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An efficient micropropagation protocol for teak (*Tectona grandis* L.) using nodal explants and ex vitro rooting

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Abstract

Teak (*Tectona grandis* L.) is one of the most valuable tropical hardwood species; however, large-scale propagation of elite genotypes is constrained by poor seed germination and limited vegetative multiplication. The present study describes an efficient micropropagation protocol using nodal explants collected from a 20-year-old elite teak plus tree (var. Teli). Approximately 80% aseptic culture establishment was achieved on Woody Plant Medium (WPM) supplemented with 0.5 mg L⁻¹ 6-benzylaminopurine (BAP), with axillary bud break occurring within 3–4 days. Shoot multiplication was significantly influenced by growth regulator concentration, with the highest mean shoot number (3.92) recorded on WPM containing 5.0 mg L⁻¹ BAP, although optimal shoot quality and elongation were obtained at 0.5 mg L⁻¹ BAP. Ex vitro rooting was successfully induced by pulse treatment of microshoots with 1.0 mg L⁻¹ indole-3-butyric acid (IBA), followed by direct transfer to potting substrates. A soil mixture containing soil, sand, cocopeat, and vermicompost resulted in the highest survival rate (90%) during hardening. The protocol offers a simplified, cost-effective, and reliable method for rapid clonal propagation of mature teak trees, suitable for large-scale plantation forestry.

Keywords: *Tectona grandis*, micropropagation, nodal explants, BAP, ex vitro rooting

Introduction

Tectona grandis (L.) belongs to family *Verbenaceae* and commonly called teak, Indian oak, Bangkok teak, etc. (Tiwari *et al.* 2002) ^[1]. Teak is a valuable hard wood tree of the tropics which is considered to be the best timber available for a wide variety of uses. International demand, which is getting stronger and stronger for good quality teak, has resulted in an ever increasing depletion of both natural and plantation teak resources. Teak is conventionally reproduced through seeds (in natural conditions) but germination is often difficult because the hard seed coat limits the production of a large number of seedlings in a defined time. The propagation of teak via cutting has been reported but the limitation to it is that from selected individuals few propagules are produced. Currently *in vitro* propagation is being viewed as an alternative method of clonal propagation. Over-exploitation caused destruction of natural teak forests which warranted urgent need to develop an alternative technique like tissue culture (Gupta *et al.* 1980) ^[4] to accelerate the production of plantation propagules. Gangopadhyay *et al.* (2002) ^[2] utilized the tissue culture technique for the production of true to type plant and established genetic fidelity of the regenerants. However, micropropagation of teak from mature trees has remained problematic. Poor explant response and phenolic exudates from cut ends of explants taken from plus tree pose great problems for establishment of *in vitro* cultures of *T. grandis* (Shirin *et al.* 2005) ^[9]. In the present study an attempt was made to improve the regeneration system using nodal bud explants collected from the mature elite teak mother plants.

Materials and Methods

Nodal segments of 3-4 cm length of teak (*Tectona grandis*) Var. Teli were collected from 20 years old plus tree present at ICAR-CRIDA, Hyderabad, Telangana, India.

Initiation and aseptic culture establishment

Fresh single node explants were collected from elite mother plant (Var. Teli) and washed under running tap water for 15 min. Explants were soaked in 0.2% (w/v) bavistin + 0.01% streptomycin (antibiotic) solution for 30 minutes and then washed with distilled water for 2-3 times to remove all the traces of fungicide. Later the explants were treated with ethanol (70%) for 30 sec. They were surface sterilized with 0.1% (w/v) mercuric chloride solution for 10 min, followed by five rinses with sterile distilled water. After trimming both ends, explants were inoculated onto initiation media i.e. WPM supplemented with 0.5 mg/l BAP and cultured for 4 weeks under growth racks. Observations on type of contamination, percentage of explants without contamination and number of days taken for the shoot proliferation on each medium were recorded.

Shoot Multiplication

For multiplication, single node segments were prepared from four week old shoots proliferated on initiation media by making a cut at the inter nodes and leaf lamina by leaving leaf petiole intact to the node and transferred onto shoot multiplication media i.e. WPM basal medium supplemented with 0.5 to 5.0 mg/l BAP alone and 0.5 mg/l BAP in combination with 0.5 mg/l Kn & 0.5 mg/l IBA and cultured for six weeks. Percentage of regeneration efficiency of explant in each treatment was recorded for every 3days interval for 30days whereas mean shoot length, shoot number was noted after 45d of inoculation.

Culture Conditions

In all the above experiments, the pH of the medium was adjusted to 5.8 with 1 N NaOH or HCl before autoclaving. The cultures were maintained at $25 \pm 2^\circ\text{C}$ under a 12/12 h photo period with light provided by white fluorescent tubes at an intensity of $85 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. In each treatment minimum of 15 explants were used with three replicates and each experiment was repeated three times.

Ex vitro Rooting and Hardening

For *ex vitro* rooting, fully grown shoots with 4-5 distinct nodes were prepared by making a slant cut across the basal node and pulse treated the cut ends of shoots with IBA solution (1.0 mg/l) for 10 min. Transferred the pulse treated shoots into poretrays filled with different hardening material (cocopeat, soil and soil mixture (soil+sand+cocopeat+ vermicompost)) and kept under poly tunnel at above 80% relative humidity to minimize transpiration losses for 30 days. Later these plantlets were slowly acclimatized by gradually exposing them to the open environment and decreasing the humidity for 1-2 weeks. These acclimatized plantlets were transferred into nursery covers filled with soil mixture and kept for secondary hardening under 75% green shadenet for another 4 weeks.

Results and Discussion

Initiation and aseptic culture establishment

Approximately 80% of the node explants remained aseptic. Three to four days after incubation on establishment medium, the axillary buds started to burst (Fig 1d). At least, one axillary shoot developed per node, rarely two, however only one was able to elongate. The process of teak culture establishment in this experiment coincided with Devi *et al.* (1994), who reported that axillary shoots developed after 7–10 days. New shoots from the node grew to 5-6 cm within 6 weeks. Each new shoot had 4–5 nodes (Fig 2b).

Shoot Multiplication

In the present study, the percentage of shoot proliferation is highly affected by the nutrient media composition. On WPM basal medium, bud break and shoot proliferation was observed with lowest mean shoot number (1.67). Inclusion of BAP at 0.5 mg/l significantly improved the shoot proliferation rate and explants shown 2.44 mean number of shoots. BAP has been the most commonly used in micropropagation of teak alone or with kinetin (Gupta *et al.* 1980; Goswami *et al.* 1999) [4, 3]. Further increase in BAP concentration to 2.0 mg/l and 5.0 mg/l, shoot proliferation rate and mean shoot number got increased (Fig 4c,d), but at the cost of shoot length. Highest mean number of shoots (3.92) was recorded on WPM+5.0 mg/l BAP (Table 1). However, high concentrations of BAP can pose some risks of unexpected abnormalities of new shoots (Debergh, 1983) [1]. Addition of lower concentrations (0.5 mg/l) of Kinetin and IBA did not favour increase in shoot number (Table 1). WPM + 0.5 mg/l BAP medium can be considered as the best for shoot multiplication as each shoot on this medium have 4-5 distinct nodes and serve as nodal explants in next round of shoot multiplication.

Ex vitro Rooting and hardening

In vitro developed micro-shoots planted in a potting mixture produced new leaves within twenty days. Treating shoots with 1.0 mg/l IBA for 10 min resulted in rooting response (Fig 3b). Shoots surviving one month after planting were assumed to be rooted and the rooting percentage for each treatment was estimated. Only 20% of the plantlets survived in cocopeat after 30 days (Fig 5a). This lowest survival could be due to lack of nutrients as cocopeat is an inert material. Survival percentage was increased (60%) when soil used as hardening material (Fig 5b). Among the three different potting mixtures tested soil mixture containing red garden soil, sand cocopeat and vermicompost resulted in highest percent (90%) of survival of the plantlets (Table 2, Fig 5c). Shekhawat *et al.*, (2015) [8] observed better rooting response through *ex vitro* than *in vitro* in *Passiflora foetida*. *Ex vitro* rooting is attractive to micropropagators because of simultaneous rooting and hardening of plants (Nemeth, 1986; Tiwari *et al.*, 1997) [7, 10]. It also cuts the cost of production significantly. Further, such plants gave a high percentage of field survival (Moncousin, 1991) [6]. Major improvement over previous protocols (Mascarenhas *et al.*, 1987) [5] has been achieved for percentage of culture establishment, multiplication and *ex vitro* rooting which reduces the number of steps required in micropropagation of teak.



Fig 1: Different stages of teak micropropagation; a) Mother plant selection, b) Collection of nodal buds, c) Processed nodal buds, d) Bud initiation from nodal bud explant.

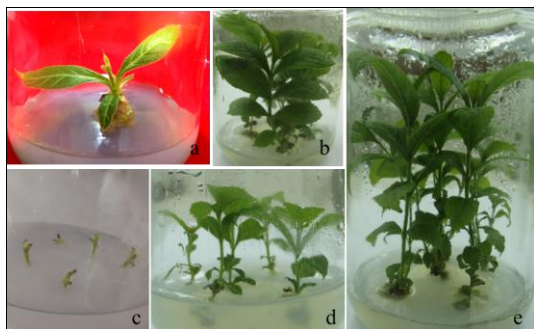


Fig 2: Different stages of teak micropropagation; a) Bud initiation on WPM+1.0 mg/l BAP medium, b) Bud elongation, c), d), e) Different stages of shoot multiplication from node explants.



Fig 3: Different stages of teak micropropagation; a) Primary hardening under poly tunnel, b) Plantlets showing root formation *ex vitro*, c) Secondary hardening under shade net, d) Completely hardened teak plant ready for plantation.



Fig 4: Shoot regeneration after six weeks on different multiplication media. a) WPM without hormones, b) WPM+0.5 mg/l BAP, c) WPM+2.0 mg/l BAP, d) WPM+5.0 mg/l BAP, e) WPM+0.5 mg/l BAP+0.5 mg/l Kinetin, f) WPM+0.5 mg/l BAP+0.5 mg/l IBA.



Fig 5: Hardening using different hardening material. a) Cocopeat, b) soil, c) Cocopeat+Soil+ sand + vermicompost, d) completely hardened plantlets transferred to nursery covers containing soil mixture.

Table 1: Effect of different concentrations of growth regulators on shoot multiplication from node explants of teak

BAP (mg/l)	Kinetin (mg/l)	IBA (mg/l)	Mean shoot number	Mean shoot length (cm)
0	-	-	1.67±0.08	6.41±0.35
0.5	-	-	2.44±0.16	7.71±0.15
2.0	-	-	2.92±0.44	7.47±0.28
5.0	-	-	3.92±1.02	5.61±0.49
0.5	0.5	-	1.25±0.14	8.07±0.78
0.5	-	0.5	1.28±0.15	5.19±0.56

Table 2: Effect of different potting mixture on survival of shoots during primary hardening

S. No.	Potting Mixture	% survival
1	Cocopeat	20
2	Soil	60
3	Soil mixture (Soil+sand+ cocopeat+vermicompost)	90

Conclusion

The present study successfully established a reliable and efficient micropropagation protocol for *Tectona grandis* using nodal explants derived from mature elite trees. High-frequency aseptic culture establishment and rapid axillary shoot initiation were achieved on WPM supplemented with low concentrations of BAP. Although higher BAP levels enhanced shoot proliferation, 0.5 mg L⁻¹ BAP proved optimal for producing healthy, elongated shoots suitable for repeated subculturing. The adoption of *ex vitro* rooting through a brief IBA pulse treatment effectively combined rooting and acclimatization, significantly reducing time, labor, and production costs. Among the hardening substrates tested, a composite soil mixture containing soil, sand, cocopeat, and vermicompost ensured maximum

survival and vigorous plantlet growth. Overall, this protocol represents a significant improvement over earlier methods by minimizing *in vitro* steps while maximizing survival and field readiness. The approach is well suited for mass clonal propagation of superior teak genotypes and has strong potential for application in commercial forestry and conservation programs.

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