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Research Article

AN IMPROVED METHOD OF MICROPROPAGATION OF TEAK *(TECTONA GRANDIS)*

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ABSTRACT
An efficient method of Micropopagation of teak has been developed. For the shoot multiplication the explants were collected from apical nodes of a mature teak tree, planted in Shree Ganesh Biotech India ltd, a Private laboratory situated in Birbhum, (WB). Four different trails were done with different combination of both Kinetin, BAP for multiplication of shoots. Among them the best result was found in the combination containing MS Basal medium+ 0.5mg/l KIN + 0.5mg/l BA+ 0.1mg/l IAA. For rooting different concentration of NAA + MS medium were used in different four trails. Among them culture supplemented with ½ MS +1mg/l NAA were resulted the highest percentage of roots. Different potting mixtures were used for efficient acclimatization. The best results were found in jiffy bags with 98% of survival rate.

Keywords: *Micropropagation, Tectona Grandis, BAP- Benzyle Amino Purine, Kinetin*

INTRODUCTION
TEAK (*TECTONA GRANDIS*) is a tropical hardwood species placed in the family Verbanaceae. It is highly sought for ship building as well as interior and exterior luxury furnishings. It has gained worldwide reputation for high quality timber due to aesthetic appeal and wood durability. The attraction of this species is the mechanical and physical quality of its timber which is also resistant to termite attack due to the presence of Sesquiterpenes, (Anonymous, 1996; Maleskar, 1983). At present, teak ranks among the top five tropical hardwood species in terms of plantation area established worldwide (Dah and Baw, 2001). Due to poor capacity of shoot proliferation, high susceptibility of shoots to vitrification and browning, low survival rates in primary as well as secondary hardening an improved method has been developed for efficient acclimatization of teak.

MATERIALS AND METHODS
A. Collection of the Ex-Plants & Sterilization
The new immerging buds of *Tectona Grandis of* 35 year old matured trees were collected for the culture initiation. Mostly the explants were collected in the month of February to march. The auxiliary buds were considered for inoculation.
The collected buds were treated with different sterilizing agent. Initially after the fresh collection of buds, the buds were washed properly in detergent water and were dipped in 1% solution of Bavastin and m45 for two hours. Then after again buds were washed 4 to 5 times in double distilled water and finally buds were taken inside the laminar flow bench for further sterilization. Finally, buds were treated with 0.5% mercuric chloride for 8 minutes followed by 5-6 times double distilled water wash. Strictly autoclaved distilled water has been taken for all the procedures. After proper sterilization the cultures were again treated with alcohol (97 %) for 30 second. At last cultures were washed properly with 5-10 times by distilled water before inoculation into the medium.
B. Culture Initiation
Finally, cultures were trimmed slightly from the ends and were inoculated in culture initiation medium supplemented with MS basal medium (Murashige & Skoog, 1962) + 0.1 mg/l Kinetin +0.1 mg/l BA+ 0.1 mg/l IAA. The ph was adjusted to 5.8 before autoclaving. The cultures were incubated for 4 week in 25±2 ºC under 60% relative humidity. After four weeks the cultures were observed and the successful initiated contamination free cultures were taken for further shoot multiplication.

C. Shoot Multiplication

For the shoot multiplication, the cultures were transferred to four different trails with varying concentration of Kinetin, BAP & IAA. i.e., S1 (MS+ 0.5mg/l KIN +0.5 mg/l BA+0.1 mg/l IAA), S2 (MS +1mg/l KIN + 1mg/l BA+0.1 mg/l IAA), S3 (MS + 1.5 mg/l KIN + 1.5 mg/l BA+0.1 mg/l IAA) and S4 (MS + 2 mg/l KIN + 2 mg/l BA+0.1 mg/l IAA). After inoculations the cultures were kept in growth room. The temperature was maintained to 25 – 27º C. Cultures were incubated for four weeks.

D. Root Induction

After successful shoot multiplication, the cultures with well morphological characteristics having healthy shoots and good inter nodal gap were selected for root induction. For induction of roots, the shoots of length 3-4 cm were taken & dissected and inoculated in different media composition of NAA (Table 2). After inoculation the cultures were kept for 16 hour photoperiod and 25 – 27º C temperatures. Each bottle contains 6 cultures. Successful rooting percentages were recorded after 30 days.

E. Acclimatization

After successful root induction the plants were transferred to greenhouse for acclimatization. Four types of potting media were used for the primary hardening i.e. H1 (coco-peet+vermiculite+soil), H2 (Coco-peet +sand +vermiculite), H3 (vermiculite+ sand soil), and H4 (jiffy bag), in the ratio of (2:1:1), (1:1:2), and (2:1:1) respectively (table 3). The cultures were kept in greenhouse under relative humidity of 90% with mist chambers. Result was observed after 45 days of plantation. During these periods plants were intermittently treated with ½ strength of MS (Murashige & Skoog, 1962). After successful primary hardening plants were transferred to another potting media in poly bags for the secondary hardening (red soil+ vermiculite). During secondary hardening plants were kept in 50% net house.

RESULTS & DISCUSSION

a. Shoot Multiplication

In the present study the morphological responses of explants were recorded in different concentration of Kinetin & BA (Benzyl adenine) (table 1). The cytokinins are indispensable for sprouting of auxiliary bud (Purohit & Dave, 1996). The cultures contain S1 (MS +0.5 mg/l KIN + 0.5 mg/l BA+0.1 mg/l IAA) Resulted the best response with 5-6 mean number of shoots. The inter nodal gaps and morphology was found excellent (figure 1).

25 replications were conducted for each trial and each trial was conducted twice to find out the repeatability. Surrender & Narender (2009) reported induction of multiple shoots on MS (1962) medium supplemented with various cytokinins and auxins. Rout *et al.*, (2008) reported that addition of BA, ADS, & IAA, in medium proved as the most effective for promoting shoot multiplication. As a general principle, high concentration of cytokinin & low concentration of auxins in a medium promotes the induction of shoot morphogenesis in vitro culture by Chitra *et al.*, (2009). The present study showed active induction of axillary bud in various concentrations of KIN. BAP & IAA.

A. Induction of Roots

For *In vitro* rooting single shoots with 2 or 3 nodes were taken with subjected to different combinations of NAA (Table 2). The best result was found in cultures containing media R1 (½ MS+ 1mg/l NAA) where 98 % of roots were recorded (Figure- 4). The percentage of rooting was recorded after four weeks. As the use of either IAA or IBA in the culture medium influences the higher rate of root induction (Rout *et al.*, 2008). But this study showed that maximum induction of roots in media containing 1mg/l NAA (Naphthalene acetic acid).

B. Acclimatization

The healthy shoots with well developed roots were taken for further acclimatization in green house condition .The well rooted plants (Figure-03) were placed in four different trails of potting mixture ie.H1,H2,H3,H4. (Table 3) in mist chambers with 90% of relative humidity. The result was observed after 45 days of plantation. The best result was found in jiffy bag with 98% of survival (figure 5). The humidity was maintained with the covering the plants with polythene. Gradual removal of the polythene covering day by day during the acclimatization was also reported in Verbena tenera (Hosaki and Katanira, 1994)

for better survival. In initial period plants were treated with half strength of MS medium (Murashique & Skoog) for the intermittent basis. After 45 days hardened plants were transferred to poly bags containing red soil and vermiculite with the ratio of 1:1. Now the plants were kept in 50% net-house for 45 days. During secondary hardening plants were regularly treated with NPK (19: 19:19) 2gm/l and Bavastin 1 gm/l. It was noted that the plants were fully acclimatized in 3 months (Figure-6) and ready for the field plantation.

Table 1: Shoot Induction under Different Concentration of the Kinetin & BAP

Code	Kinetin (mg/l)	BA (mg/l)	IAA(mg/l)	Mean No. of Shoots	Cultures Status
S1	0.5	0.5	0.1	5-6	Healthy shoots
S2	1	1	0.1	3-4	Healthy shoots
S3	1.5	1.5	0.1	2-3	Healthy shoots
S4	2	2	0.1	7-8	vitrified

Table 2: Root Induction under the Varying Concentration of NAA

Trail Code	NAA mg/l	% of Rooted Shoots	Leaves	Growth
R1	1	98	Opened & Healthy	Fast
R2	0.5	40	Opened & Healthy	Normal
R3	1.5	60	Opened, Not Healthy	Slow
R4	2	49	Not Opened, Vitrified	Shunted

Table 3: Acclimatization in Different Potting Mixtures

Trail Code	Potting Media	Ratio	Fertilizers	% of Survival
H1	Cocopeet+Vermiculite+soil	1:1;1	NPK	40
H2	sand+ vermiculite	1;1	NPK	41
H3	Cocopeet+vermiculite+sand	2;1;1	NPK	50
H4	jiffy bags	Filled with only coco peat	1/2 MS	98

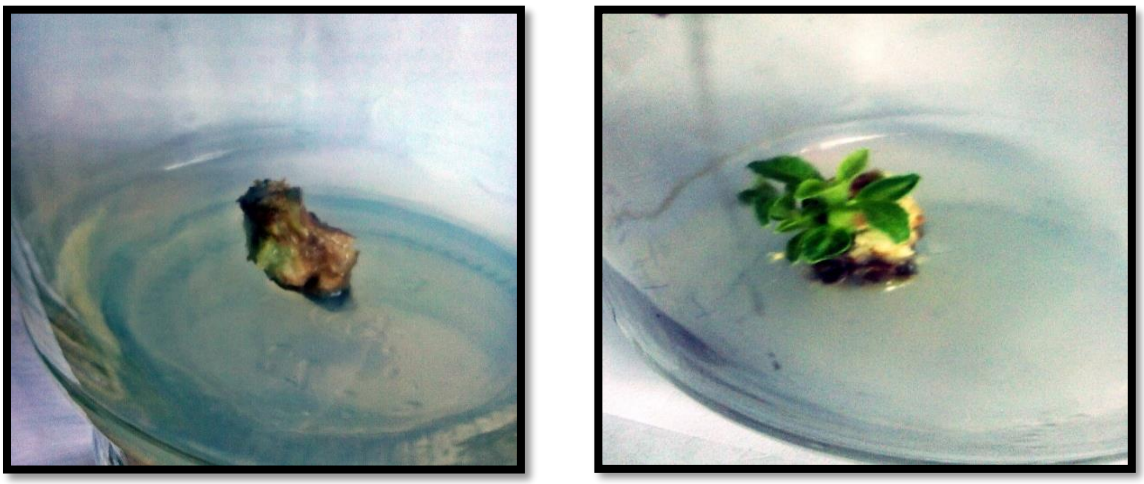

Figure 1: Initiation (MS Basal +0.1mg/l BA+0.1mg/l Kin+0.1mg/l IAA)

Figure 2: Shoot Induction (MS Basal+0.5 mg/l BAP+0.5 mg/l Kin+0.1 mg/l IAA)

Figure 3 & Figure 4: Rooted Teak Plants (1/2MS Basal +1mg/l NAA)

Figure 5: Primary Hardened Plants in Jiffy

Figure 6: Healthy Root Development in Jiffy

Figure 7: Well Developed Secondary Hardened Plants Ready for Field Transfer

Conclusions

The present work is Highly efficent for the mass propagation of the Teak Planting materials in minimum cost & less time. It was noted that the plants were healthy after tasplantatio in th field & were free from any variation.

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