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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 2056-2060
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www.thepharmajournal.com
Received: 09-09-2023
Accepted: 11-10-2023

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In vitro shoot development from callus and other explants in teak (*Tectona grandis* Linn. F.)

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Abstract
The present investigation on "*In vitro* shoot development from callus and other explants in Teak (*Tectona grandis* Linn. F.)" was carried out at the Plant Biotechnology Centre, College of Agriculture, Dapoli, Dist. Ratnagiri 415 712 (Maharashtra). Among the matured and juvenile explants used, apical buds and nodes from juvenile plants were found best for shoot induction. T_{11} (Ethyl Alcohol 70% for 10 sec. + $HgCl_2$ 0.05% for 5 min.) achieved the highest aseptic culture establishment rate (70.60%) and the highest survival rate (72.00%) for surface sterilization of these explants. Browning of the media was prevented by using PVP (100 mg l^{-1}) as an antioxidant and dark incubation for three days, resulted in aseptic cultures. Among the various treatments, the MS medium with 2.0 mgl^{-1} BAP shown the best for teak explant establishment and shoot induction. In MS media supplemented with 1.0 mg l^{-1} BAP and 2.0 mg l^{-1} NAA, callus induction was better in leaf explants of young teak plants while shoot induction was better in apical buds and nodes.

Keywords: Teak, sterilization, callus induction, *Tectona grandis*, and shoot regeneration

Introduction
Teak (*Tectona grandis* Linn. f.) is one of the most valuable timber producing species in the world. It is a member of the Verbenaceae (2n=36) family and is found primarily in tropical or subtropical regions. The name *Tectona* is derived from the Portuguese term teca, which is a derivative of the Greek word tekton, which means carpenter and *grandis* is Latin for large (Cowen, 1965) [6]. Teak is a sun-loving, deciduous tree that grows well in any well-drained soil. It takes 15-25 years to mature. It is one of the most important wood trees in the tropics, distinguished by a tall clean trunk and a rounded crown (Tiwari *et al*., 2002) [20]. It is a major plantation species in Southeast Asia's tropical and subtropical climates, including India.

Teak constitutes about 75 percent of the world's high quality tropical hardwood plantation (Reddy *et al*., 2014) [18]. In order to produce timber, it is frequently advised for plantation programmes in dry tropical areas. India is home to over 44% of the world's teak plantations (Ball *et al*., 1999) [4]. About 5.82 million hectares (4%) of the projected 142 million hectares of plantations worldwide in 2005 were teak plantations (Reddy *et al*., 2014) [18]. According to estimates, there are 3 million ha of teak plantations worldwide, 94% of which are in Tropical Asia, with the largest shares being in India (44%) and Indonesia (31%), followed by Thailand (7%) and Myanmar (6%) (Ball *et al*., 2000) [4].

The main source of teak's long history and widespread renown is the ship building industry. It is a crucial building material in its native countries, used for making bridges, wharves, piles, furniture, cabinets, railcars, waggons, wheel spokes and general woodwork. Teak has a reputation for having an unrivalled combination of properties, including resistance to termites, fungi, and weather, lightness and strength, beauty, workability and seasoning without splitting, cracking, wrapping or significantly altering shape. In India, the mechanical, physical and durability characteristics of teak are used as a benchmark when evaluating the quality of other types of wood.

Because teak is widely available and is grown across the tropics, its durability and usability were recognized in our nation. Teak also has therapeutic benefits; the bark is a bitter tonic that is said to be effective in treating fever. Additionally helpful for treating headaches and gastrointestinal issues, teak bark or wood may improve digestion. Teak oil, which is produced from trees grown in humid deciduous forests, has a powerful and recognizable aroma. In addition to the paste made from powdered teak wood, which is regarded an astringent diuretic, hepatic stimulant, sedative, and local refrigerant, the tree's juice from its leaves can be used as fabric dye. Furthermore, it is advised for lowering inflammatory swelling and toothache.

Materials and Methods

The investigation named "*In vitro* shoot development from callus and other explants in Teak (*Tectona grandis* Linn.f.)" was carried out in the tissue culture laboratory of the Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist: Ratnagiri (MS) during the academic year 2022-2023.

The experimental material of the present investigation was collected from the nursery of College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Juvenile phased explants were taken from 1-2 year old plants, propagated through seeds and maintained under protective conditions of polyhouse in polybags and callus explants were procured from previous maintained cultures. The type of explants along with their age is mentioned in Table No. 1.

Table 1: Explants and their age

Phase	Age of mother plant	Type
Juvenile phase	1-2 year old plants	1. Apical buds/ nodes 2. Leaf
Matured phase	70 years old trees	1. Apical buds/ nodes 2. Leaf
Callus	_	1. Callus

Methodology

1. Collection and preparation of explants

Prophylactic sprays of the systemic fungicide, Bavistin 50% W.P. (Carbendazim) were given on alternate days to the mother plants except in rainy season for prevention from microbial inoculum. Young leaves and stem segments with apical buds and 2-3 nodes were excised from mother plant with the help of secateurs. The stem cuttings were brought to the lab, defoliated and converted into apical buds of 1-2 cm dimension and nodal segments of dimension (3-4 cm). The segments were then washed under running tap water for 20 minutes. After this they were washed thoroughly with a solution of Cetrimide (0.5%) and Tween- 20 (1%) for 15 minutes. After this they were washed thoroughly with teepol to remove all extraneous materials adhered to the segments.

2. Surface sterilization of explants

The process of surface sterilization was carried out in a laminar airflow cabinet, which was made sterile by the incessant exposure of germicidal U.V. rays for half an hour before use. In the laminar airflow cabinet, fresh treatment of Carbendazim (2%) to buds/nodes and Carbendazim (1%) to leaves was given for 45 minutes. Explants were washed two times with SDW. The explants were sterilized with 70% ethanol, $HgCl_2$, Sodium hypochlorite followed by washing with sterilized double distilled water in laminar airflow chamber. At the end of surface sterilization treatment, explants were treated with a solution of PVP (0.7%) and sucrose (2%) to minimize the browning which may be caused due to various surface sterilizing agents on explants. Each treatment had three replications, each of which contained seven explants. The information was gathered at the end of the fourth week.

3. Culture media used

The explants were cultured on agar solidified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), Woody Plant Medium (WPM) (Llyod and McCown, 1980).The chemical compositions of the different media are given in Table 2.

Table 2: Chemical composition of Murashige and Skoog (MS) medium and Woody Plant Medium (WPM)

Compound	Quantity in mg	
	MS	WPM
Major salts		
Ammonium nitrate (NH_4NO_3)	1650.00	400.00
Calcium chloride ($CaCl_2.2H_2O$)	440.00	96.00
Calcium nitrate ($Ca(NO_3)_2.4H_2O$)	0.00	556.00
Potassium orthophosphate (KH_2PO_4)	170.00	170.00
Potassium sulphate (K_2SO_4)	0.00	990.00
Magnesium sulphate ($MgSO_4.7H_2O$)	370.00	370.00
Minor salts		
Boric acid (H_3BO_3)	6.20	6.20
Manganese sulphate ($MnSO_4.4H_20$)	22.30	22.30
Sodium molybdate ($Na_2MoO_4.2H_20$)	3.25	0.25
Zinc sulphate ($ZnSO_4.4H_2O$)	0.60	8.60
Zinc nitrate $Zn(NO_3)_2.6H_2O$	0.83	0.00
Potassium iodide (KI)	0.025	0.00
Cobalt chloride ($CoCl_2.6H_2O$)	0.025	0.00
Copper sulphate ($CuSO_4.5H_2O$)	0.025	0.025
Chelating agent		
Ferrous sulphate ($FeSO_4.7H_2O$)	27.80	27.80
Sodium ethylene diamine tetra acetic acid (Na_2 EDTA. $2H_2O$)	37.30	37.30
Vitamins		
Thiamine HCl	0.10	0.10
Nicotinic acid	0.50	0.50
Pyridoxine HCl	0.50	0.10
Glycine	2.00	2.00
Myo- inositol	100.00	100.00
Other Compounds		
Sucrose (in percent w/v)	3.00	2.00
Agar (in percent w/v)	0.70	0.70

The data recorded for different parameters was subjected to completely randomized design (Gomez and Gomez, 1984) [8]. The statistical analysis based on mean values per treatment was made using analysis of variance technique for CRD.

Result and Discussion

Effect of sterilization treatments

Table 1: Effect of sterilization treatments on apical buds and nodes from juvenile plants

Treatment	Concentration	Time (min.)	Browning (appearance in days)	% of cultures survived	% of aseptic cultures
$HgCl_2$	0.05%	5 min.	1 (+)	83.62	41.80
NaOCl	1%	1 min.	immediate (+++)	6.35	2.56
Ethyl Alcohol	70%	10 sec.	3 (+)	77.15	48.21
Ethyl Alcohol	70%	10 sec.	5 (++)	72.00	70.60
$HgCl_2$	0.05%	5 min.			

In apical buds and nodes of juvenile plants (Table 1), the treatment of Ethyl Alcohol 70% for 10 seconds followed by $HgCl_2$ 0.05% for 5 min. has recorded 70.60% aseptic culture establishment with 72.00% survival rate showing high frequency of media browning 5 days after inoculation and was found to be best which are similar to the findings of Tiwari *et al.* (2002) [20] in axillary buds of teak.

The treatment of NaOCl 1% for 10 minutes have caused very high browning of media immediately after inoculation. The concentration of the sterilizing agents used, the length of the treatments with different sterilizing agents, and the order of the treatments all affect how effectively surfaces are sterilized. The use of NaOCl has shown damaging effects during sterilization treatments even at low concentration and duration leading to excessive polyphenol exudation causing media browning and cell death. Typically, many sterilizations and sterilizing agents were shown to be beneficial.

Phenol alleviation

The explants showed rapid exudation of phenols *In vitro* during sterilization treatments due to the damaging effects of sterilants. Media and explants turned reddish-brown within 24 h which are at par with the observations recorded by Tiwari *et al.* (1997) [19].

Table 2: Effect of different treatments on phenol elevation

Treatment	Ascorbic acid (150 mg l-1)	Activated Charcoal (1.0 g l-1)	PVP (100 mg l-1)	Activated Charcoal (1.0 g l-1) and PVP (100 mg l-1)	PVP (100 mg l-1) and dark incubation for three days
Percentage of cultures showing phenol alleviation	24.60	63.55	77.26	86.45	96.89

When cultures were given dark incubation for the initial 3 days along with PVP treatment (Table 2), 96.89% of cultures showed phenol alleviation. This finding indicates that light had a significant impact on phenol exudation. Preper and Zimmer (1986) [17] also found a decrease in phenol exudation when cultures were kept in the dark. Browning was lesser when, the axillary buds of teak were cultured in media containing 0.5 g l^{-1} charcoal (Antony *et al.*, 2015) [2]. A combination of activated charcoal and PVP has also shown good phenol alleviation of 86.45% (Fig. 1). PVP was reportedly used by Gupta *et al.* (1980) [9] to control phenol in teak. Dhawan (1993) also demonstrated that the inclusion of antioxidants in media has become mandatory in order to overcome the browning problem. According to Weather head *et al.* (1979) [21], activated charcoal absorbs chemicals that hinder growth and so fosters growth.

Effect of basal media and various growth regulators on culture establishment and growth

A comparative study of the effect of various media on culture establishment and growth of apical buds, nodes and leaves was carried out (Table 3). The percentage of bud break and leaf initiation was found highest in MS and WPM. The number of days taken for bud break and leaf initiation was also lesser in these media. All three types of basal media were supplemented with various concentrations of BAP and IBA individually. Results show that maximum bud break, leaf initiation, mean shoot length and mean number of shoots per bud sprout per explant occurred with 0.5 and 1 mgl^{-1} BAP in all two types of basal media. With the increase in BAP to 3 mgl^{-1}, these parameters showed minimum values for each type of basal media. However, a significant increase in these values was observed with the addition of 0.5 mgl^{-1} IBA. In general, BAP has been utilized more often than other cytokinins to promote greater shoot growth and multiplication, especially in tree species, according to Ahmed (1990) [1]. An increase in BAP concentration decreased the number of responsive cultures and shoot length, according to research by Kannan (1995) [10]. It was observed that a lower concentration of BAP leads to a higher percentage of bud initiation, mean shoot length and mean number of shoots per explant. Numerous authors have found that species like *Eucalyptus globulus* (Pattanaik and Vijayakumar, 1997) [16], *Morus alba* (Kanwar *et al.*, 1991) [11], *Daubanga grandiflora* (Kumar and Kumar, 1997) [12], *Ailanthus triphysa* (Natesha, 1999) [15], and *Betula pendula* exhibit the best growth of shoots and leaves when BA concentrations are lower (Durkovic, 1997) [7].

Bud sprouting and leaf initiation in teak

Vitrification was also observed in shoots, the reason for which maybe high levels of cytokinins, low concentration of gelling agents or excess mineral elements.

Bud sprouting and leaf initiation in teak

Details	Percentage of		Time taken (in days) for		Mean shoot length (cm)	Mean no. of shoots/bud sprout per explant
	Bud break	Leaf initiation	Bud break	Leaf initiation		
MS + 0.5 mgl^{-1} BAP	80.45	63.00	14	27	5.03	3.33
MS + 1 mgl^{-1} BAP	70.95	39.47	18	32	3.91	1.93
MS + 3 mgl^{-1} BAP + 0.5 mgl^{-1} IBA	45.81	41.35	21	30	2.59	1.95
WPM + 1 mgl^{-1} BAP	79.12	63.33	16	26	4.77	3.30
WPM + 1.5 mgl^{-1} BAP	70.81	40.62	17	32	3.73	1.68
WPM + 3 mgl^{-1} BAP + 0.5 mgl^{-1} IBA	46.05	45.20	25	39	2.51	1.96

Varying shoot lengths observed in teak for different basal medium supplemented with 1 mgl^{-1} BAP

Various scholars have noted the induction of multiple shoots in species like *Acacia mearnsii* (Beck *et al*., 1998) [5] and Aegle marmelos on medium supplemented with BA (Arumugam and Rao, 1996) [3].

Shoot induction through axillary bud sprouting

Media composition for shoot induction from callus with MS basal media

Tr. No.	Treatment Details	Shoot induction (%)	Mean Shoot length
1	MS + 2.0 mgl^{-1}BAP + 0.5 mgl^{-1} NAA	18	7.33
2	MS + 2.5 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	15.33	5.50

Conclusion

In the present investigation, it was observed that, apical buds and nodes from juvenile plants of teak were successfully made aseptic for nearly 72% cultures with satisfactory survival rate after surface sterilization of these explants. Browning of media was prevented by imposing the antioxidant treatment of PVP (100 mg l^{-1}) and dark incubation for three days which resulted in aseptic cultures. MS and WPM Medium with 0.5 and 1 mgl^{-1} BAP found to be best for establishment and shoot induction also 2 mgl^{-1} BAP found effective for shoot development from calli in teak explants among the other treatments. Thus, it can be concluded that an efficient method has been established for successful sterilization and shoot induction in teak.

Acknowledgements

Author is thankful to, Department of Agricultural Botany, Plant Biotechnology Centre, College of Agriculture, and College of Forestry, Dapoli, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli-415712, Ratnagiri-district, Maharashtra (India), for providing necessary facilities and valuable suggestions during the study.

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