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Rapid *in Vitro* regeneration of *Teakwood*, An important timber and wood plant

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Abstract

Tectona grandis L. commonly known as teak is a commercially important, tropical hardwood tree species that has several applications in the lumber industry. In this experimental work of *in vitro* clonal propagation, the protocol has been established for regeneration of teakwood (*Tectona grandis* L.) plants with MS media beside with the supplementation of various plant growth hormones. Nodal and leaf explants of teakwood were used for callus induction and plantlet regeneration. Maximum percentage of callus formation was observed in MS medium supplemented with 2,4-D at a concentration of 2.0 mg l⁻¹. Multiple shoots were observed from nodal segments through axillary bud proliferation. Maximum shoot multiplication percentage was achieved on MS medium supplemented with 6-benzyladenine (BAP) (2.5 mg l⁻¹) and α -naphthalene acetic acid (NAA) (1.0 mg l⁻¹). Cultures have been continuously subcultured to new media supplemented with same hormone treatment after every 3-4 weeks. Multiple shoots of high quality were produced *in vitro* from node explants. An average of about 7 shoots/ uninodal explants was obtained in 3.5 weeks of culture on MS medium. When shoots were rooted by supplementation with NAA (2.5 mg l⁻¹) in combination with kinetin (0.2 mg l⁻¹) the frequency of rooting of shoots was better than any other combinations. More than 95% of shoots were developed strong and highly ramified roots on MS medium containing NAA (2.5 mg l⁻¹) with Kinetin (0.2 mg l⁻¹). *In vitro* raised plantlets were regenerated on MS medium supplemented with 0.5 mg l⁻¹ of NAA and acclimatized on vermiculate before transferring them to field for hardening. Shoots were successfully rooted and acclimatized to greenhouse conditions. *In-vitro* plantlets were planted in pots containing autoclaved mixture of soil: sand: farm yard manure (1:1:1) and grown in the green house for hardening.

Keywords: *Tectona grandis* L., callus, 6-benzyladenine, acclimatization, farm yard manure

Introduction

Tectona grandis L. f. (*Verbinaceae*) is one of the most valuable structural timber species which has lot of applications in lumber industries. It is indigenous to Burma, Thailand and Central and South India. Teak has been introduced into all agro-climatic regions of Central and South India has naturalized in almost all of these regions [1]. In Maharashtra it is grown in Gadchiroli, Chandrapur, Nagpur, Bhandara, Amaravati, Nanded, Vardha, Yawatmal, Dhule, Nasik and Raigadh. The tree is characterized by a tall clear and rounded crown. Central characteristics of its wood include resistance to weathering, strong fiber, and special oil content which produces premium timber [2] makes teak one of the world's most valuable hardwood species [3]. Teak wood is also used for making fine furniture, shipbuilding, and decorative objects [4]. The Teakwood plant is conventionally reproduced through seeds, but germination is often difficult because the hard seed coat limits the production of a large number of seedlings in a defined time. Poor germination rate leading to a low production of seedlings further contributes to the paucity of planting material [5, 6]. The propagation of teak via cuttings and other techniques have also been reported [7, 8], but this method has several limitations and only provides a few propagules from selected individuals. The application of *in vitro* techniques is considerably more difficult in tree species than in herbaceous plants. However over the last two decades the number of tree species that have been micropropagated has been increasing rapidly. In India micropropagation of forest trees has been relatively successful [9]. *In vitro* propagation technique has become an efficient way for producing plants as uniform as possible on a large scale and in a short time for the plantation industry. However, although numerous authors have been experimenting to establish an efficient, reproducible and simple system for Micropropagation of teak [10]. Tissue browning *in vitro* can be a serious problem when mature shoots of trees are used as a source material for Micropropagation studies [11]. Tissue culture attempts of this plant have had limited success due to the oxidative browning of explants. This paper describes a method for the micropropagation of teak using node and shoot tips explants in different media such as MS medium along with the supplementation of plant growth hormones.

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Materials and Methods

Fresh and young plant seedlings maintained in the Yash Biotech nursery at Nashik, Maharashtra were used as a mother plant for *in-vitro* regeneration. Mature nodal segments (NS) and leaflets of the naturally grown healthy plant of *Tectona grandis* L. were used as the source of explants material for the present study. Single nodes and leaflets of young twigs were excised and they were initially surface sterilized with ethanol (70% v/v) for 30 s and then dipped in 5-10% commercial bleaching solution containing 5% sodium hypochlorite (NaClO) and a few drops of Tween-80 for 5-10 min. After rinsing three times with sterile distilled water, the explants were dipped in a 0.20% mercuric chloride solution for 5-7 min, followed by three rinses in sterile distilled water. After sterilization the explants were placed on tissue paper for removal of moisture on the surface and then the nodal segments and leaflets were trimmed at both the ends to appropriate size (1-1.5 cm) and cultured on sterile media. Murashige and Skoogs medium [12] for plantlet development different organic additives were added to the basal medium to determine the factors affecting the morphogenic response at different stages of the culturing process. The node and leaf explants were cultured in glass bottles with MS medium containing 3% sucrose and solidified with 0.8% agar, supplemented with 6-benzylamino purine (BAP) and α -naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and indole acetic acid (IAA) in different concentrations. The PH of the medium was adjusted to 5.8 before autoclaving. All inoculations were carried out in a laminar air flow cabinet cultures then inoculated in a culture room at 25°C and a 16 hrs light 8 hrs dark period, observations were taken at regular interval for growth and contamination, all cultures were renewed by sub culturing every 4 weeks and the contaminated cultures were discarded. The explants derived shoots were transferred to MS medium supplemented with different concentrations of cytokines in combination with auxin (BAP+NAA) using the conditions described for shoot induction. Elongated shoots derived from nodal explants transferred to MS medium supplemented with different concentrations of NAA and Kin for their rooting. In general, from each shoot 4-5 nodes were obtained. The cultures were initially maintained for 6 days under dark and then exposed to light and temperature. Multiple shoots were rooted by following the procedure of Akram and Aftab [13]. The rooting medium consisting MS with 3% sucrose and solidified with 0.8% of agar. NAA was tested either alone (at 0.5, 1.0, 1.5, 2.0 mg l⁻¹) or in combination with Kinetin (0.2 mg l⁻¹). The aseptic shoots were cut into single nodes with their respective 2-3 leaves and placed, randomly distributed, into the different proliferation media. The regenerated young plants were removed from the culture vials and washed

thoroughly with tap water. They were acclimatized in a culture room a mixture of soil, sand, and farmyard manure (1:1:1 v/v) for a period of two weeks.

Results and Discussion

A protocol for rapid propagation of *Tectona grandis* (L) through nodal and leaf explants had been established. Nodal and leaf explants were cultured on MS medium containing various concentrations and combinations of hormones. From these result it was understood that 2,4 D at a conc. of 2.0 mg/l gives better response for callus induction (Table 2, Fig. 1B) from nodal explants. The leaf explants also led to induction of callus on the medium containing BAP (2 mg l⁻¹) in combination with 2,4 D (2 mg l⁻¹). However, culture browning and therefore necrosis remains a serious threat during micropropagation of teak using tissues derived from mature trees. The callus initiations were subjected to the regeneration on the different media and the growth was observed after 4 weeks of sub culturing. Also from the observations it was found that maximum shoot induction is found from the nodal explants through the proliferation or sprouting of bud (Table 3, Fig. 1A). The combination of BAP and NAA showed better response for shoot initiation and multiplication and maximum no of shoot i.e 7 shoots/ nodal explants were found in media supplemented with 6-benzyladenine (BAP) (2.5 mg l⁻¹) and α -naphthalene acetic acid (NAA) (0.5 mg l⁻¹). Highest number of multiple shoots on BAP and NAA using nodal explants from mature teak shoots (Table 3, Fig. 1E). From the results of the present investigation, it is evident that shoots from large stem segments is a strong possibility for recalcitrant woody plant species of tropical origin. It is well known that the woody tree species of temperate or tropical origin respond differently at different temperatures and the optimum conditions including temperature may vary from species to species. Usually, the optimum temperature is lower for temperate or higher for tropical tree species [14]. Gangopadhyay *et al.* [15]; Nissen and Sutter [16] carried out rooting in a liquid medium supplemented with IBA and IAA, but the percentage of the survival of plants after transplantation was relatively low. In the present investigation shoots were rooted by supplementation with NAA 2.5 mg l⁻¹ in combination with Kinetin (0.2 mg l⁻¹) the frequency of rooting of shoots was better than any other combinations i. e. 8 roots /explants (Table 3, Fig. 1F). Rooting was significantly affected with the increasing concentration of either kinetin or NAA. Finally the *in-vitro* regenerated whole plants were primary hardened in plastic pots containing autoclaved mixture of soil: sand: farm yard manure (1:1:1) and put in the green house survival. Higher survival rate (70 %) was observed when planted in field.

Table 1: Effect of different concentration BAP and 2, 4-D on callus induction from leaf segment of *Tectona grandis* L. Data was recorded after four weeks of culture.

Supplement (BAP mg/l)	Supplement (2, 4-Dmg/l)	Type of explants inoculated	No. of explants inoculated	No. of explants regenerated	% of explants induced callus	Weight of callus (mg)	Days of shoot initiation
0.5	0.5	Leaf segment	10	0	0	-	-
1.0	1.0	Leaf segment	10	1	10	-	-
1.5	1.5	Leaf segment	10	4	40	41.0	29
2.0	2.0	Leaf segment	10	5	50	62.2	28
2.5	2.5	Leaf segment	10	3	30	32.2	29

Table 2: Effect of different concentration BAP and 2, 4-D on callus induction from nodal explants of *Tectona grandis* L. Data was recorded after 4 weeks of culture.

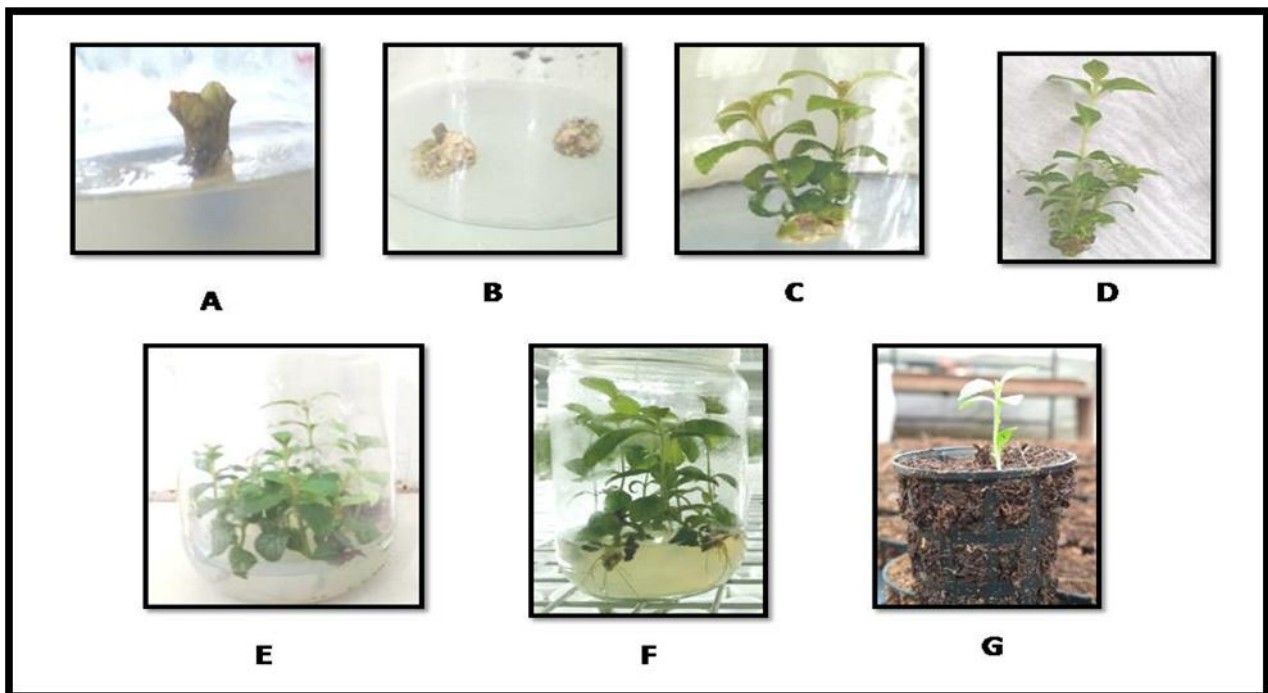
Supplement (2, 4-Dmg/l)	Type of explants inoculated	No. of explants inoculated	No. of explants regenerated	% of explants induced callus	Weight of callus (mg)	Days of shoot initiation
0.5	Nodal	10	2	20	35.0	28
1.0	Nodal	10	4	40	65.0	26
1.5	Nodal	10	6	60	72.0	26
2.0	Nodal	10	8	80	134.5	26
2.5	Nodal	10	7	70	83.3	26

Table 3: Observations of various combination of hormone BAP and NAA on shoot formation by using callus culture of *Tectona grandis* L. after 4 weeks.

Supplement (BAP + NAA mg/l)		Type of explants inoculated	No. of shoots	Length of shoot (cm)	Performance
BAP	NAA				
0.5	0.5	Callus of <i>Tectona grandis</i> L.	-	-	-(Poor)
1.0	0.5		1	2.1	+(Fair)
1.5	0.5		1	2.6	+(Fair)
2.0	0.5		2	4.2	++(Good)
2.5	0.5		7	6.4	+++ (Very good)
3.0	0.5		3	4.0	++(Good)

Table 4: Observation of various combination of hormone NAA combine with kinetin on root formation of *Tectona grandis* L. after 3 weeks.

Sr. No.	Source of culture	MS + concentration of growth hormones (mg/l)		No. of root	Length of root (cm)	Performance
		NAA	Kinetin			
	Regenerated shoots of <i>Tectona grandis</i> L.			-	-	-
1		1.0	0.2	3	2.0	+(Fair)
2		1.5	0.2	3	2.2	+(Fair)
3		2.0	0.2	4	3.1	+(Fair)
4		2.5	0.2	8	4.5	+++++(Excellent)
5		3.0	0.2	5	3.2	+(Fair)

**Fig. 1:** *In-vitro* Plant Regeneration of *Tectona Grandis* L.– (A) Callus initiation from the nodal explants on MS medium (B) Friable callus formation on MS media supplemented with BAP and 2, 4-D (C), (D) Shoot Induction starts from friable callus (E) Shoot multiplication on the MS medium with BAP and NAA (F) Shoots with well developed roots on the MS medium with NAA and kinetin (G) Primary hardening of regenerated plants in a medium containing Soil: Sand: FYM (1:1:1)**Reference**

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