Original Article

Journal of Phytology 2017, 9: 7-10
http://scienceflora.org/journals/index.php/jp/
doi: 10.19071/jp.2017.v9.3070

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

D. Rathnaprabha, N. Muralikrishna, E. Raghu, V. Yashodhara, A. Sadanandam*

Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

Received: 24.06.2016
Accepted: 03.07.2016
Published: 12.07.2016

***Address for correspondence:**
A. Sadanandam, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India.
E-mail: nandamas@rediffmail.com

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 cotyledonary 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*, cotyledonary 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 Sircilla 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 cotyledonary 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 cotyledonary 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 cotyledonary 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 remainant 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 cotyledonary 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 cotyledonary node as suitable experimental material, including *Acacia nelotica* (Dewan *et al*., 1992), *Dalbergia sissoo* (Pradhan *et al*., 1998), *Sterculi aurens* (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

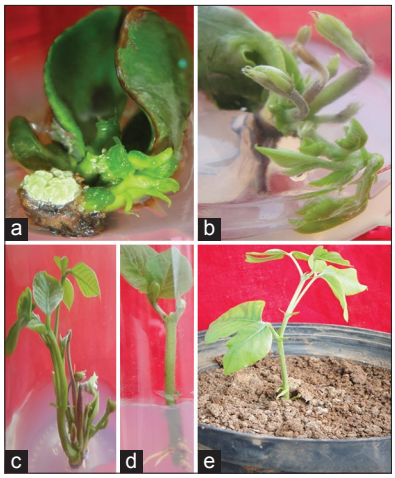

Figure 1: *In vitro* plant regeneration of *Butea monosperma* (Lam.) Taub. Var. *lutea* via multiple shoot proliferation from cotyledonary nodes. (a) Induction of multiple shoots from cotyledonary node explants, (b) proliferation of multiple shoots on Murashige and Skoog (MS) medium with 2 mg/L benzylaminopurine after 3-4 weeks of culture, (c) elongation of shoots on same proliferation medium, (d) rooting of shoot grown in MS medium supplemented with 1 mg/L indole-3-butyric acid, (e) complete plantlet acclimatized in soil

Ramawat, 1993) (Figure 1c). The frequency of axillary shoot induction in many woody tree species with TDZ as a potent cytokinin for micropropagation is not in accordance with the present finding where there are lower comparable rates of shoot growth and elongation (Husain *et al*., 2007).

IBA 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 per 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 variegate* (Mathur and Kumar, 1996), *Dalbergia latifolia* (Swamy *et al*., 1992), *Phellodendron amurense* (Azad *et al*., 2005), *Syzygium cuminii* (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*

Cytokinin (mg/L)	Mean±SE		
	Percentages shoot proliferation	Number of shoots/explants	Shoot length (cm)
BAP			
0.5	23.75±1.21	2.15±0.22	1.35±0.30
1.0	74.75±1.69	6.55±0.38	3.05±0.21
2.0	91.9±1.79	8.35±0.32	4.15±0.28
3.0	43.15±1.81	4.25±0.28	2.05±0.18
KN			
0.5	21.2±0.66	1.65±0.22	0.95±0.06
1.0	53.25±1.89	5.65±0.35	2.75±0.39
2.0	34.25±1.14	3.35±0.20	1.65±0.26
3.0	23.65±0.68	2.15±0.18	1.35±0.18
TDZ			
0.5	19.25±0.90	1.85±0.22	0.75±0.13
1.0	33.75±1.21	3.55±0.35	2.25±0.38
2.0	28.25±1.34	2.7±0.40	1.35±0.25
3.0	25.25±1.17	2.55±0.33	1.15±0.18

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*

Auxin (mg/L)	Root induction (%)	Number of shoots/explants	Length of roots (cm)
IBA			
0.5	21.75±0.75	0.85±0.15	1.05±0.15
1.0	84.5±0.98	3.75±0.59	2.45±0.36
2.0	43.5±0.88	1.45±0.30	1.35±0.25

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, acclimatized 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 cotyledonary 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

Rathnaprabha would like to acknowledge the financial assistance provided by the University Grants Commission-Rajiv Gandhi National Fellowship (No.F.17-1(ST)/2012-2013 (SA-III)).

REFERENCES

Ahuja M. Micropapagation of Woody Plants. Dordrechet, The Netherlands: Kluwer Academic Publ.; 1993. p. 507.

Azad MA, Yokota S, Ohkubo T, Andoh Y, Yahara S, Yoshizawa N. *In vitro* regeneration of the medicinal woody plant *Phellodendron amurense* Rupr. through excised leaves. Plant Cell Tissue Organ Cult 2005;80:43-50.

Barik DP, Naik SK, Mudgal A, Chand PK. Rapid plant regeneration through *in vitro* axillary shoot proliferation of butterfly pea (*Clitoria ternatea* L). A twinning legume. *In vitro* Cell Dev Biol 2007;43:144-8.

Beck SL, Dunlop RW, Staden JV. Micropropagation of *Acacia mearnsii* from *ex vitro* material. Plant Growth Regul 1998;26:143-8.

Bonga JM, von Aderkas P. *In vitro* Culture of Trees. Dordrechet, The Netherlands: Kluwer Academic Publ.;1992. p. 236.

Chand S, Singh AK. *In vitro* shoot regeneration from cotyledonary node explants of a multipurpose leguminous tree, *Pterocarpus marsupium* Roxb. *In vitro* Cell Dev Biol Plant 2004;40:464-6.

Dewan A, Nanda K, Gupta SC. *In vitro* micropropagation of

Acacia nilotica subsp. indica Brenan via cotyledonary nodes. Plant Cell Rep 1992;12:18-21.

Hitesh P, Patel RS. *Butea monosperma* (Lam.) var. *lutea* (Witt) (*Fabaceae*): A new species record from Gujarat state, India. Int J Curr Microbiol Appl Sci 2015;4:867-9.

Husain MK, Anis M, Shahzad A. *In vitro* propagation of Indian Kino (*Pterocarpus marsupium* Roxb.) using Thidiazuron. *In vitro* Cell Dev Biol Plant 2007;43:59-64.

Jadhav SN, Ved DK, Ghate U, Reddy KN, Reddy CS. Proceedings of the Workshop on Conservation Assessment and Management Planning for Medicinal Plants of Andhra Pradesh. Bangalore: FRLHT; 2001.

Jha AK, Prakash S, Jain N, Nanda K, Gupta SC. Micropropagation of *Sesbania rostrata* from the cotyledonary node. Biol Plant 2004;48:289-92.

Mahender A, Damodar MM, Murthy EN. *In vitro* seed germination and development of *Butea monosperma* (Lam.) Taub. Var. *lutea* (Witt.): A step for rehabilitation. Int J Multidiscip Curr Res 2014;2:297-301.

Mathur J, Kumar SM. Micropropagation of *Bauhinia veriegata* and *Parkensonia aculeata* from nodal explants of mature trees. Plant Cell Tissue Organ Cult 1996;28:169-75.

Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 1962;15:473-97.

Nandwani D, Ramawat KG. *In vitro* plantlet formation through juvenile and mature explants in *Prosopsis cineraria*. Indian J Exp Biol 1993;31:156-60.

Pradhan C, Pattnaik SK, Chand PK. Propagation of *Dalbergia sissoo* Roxb through *in vitro* shoot proliferation from cotyledonary nodes. Plant Cell Rep 1998;18:122-6.

Purohit SD, Dave A. Micropropagation of *Sterculia urens* Roxb - An endangered tree species. Plant Cell Rep 1996;15:704-6.

Reddy CS, Murthy EN, Reddy KN, Raju VS. *Butea monosperma* (Lam.) Taub var. *lutea* (Witt): A little known taxon of India needs immediate conservation. Proceeding A.P. Akademi Sci 2008;12:293-5.

Reddy CS, Reddy KN, Jadhav SN. Threatened Medicinal Plants of Andhra Pradesh. Hyderabad: EPTRI; 2001.

Shyamkumar B, Anjaneyulu C, Giri C. Multiple shoot induction from cotyledonary node explants of *Terminalia chebula*. Biol Plant 2003;47:585-8.

Sujatha M, Dhingra M. Rapid plant regeneration from various explants of *Jatropha integerrima*. Plant Cell Tissue Organ Cult 1993;35:293-6.

Swamy BV, Himabindu K, Sita GL. *In vitro* micropropagation of elite rosewood (*Dalbergia sissoo* Roxb.). Plant Cell Rep 1992;11:126-31.

The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Publication and Information Directorate. Vol. II. New Delhi, India: CSIR; 1988.

Thirupataiah A. Isolation of New flavonoides and their Antimicrobial Activity from Medicinal Plants of Telangana Region. 2007. Ph.D. Thesis, Kakatiya University, Warangal, India.

Widiyanto SN, Sari MD, Irwanto RR. Effect of cytokinins and carbenicillin on *in vitro* axillary-shoot growth of *Albizia* (*Albizia falcataria* (L.) Fosberg). J Mat dan Sains 2008;13:43-9.

Yadav U, Lal M, Jaiswal VS. *In vitro* micropropagation of tropical fruit trees *Syzygium cuminii* L. Plant Cell Tissue Organ Cult 1990;21:87-92.