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In vitro Propagation of Shoot and Callus Culture of *Tectona grandis* (L.)

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Abstract: Tissue culture in forestry is one of the main attractive subjects for research and development and hence the present study was initiated to induce the callus and shoot induction from leaf and node explants of *Tectona grandis* (L.). Of the selected different concentrations of benzylaminopurine (BAP) and Kinetin (KN) treatments, the maximum (7.5cm) shoot length was identified with the 3.0mg l^{-1} concentration of BAP treatment. It can be concluded from the present findings that, the addition of 3.0mg l^{-1} of BAP can be used as a suitable ingredient for the prominent growth of *Tectona grandis* (L.) tissue culturing.

Abbreviations: BAP = Benzylaminopurine, KN = Kinetin, MS = Murashige and Skoog medium
Key words: Benzylaminopurine · Kinetin · Murashige and Skoog medium · *Tectona grandis*

INTRODUCTION

Tectona grandis (L.) belongs to plant family verbenaceae, commonly known as teak. It is valued as a prized timber due to its versatility, durability and attractiveness of its wood. It's uses included flooring and parquet, ship building, furniture-making and in building construction. Teak is grown naturally in India, Myanmar, Thailand and Laos. Teak is traditionally reproduced through seeds, but in most cases, germination is tissue culture due to the hard seed coat, low seed quality and late seed production. Poor germination rate leading to a low production of seedlings further contributes to the paucity of planting material [1, 2]. At present, teak ranks among the top five tropical hardwood species in terms of plantation area established worldwide [3, 4]. In this consideration of delayed seed germination, *in vitro* micro propagation can be considered as a commercially feasible method for teak cultivation [5]. However, numerous authors have been experimenting to establish an efficient, reproducible and simple system for micro propagating teak, [2, 6-8]. Even though, it still remains problematic due to the poor capacity of shoot proliferation, high susceptibility of shoots to verification and browning and the low frequency of *in vitro* rooting. None of the published protocols fully satisfy the requirements for commercial application in spite of the progress made on tissue culture of teak since the 1970s [9-11]. In this connection, the present work was aimed to improve the efficiency of shoot proliferation and to accelerate the plant growth.

MATERIALS AND METHOD

Plant Material and Explants: Nodal explants were collected from botanical and research garden of A.V.V.M. Sri Puspham College, Poondi (Lat: 13° 63'N and Lang 79° 84'E). The collected sample was washed thrice with tap water to remove the adherent dust particles and other contaminants. The washed sample was transferred to the laboratory condition by using unused polythene bags. The explants were prepared with fresh young leaves (1x1 cm) by using sterile scalpel and the samples were washed with sterile distilled water for 3 times. The washed explants were subjected for surface sterilization with ethanol: distilled water (7:1 v/v) mixture for explants and dipped in 0.1% mercuric chloride for about 5-10 min. After rinsing three times with sterile distilled water, explants were dipped in 5- 10% sodium hypochloride and a few drops of Tween-80 for 5-10 min, followed by three rinses in sterile distilled water and were then kept in a laminar air flow chamber.

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Medium and Culture Conditions: The explants were cultured on Murashige and Skoog (MS) medium [7] containing 3% (W/V) sucrose was used in all the experiments. The pH of the medium was adjusted to 5.8 prior to the addition of 0.8% (w/v) agar (HiMedia Laboratories Private Limited Mumbai, India). Molten medium was dispensed in 15 ml aliquots into culture tubes (25x150mm) and closed with non-absorbent cotton plugs. The medium was autoclaved at 1.1 kg/cm^2 pressure and 121°C temperature for about 15 min. All the cultures were incubated in a culture room maintained at 25±2°C and 55 to 65% RH fewer than 12 h photoperiod of 50-60 µmol m^{-2} s^{-1} light intensity provided by cool white fluorescent tubes.

Culture Establishment: The nodal explants were then singly placed in test tubes (25x150mm) with basal MS [2] medium supplemented with 6-benzyl amino purine (1.0mg L^{-1}) and adenine sulphate (20mg L^{-1}), sucrose (3%) and solidified with 0.6% agar supplied by Duchefa Bio-chemie (NL) to induce bud support and to select sterile shoots. Activated charcoal (Sigma, USA), 3g L^{-1}, was added to the medium to reduce browning. Medium pH was adjusted to 5.8 before autoclaving. After 30 days, the aseptic auxiliary shoots were transferred to a fresh medium of the same composition for 3 subcultures to produce a large number of shoots.

Shoot Multiplication: MS basal medium supplemented with various concentrations (0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of plant growth regulators such as benzylaminopurine (BAP) and Kinetin (KN) were added with adenine sulphate individually. All the cultures were transferred to fresh medium after 2-3 weeks duration. The mean number of shoots and their length and basal callus fresh weight were calculated after 6 weeks of inoculation.

RESULTS AND DISCUSSION

The results of callus induction and shoot propagation were tabulated in Table 1 and Figure 1 and 2. In all the BAP treatments, the explants were produced the bud formation, but 1.0 mg ml^{-1} concentration of BAP treatment did not show any bud formation. Similarly, several authors have reported the bud formation in teak plants with different concentrations of growth *viz.,* Devi *et al.* [12] reported the auxiliary shoot formation in 10 day incubation. Tiwari *et al.* [13] also observed the auxiliary shoot formation after 6 weeks. Similarly Goswami *et al.* [14] reported the maximum shoot length 4.7 cm in 8 weeks incubation. But in the present study, the maximum (7.5 cm) shoot average was identified within 30 days (Table 1). In all the BAP treatments, the maximum (7.5 cm) shoot length was identified with the 3.0 mg/L concentration. Increased shoot length formation was identified with increased concentration of BAP treatments, but no callus formation was observed in 1.0 mg/L concentration of BAP treatment. In the KN treatments, the maximum (0.7 cm) shoot length was identified with 2.0 mg/L concentration, but all the other treatments were not showed any shoot induction. Likewise, Gangopadhyay *et al.* [5] also reported the stunning growth properties with KN treatments and enhanced growth properties with BAP treatments. According to the literature, BAP and KN is the most commonly used cytokinins for microprpagation of teak plants, but high concentration of KN did not show any

Table 1: Effect of BAP and Kn on shoot formation from the nodal explants of *Tectona grandis* on MS medium

Treatments	Growth regulators concentrations (mg/L)	Presence of callus (C) and shoot (S) formation	Days to callus and shoot formation	Height of shoots (cm)	Morphology and colour of callus
	BAP				
T 1	0.5	S	7	Live	Shoot only
T 2	1.0	S + C	14	Live	Shoot only
T 3	1.5	S + C	21	1.3	Yellow Green + S
T 4	2.0	S + C	28	3.3	Brown Yellow + S
T 5	2.5	S + C	35	4.8	Light Yellow + S
T 6	3.0	S + C	42	7.5	White, Light Greenish + S
	KN				
T 7	0.5	C	7	Live	Brown
T 8	1.0	C	`14	Live	White light Yellow
T 9	1.5	C	21	Live	Light Greenish
T 10	2.0	C + S	28	0.7	Greenish white+ S
T 11	2.5	C + S	35	0.5	Yellow + S
T 12	3.0	C	42	Live	Yellow

De notes - = No growth; S = Shoot; C = Callus; T = Treatment; BAP = Benzylaminopurine; KN = kinetin

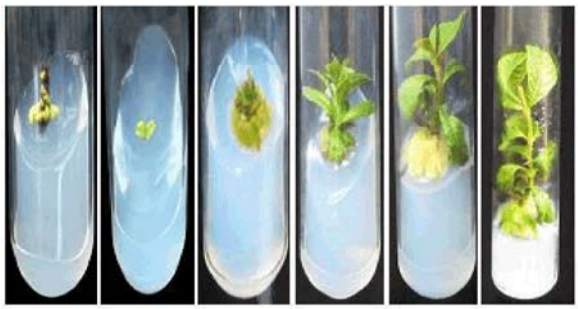

Fig. 1: Picture showing the shoot formation
1. Initiation of Burst from the nodal explants on MS medium fortified with 0.5 mg/L BAP with KN after 1 weeks; 2.Initiation of shoot in explants MS medium supplemented 1.0 mg/L BAP after 2 weeks; 3. Induction of basal callus from the micro shoots was observed on MS medium supplemented with 1.5mg/L KN after 3 weeks; 4.Formation of shoots from nodal plants on MS medium supplemented 2.0mg/L BAP after 4 weeks; 5.Formation of quality of shoot from Explants on supplemented 2.5mg/L KN after 5 weeks; 6. Developing of shoot from nodal plants on supplemented 3.0mg/L B AP with KN after 6 weeks.

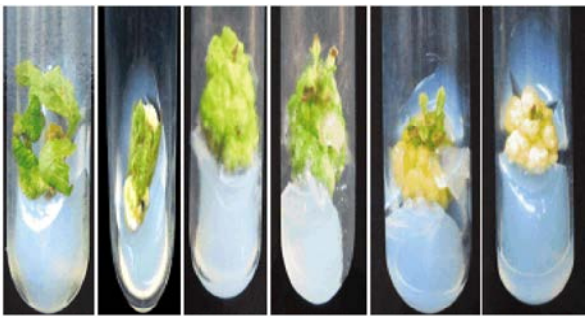

Fig. 2: Picture showing the formation of root nodels from explants
7. Initiation of callus from the leaf on MS medium fortified with 0.5 mg/L KN after 2 weeks. 8. Formation of callus in leaf MS medium supplemented 1.0mg/L BAP after 3 weeks. 9. Induction of basal callus from the micro shoots was observed on MS medium supplemented with 1.5mg/L KN after 3 weeks. 10. Formation of callus from leaf on MS medium supplemented 2.0mg/L BAP after 4 weeks. 11. Formation of quality of callus with micro shoots from Explants on supplemented 2.5mg/L KN after 5 weeks. 12. Maturity of callus from leaf on supplemented 3.0mg/L BAP, KN with cytokinin after 6 weeks.

formation of the callus indication and this might be due to the inhibitory properties of auxiliary bud sprouts [15]. Phenolic compounds cause necrosis and death in *in vitro* tissues. It is, hence, a prerequisite to remove these compounds from explants before culturing to avoid medium darkening that is usually a result of phenolic exudation [16]. In some instances the blackening of explants were observed in the beginning stage of budding formation and this might be due to the higher exudation of phenolic [17], further the phenolic content was reduced by the addition of the charcoals and this might be due to the absorption properties of the charcoal [18]. The present study provides an efficient *in vitro* propagation method which could be commercially feasible for teak, by providing a protocol for producing genetically similar plants from selected genotypes. This work showed the highest number of micropropagated shoots reported for teak, up to now, in the available literature and in a relative short period of time, producing about quality of shoot within 6 weeks. Some steps were important for improving quality of shoots, aimed at improving shoot length percentage, by MS medium composition in salt balance.

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