). There are a few other similar successful reports on *in vitro* multiplication of different forest tree species using cotyledonal node explants (Chand and Singh, 2004; Barik et al., 2007). Thus, the authors described a simple, reproducible, and improved *in vitro* regeneration protocol of *B. monosperma* var. *lutea* through cotyledonal node explants derived from axenic seedlings.

**MATERIALS AND METHODS**

**Plant Material**

The dried pods were collected from 10-year-old *B. monosperma* (Lam.) Taub. Var. *lutea* located at Kothakonda village of Karimnagar district, Telangana. The seeds were
removed from the pods and washed thoroughly under running tap water for 15 min followed by treatment with an aqueous solution of Tween-20 for 10 min and rinsed five times with double-distilled water. The seeds were then surface sterilized with an aqueous solution of 0.1% (w/v) HgCl\(_2\) for 7 min and rinsed thoroughly with autoclaved distilled water. The disinfected seeds were inoculated in 300-ml screw-capped glass jars containing Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) and 8.0 g/L of agar.

**Multiple Shoot Induction**

12-day-old axenic seedlings were used as the source of explants. After removal of the radicle and primary shoots, the cotyledonal nodes were inoculated in 300 mL screw capped glass jars (one explant per vessel) containing MS medium supplemented with 0.5-3.0 mg/L of N\(^6\)-benzylaminopurine (BAP), kinetin (KN), and thidiazuron (TDZ). The pH of the media was adjusted to 5.8. All the cultures were maintained under similar conditions at 24 ± 2°C under 16-h light/8-h dark cycle at an intensity of 60 μ mol/m\(^2\)/s. The cultures were shifted to fresh medium at regular intervals of 21 days. For shoot development experiment, each treatment consisted of 20 replicates (culture vessels), each containing one explant. The percentage of shoot proliferation, number of shoots per explants, and shoot length were recorded after 4 weeks of culture.

**Rooting and Acclimatization**

Well-developed shoots (4.5-5.0 cm) with fully expanded leaflets were excised and inoculated in MS medium gelled with 0.8% (w/v) agar. The medium was supplemented with 0.5-3.0 mg/L indole-3-butyric acid (IBA), and roots were initiated within 3 weeks of culture. In the rooting experiment, each treatment consisted of 15 replicates (culture tubes) each with one explant and the results of root length and number were recorded after 4 weeks of culture. Plantlets of 3-4 week old were removed from culture medium, and the roots were washed gently under running tap water to remove the remnant traces of agar. The plantlets were then transferred to the pots containing autoclaved vermicompost mixed with soil (1:1) and moistened with sterile water, and the acclimatized plantlets were transferred to the greenhouse.

**RESULTS AND DISCUSSION**

In the present investigation, an easy, reproducible protocol was developed for direct multiple shoot regeneration from cotyledonal nodes derived from axenic seedlings of mature seeds. Recently, the application of micropropagation techniques to a range of plant species has been increased with interest to excised cotyledonal node as suitable experimental material, including *Acacia nilotica* (Dewan et al., 1992), *Dalbergia sissoo* (Pradhan et al., 1998), *Sterculia auriculata* (Purohit and Dave, 1996), and *Sesbania rostrata* (Jha et al., 2004). The type and concentrations of cytokinin used will significantly affect the percentage of shoot regeneration, number, and length of shoots, respectively. Among the three different cytokinins tested (BAP, KN and TDZ), BAP at 2 mg/L was found to be most effective which induced proliferation percentage of 91.9 ± 1.79 and 8.35 ± 0.32 shoots per explant with an average length of 4.15 ± 0.28 cm (Table 1 and Figure 1a and b). Further higher concentrations of BAP did not improve shoot regeneration. Continuous presence of BAP in the medium did not affect the shoot proliferation or its elongation in any manner. Among the KN and TDZ concentrations tested, percentage shoot regeneration was optimal at 1.0 mg/L with 53.25 ± 1.89 and 33.75 ± 1.21, respectively. The comparative studies with different plant growth regulators for multiple shoot formation were effectively demonstrated in *Jatropha integerrima* (Sujatha and Dhingra, 1993), *Pterocarpus marsupium* Roxb. (Chand and Singh, 2004), and *Albizia falcataria* (Widiyanto et al., 2008). The superiority and differential effectiveness of BAP over KN and *viz.* on axillary shoot proliferation might show their different modes of action on different plant species (Shyamkumar et al., 2003; Nandwani and...
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1.35 ± 0.18
25.25 ± 1.17
1.85 ± 0.22
23.65 ± 0.68
3.35 ± 0.20
4.15 ± 0.28
21.75 ± 0.75
43.15 ± 1.81
3.75 ± 0.59
Clitoria ternatea
43.5 ± 0.88.

I BA was the most effective in percentage response for rooting of regenerated shoots at 84.5 ± 0.98. Root formation occurred on MS medium supplemented with 1 mg/L IBA after 25-30 days of culture with 3.75 ± 0.59 number of roots per explant with a root length of 2.45 ± 0.36 cm (Table 2 and Figure 1d). The promotive effect of IBA on in vitro rooting has also been reported in several plant species, including Bauhinia variegata (Mathur and Kumar, 1996), Dalbergia latifolia (Swamy et al., 1992), Phellodendron amurense (Azad et al., 2005), Syzygium cumini (Yadav et al., 1990), and Acacia mearnsii (Beck et al., 1998).

Table 1: Effect of different plant growth regulators on direct shoot regeneration from cotyledonary nodes of Butea monosperma (Lam.) Taub. Var. lutea

<table>
<thead>
<tr>
<th>Cytokinin (mg/L)</th>
<th>Mean ± SE</th>
<th>Plantlet Induction (%)</th>
<th>Number of shoots/explant</th>
<th>Shoot Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BAP</strong></td>
<td></td>
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</tr>
<tr>
<td>0.5</td>
<td>23.75 ± 1.21</td>
<td>2.15 ± 0.22</td>
<td>1.35 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>74.25 ± 1.69</td>
<td>6.55 ± 0.36</td>
<td>3.05 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>91.9 ± 1.79</td>
<td>8.35 ± 0.32</td>
<td>4.15 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>43.15 ± 1.81</td>
<td>4.25 ± 0.28</td>
<td>2.05 ± 0.18</td>
<td></td>
</tr>
<tr>
<td><strong>KN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>21.2 ± 0.66</td>
<td>1.65 ± 0.22</td>
<td>0.95 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>53.25 ± 1.89</td>
<td>5.65 ± 0.35</td>
<td>2.75 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>34.25 ± 1.14</td>
<td>3.35 ± 0.20</td>
<td>1.65 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>23.65 ± 0.68</td>
<td>2.15 ± 0.18</td>
<td>1.35 ± 0.18</td>
<td></td>
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<tr>
<td><strong>TDZ</strong></td>
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<td></td>
</tr>
<tr>
<td>0.5</td>
<td>19.25 ± 0.90</td>
<td>1.85 ± 0.22</td>
<td>0.75 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>33.75 ± 1.21</td>
<td>3.55 ± 0.35</td>
<td>2.25 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>28.25 ± 1.34</td>
<td>2.7 ± 0.40</td>
<td>1.35 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>25.25 ± 1.17</td>
<td>2.55 ± 0.33</td>
<td>1.15 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± SE represents the number of shoots/explant of 20 replicates (culture vessels) per treatment and each vessel containing one explant. The data were recorded after 3-4 weeks of culture. SE: Standard error, TDZ: Thidiazuron, KN: Kinetin, BAP: Benzylaminopurine

Table 2: Influence of different concentrations of IBA on root induction in in vitro regenerated shoots of Butea monosperma (Lam.) Taub. Var. lutea

<table>
<thead>
<tr>
<th>Auxin (mg/L)</th>
<th>Root Induction (%)</th>
<th>Number of shoots/explant</th>
<th>Length of roots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>21.75 ± 0.75</td>
<td>0.85 ± 0.15</td>
<td>1.05 ± 0.15</td>
</tr>
<tr>
<td>1.0</td>
<td>84.5 ± 0.98</td>
<td>3.75 ± 0.59</td>
<td>2.45 ± 0.36</td>
</tr>
<tr>
<td>2.0</td>
<td>43.5 ± 0.88</td>
<td>1.45 ± 0.30</td>
<td>1.35 ± 0.25</td>
</tr>
</tbody>
</table>

Data were collected after 3-4 weeks of culture, and the values are given as mean ± SE represents the number of roots/shoot of 15 replicates (culture tubes) per treatment. SE: Standard error, IBA: Indole-3-butyric acid

Hardening is a vital step after successful rooting of microshoots, attempts were taken to establish regenerated plantlets onto soil. The rooted plantlets were transferred to pots containing soil mix and compost (1:1) (Figure 1e). The survival rate of these plants after transfer was 75%. Hardened plants were finally transferred to clay pots after 28 days. After 2 months, aclimatized plantlets were transferred to the greenhouse. All the established plants showed a high degree of uniformity without any detectable morphological variation and growth characteristics when compared to donor mother plant.

CONCLUSION

In conclusion, the in vitro protocol reported in this study for regenerating plantlets of B. monosperma var. lutea using cotyledonal nodes of axenic seedlings is an improved method and may open up the challenging opportunities for large scale in vitro propagation and its conservation. Future research in B. monosperma var. lutea may direct toward progressive development in genetic transformation studies of various useful traits.

ACKNOWLEDGMENTS

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