DIRECT SHOOT REGENARATION OF *TECTONA GRANDIS* (TEAK) FOR PRODUCTION OF PLANTING MATERIAL

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ABSTRACT

Tectona grandis or commonly known as teak is ranked among the top five tropical hardwood species planted worldwide. Its properties make it suitable to be used as furniture and raw material for the construction industry. To cater to high market demand, mass propagation of teak for plantation is one of the better ways to provide planting materials. Tissue culture is the best method for mass propagation to produce uniform and quality plantlets. In this study, nodal segments from a young mother plant were used as explants, and plant growth regulators were used to look at their effect on growth and rooting. To look at the effect of plant growth regulator on growth, benzyl aminopurine (BAP) was used. The highest shoot multiplication was achieved in MS basal medium supplemented with 0.25 mgL-1 BAP, while MS basal medium added with 0.1 mgL-1 BAP produced the highest mean shoot length (4.03 ± 0.34cm). To look at the effect of plant growth regulator on rooting, indole-3-butyric acid was used. Half strength MS with 2.0 mgL-1 IBA produced the longest roots and lateral roots. For plantlets survival rate, sand was the best potting medium, followed by Jiffy 7 and baked soil. Jiffy 7 however, produced healthiest plantlets.

Key words: in vitro propagation, Tectona grandis, teak, tissue culture

INTRODUCTION

Tectona grandis which is commonly known as teak belongs to plant family Verbenaceae. It is valued as a prized timber due to its versatility, durability, fast growth and attractiveness of its wood. Its uses include flooring and parquet, ship-building, furniture-making and as construction material. At present, teak ranks among the top five tropical hardwood species in terms of plantation area established worldwide (Dah and Baw 2001).

The cultivation of teak is widespread in the tropics, and the majority of planting materials were obtained from seeds (Nautiyal et al. 1991). Gyves et al. (2007) and Slator et al. (2013) had stated that dependance on seeds as planting materials has certain disadvantages such as non-uniformity (caused by genetic variability), seeds availability, and low germination rate, usually between 20 to 25% due to tegumentary dormancy, and to overcome this problem, there is a need to adopt costly techniques for sowing. Finally, good supply of seeds can only be made possible when the trees mass fruiting.

In vitro propagation technique has become an efficient way for producing uniform plants on a large scale and in a short time without the need to wait for seeds availability (Krishnapillay, 2000). Tiwari SK et al. (2002) had found out that micropropagation of teak from mature trees has remained problematic. Poor explant response and rapid explant browning are major hurdles to a successful establishment of teak *in vitro*. Akram and Aftab (2008) also stated that in vitro propagation of teak still remains problematic due to the poor capacity of shoot proliferation, high susceptibility of shoots to nitrification and browning, and low frequency of *in vitro* rooting. None of the published protocols fully satisfies the requirement for a commercial application in spite of the progress made on tissue culture of teak since the 1970s

In timber plantation, uniform plant from selected mother plant will produce the best output. Many studies were conducted to acquire the best method to mass propagate teak. R. Srinivasan et al. (2012) tested teak shoot multiplication in different

concentration of plant growth regulators (PGR). Many more had done studies to come up with the most efficient method to micropropagate teak (Mendoza et al. 2007, and Ramesh et al. 2009). This paper highlights an alternative protocol in propagating teak plantlets through tissue culture in order to produce uniform and disease-free planting material.

MATERIAL AND METHODS

Plant material

Tectona grandis (Teak) actively growing shoots were collected from a young mother tree located at the Forest Research Institute Malaysia. The mother plant chosen were 240 cm height and 9 cm breast height diameter with healthy new shoots.

Surface sterilization

Nodal segments from young shoots were excised and used as explants. The segments were cut into 3-4 cm long and washed with Teepol (1%) for 10 minutes. For surface sterilization, the explants were soaked in 0.1% Benlate plus 1 drop of Tween 20 for 1 hour. After rinsing with sterile distilled water, the explants were dipped in 70% ethanol plus a drop of Tween 20 for 1 min and rinsed again with sterile distilled water. The explants were later soaked in 50% Clorox® with a drop of Tween 20 for 20 min and rinsed with sterile distilled water. The explants were then dissected and soaked in 0.1% citric acid for 5 min and air dried in laminar flow. MS basal media culture medium containing 0.1 mgL^{-1} benzyl-amino-purine (BAP) was used for shoot induction.

Shoots Multiplication

Axillary bud sprouts from the explants were used as explants for shoot multiplication experiment. The explants were cultured into full strength MS basal medium supplemented with BAP of five different concentration (BAP 0 mg/L, 0.1 mg/L, 0.25 mg/L, 0.5 mg/L, 1.0 mg/L and 2.0 mg/L). The medium pH was adjusted to 5.8 before autoclaving. Four bottles of medium were prepared for each treatment, and each bottle contained 5 shoots (±1.5 cm). The number of shoots induced per explant and shoots length was observed after 6 weeks of culture.

In vitro Rooting

Individual shoots from multiplication media were used for *in vitro* rooting. Half strength Murashige and Skoog (MS) basal medium was used to induce roots *in vitro*. The MS basal medium was supplemented with five different concentration of indole-3-butyric acid (IBA) i.e., 0 mg/L, 0.5 mg/L, 1.0 mg/L, 1.5 mg/L and 2.0 mg/L. Ten replicates were prepared for each treatment. The number of roots, lateral roots, root length, and lateral root length were recorded for four weeks.

Acclimatization

In vitro rooted plantlets were used for acclimatization. Plantlets of about 4 cm in height were pulled out from the culture media and washed under flowing tap water to remove remaining agar medium. The plantlets were then dipped into Thiram (fungicide) for a few seconds to disinfect the plants. The plantlets then were planted in different potting media (jiffy 7, sand and baked soil) with 15 replicates for each treatment. The plantlets were kept in a sealed glass chamber to maintain humidity. After one month, the seal was gradually released within several days to prevent shock. The plantlets were watered only when the sealed was opened. Plantlets that survived acclimatization were transferred into poly bags. The survival rates of plantlets were recorded.

Data Analysis

Data from the shoot multiplication and *in vitro* rooting experiment were analyzed using SAS ver. 9.1 software. ANOVA was applied to compare values obtained for the different measured parameters ($P < 0.01$). Mean values were compared using Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Surface sterilization and shoot induction

Surface sterilization is a critical step in tissue culture to get a clean culture before shoot induction. In this study, due to a limited number of samples, only one surface sterilization method was used. After 1 week, it was observed that new shoots started to grow. Devi et al. 1994 and Sreedevi & Damodharam, 2015 reported that axillary shoots of teak can develop after 7 to10 days of culture. Compared using seeds as explant in this experiment, it was reported that seeds have low germination rate cause by the thick pericarp which limits water and oxygen supply to the seeds, physiological immaturity and chemical inhibiters present in the pericarp (Azamal and Mohinder 2006).

Shoot multiplication

Multiplication of teak shoots was carried out on MS basal medium containing different concentrations of BAP. Table 1 showing the mean number of new shoot and mean shoot height from 6 different treatments. MS basal medium supplemented with 0.25 mg/L BAP produced the highest mean number of shoots with 2.39 ± 0.24 shoots per explants, followed by 0.5 mg/L BAP and 1

mg/L BAP with the mean number of new shoots produced were 2.05 ± 0.11 and 2.0 ± 0.26, respectively (Table 1). There was a significant difference between MS added with BAP and the control.

Table 1: Effect of different BAP concentration on teak shoot multiplication and shoot height

Media	BAP conc. (mg/L)	Mean no. of shoots per explant	Shoot height (cm)
MS	0.0	1.0 ± 0.0 ^{c}	3.29 ± 0.19^{b}
MS	0.1	1.5 ± 0.14^{b}	4.03 ± 0.34^{a}
MS	0.25	2.39 ± 0.24^{a}	2.10 ± 0.11^{c}
MS	0.5	2.05 ± 0.11^{a}	1.86 ± 0.09^{c}
MS	1.0	2.0 ± 0.26^{ab}	1.82 ± 0.12^{c}
MS	2.0	1.87 ± 0.22^{ab}	1.64 ± 0.12^{c}

In terms of shoot height, MS basal medium containing 0.1 mg/L BAP was the best medium for shoot elongation with mean shoot height of 4.0 ± 0.34 cm, followed by medium without BAP and medium with 0.25 mg/L BAP with mean shoot height of 3.29 ± 0.19 cm and 2.1 ± 0.11 cm, respectively. MS basal medium supplemented with 0.25 mg/L BAP was the best medium for shoot multiplication (Table 1). Meanwhile MS basal medium with 0.1 mg/L BAP was the best medium for shoot elongation.

For teak micropropagation, BAP was the more commonly used plant growth regulator (PGR) either alone or in combination with other PGRs such as kinetin (Gill et al. 1991, and Goswani 1999). Different cytokinin may give a different result. In spite of that, according to Tomas et al. (2001), cytokinin-deficient plants developed stunted shoots with smaller apical meristems. With regard to PGRs, as a general principle, a high concentration of cytokinin and low concentration of auxin in a medium promote the induction of shoot morphogenesis. Based on our observation, shoot induction is the best with 0.25 mg/L BAP. The shoot induction started to decrease as the concentration of BAP increases. As shown in Table 1, the shoot height decreases as the BAP concentrations increased. To produce taller plantlets during *in vitro* phase, 0.1 mg/L BAP in the medium was sufficient. It was widely reported that BAP alone was sufficient as a sole growth regulator in teak (Devi et al. 1994; Gupta et al. 1980 and Sharma et al. 2000). The use of a single PGR for shoot multiplication not only effective but also reduces the production cost for large scale plantlets production.

In vitro rooting

In this experiment, half strength MS basal medium with different concentrations of IBA was tested. Table 2 showed how ½ MS medium with different concentration of IBA affect the mean number of roots and roots length. Individual shoots sub cultured into half MS basal medium supplemented with 2.0 mg/L IBA produced the highest number of taproots and lateral roots with a mean number of roots 2.1 ± 0.23 and 5.8 ± 2.47, respectively. MS medium containing 1.0 mg/L IBA produced taproots and lateral roots with a mean number of 1.9 ± 0.28 and 2.9 ± 0.97, respectively (Table 2). In terms of root length, higher IBA concentrations produced longer roots. There was a significant difference for the number of roots between medium added with IBA and without IBA. However, in term of root length, a significant difference was observed only in medium containing 2.0 mg/L IBA.

Table 2: Effect of different IBA concentration on the mean number of roots and roots length

Medium	IBA conc. (mg/L)	Mean no. of roots	Mean no. of lateral roots	Root mean length (cm)	Lateral root mean length (cm)
½ MS	0.0	0.4 ± 0.16^{b}	0.2 ± 0.13^{b}	0.19 ± 0.08^{b}	0.02 ± 0.01^{b}
½ MS	0.5	1.6 ± 0.22^{a}	0.5 ± 0.30^{b}	0.46 ± 0.08^{ab}	0.03 ± 0.01^{ab}
½ MS	1.0	1.9 ± 0.28^{a}	2.9 ± 0.97^{ab}	0.46 ± 0.1^{ab}	0.13 ± 0.07^{a}
½ MS	1.5	1.8 ± 0.42^{a}	1.7 ± 1.25^{b}	0.32 ± 0.07^{ab}	0.02 ± 0.01^{b}
½ MS	2.0	2.1 ± 0.23^{a}	5.8 ± 2.47^{a}	0.57 ± 0.14^{a}	0.09 ± 0.03^{ab}

The findings from this study are similar to Husen and Pal (2006). They evaluated teak rooting response against NAA and IBA and recommended IBA for higher rooting percentage, regardless of the age of the mother. Gyves et al. (2006), however, showed that the use of IBA at low concentration was advisable since it did not interfere with shoot growth, root elongation and kept the basal callus small. The use of either IAA or IBA in the culture medium influences positively the rate of root induction (Rout et al. 2008).

For the rooting experiment, half MS basal medium was used rather than full strength due to the osmotic strength of the medium. Soil medium has a much lower osmotic strength compared to MS basal medium. By lowering the medium osmotic strength during the rooting process, the survival rate of *in vitro* plantlets during acclimatization stage can be increased. I*n vitro* rooting process of other timber species such as *Eucalyptus* spp. and *Dipterocarpus* spp. also used half strength basal medium (Linington 1991, and Brondani et al. 2011). The use of a half-strength basal medium may also lower the production cost.

Acclimatization

The primary purpose of acclimatization is to enable plantlets to adapt and able to survive in *ex vitro* environment. The characteristics of plantlet's leaves produced *in vitro* are different from those raised in a normal manner. I*n vitro* plantlets developed leaves that lack cuticles but have unicellular and uniseriate trichomes scattered all over the leaf surface (Yasodha et al. 2005). On the other hand, normal plantlets have branched multicellular trichomes covering the entire surface. Stomata of leaves in culture are larger in size and do not respond as normal plantlets stomata. However, after acclimatization, the physiology and stomata of new leaves are very similar to those of normal leaves.

After one-month acclimatization in S-Accha chamber, plantlets in sand medium showed the highest survival rate (80%) followed by jiffy 7 and baked soil medium. The survival rate in both jiffy 7 and baked soil medium was equal (73.3%). Although the sand medium showed the highest survival rate, plantlets in jiffy 7 were taller and with larger leaves. Nor Aini et al. (2009) found that jiffy 7 to be a better rooting medium, and Chan et al. (2009) also reported that the survival rate for *Gynura procumbens* in jiffy 7 was 100%.

In its natural habitat, teak grows best in fertile, well-drained soil and warm tropical climate with plenty of sunshine. Jiffy 7 has the highest water retention compared to other media used. However, throughout the one-month acclimatization, the plantlets were not watered. The source of water for the plantlets was from natural condensation in the acclimatization chamber. The growth of plantlets depended on the nutrient availability in the soil. It was critical to keep the substrate well-watered to restore the loss of water from transpiration.

Temperature and humidity play major roles to ensure a successful acclimatization process. Using a simple acclimatization chamber (S-Accha), the humidity in S-Accha was step by step reduced to surrounding humidity by gradually open its plastic cover. A reduction in relative humidity leads to an increase in plant transpiration with associated development of functional stomata for controlling plant water loss. By reducing the plantlets stress level, a higher survival rate can be achieved (Seelye et al. 2003). In this study, the temperature was not controlled, but maintained according to greenhouse ambient temperature.

Fig 1 (a)*Tectona grandis* in different shoot multiplication media. (b & c) *Tectona grandis* before and after acclimatization

With all the result obtained, the mass production of teak tissue culture plantlets can be started. Until the year 2000, it is widely believe that teak best be planted at the northern region of Peninsular Malaysia such as Perlis or Kedah which is drier. Further research had counter that believe as teak equally suited to be planted at other region in Peninsular Malaysia where it is wetter and hotter (Krishnapillay. 2000). Umachandran & Sawicka (2017) had concluded that increasing the plantation volumes is the only way to prevent the downfall of the timber industry as the supplies continue to decrease year after year. With this in mind, in vitro propagation of teak planting material is needed to support Malaysia's timber industry.

CONCLUSION

This study provides an efficient method to propagate *Tectona grandis* (teak) using a tissue culture method. The technique provides a uniform and disease-free plantlet that can be used as planting stock. The study shows that young and healthy shoots can be produced by using BAP as the only PGR for shoot multiplication. 100% rooting can be achieved with half MS basal medium with IBA as PGR. The acclimatization process using jiffy 7 as potting medium produced healthy plantlets after 1 month. Figure 2 showing healthy tissue culture derive teak plantlets at the nursery. Planting materials of teak had been produced using this method at FRIM to support commercial plantation.

Fig 2 Young and healthy teak plantlets in the nursery

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REFERENCES

Azamal, H and Mohinder, P. (2006). Variation in shoot anatomy and rooting behavior of stem cuttings in relation to age of donor plants in teak (Tectona grandis Linn. F.). New Forest 21:57-73.
Brondani, G.e., Dutra, L.f., Wendling, I., Grossy, F., Hansel, F.a. and Araujo, M.a. (2011). Micropropagation of a *Eucalyptus hybrid* (*Eucalyptus benthamii x Eucalyptus dunnii*), Acta Scientiarum. Agronomy. v. 33, n. 4, p. 655-663
Chan, L.K., Lim, S.Y. and Pan, L.P. (2009). Micropropagation of *Gynura procumbens (Lour.)* Merr. an important medicinal plants. J. Med. Plants Res. 3(3): 105-111.
Dah, U.S. and Baw, U.S. (2001). Regional teak marketing and trade, Proceedings of the third regional seminar on teak, Indonesia. Pp. 1-14.
Devi, Y.S., Mukherjee, B.B. and Gupta, S. (1994). Rapid cloning of elite teak (Tectona grandis L.) by in vitro multiple shoot production. Indian J. Exp. Biol. 32:668-671
Gill, S.S., Gupta, R.P. and Pandher, M.S. (1991). In vitro propagation of teak. India J. Res. 28:507-510
Goswami, H., Keng, C.L. and Teo, C.K.H. (1999). In vitro shoot multiplication of *Tectona grandis*. J. Biosci. 10:47-54.
Gyves, E.M.D., Royani, J.I. and Rugini, E. (2007). Efficient method of micropropagation and in vitro rooting of teak (*Tectona grandis L.)* focusing on large-scale industrial plantations. Annals of Forest Sciences, Springer Verlag/EDP Sciences, 64(1):73-78
Husen, A. and Pal, M. (2006). Variation in shoot anatomy and rooting behaviour of stem cuttings in relation to age of donor plants in teak (*Tectona grandis Linn. f.).* New Forest, 1:57–73
J.A. (2003). High survival rates during acclimatization of micropropagated fruit tree rootstocks by increasing exposures to low relative humidity, ISHS Acta Horticulturae 616:13
Krishnapillay, B. (2000). Silviculture and management of teak plantations, in: Unasylva, No. 201, Teak, Int. J. Forestry Forest Ind (FAO) 51- 2000/2.
Linington, I.M. (1991). In vitro propagation of Dipterocarpus alatus and Dipterocarpus intricatus. Plant Cell, Tissue and Organ Culture 27: 81-88Marin,
Nautiyal, S., Uma, S. and Gurumurthi, K. (1991). Rooting response of branch cuttings of teak (*Tectona grandis*) as influenced by season and growth hormones. Indian Forester 117: 249−254.
Nor Aini, A.S., Goh, B.L. and Ridzuan, R. (2009). The effects of different indole-3-butyric acid (IBA) concentrations, two light regimes of in vitro rooting and acclimatization of in vitro teak (*Tectona grandis L.f*) plantlets, African Journal of Biotechnology Vol. 8 (22), pp. 6158-6161
Rout, G.R., Mohapatra, A. and Jain, S.M. (2006). Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnology Advances. Pp. 1-30.
Seelye, J.F., Burge, G.K., and Morgan, E.R. (2003). Acclimatizing tissue culture plants: reducing the shock, combined proceedings international plant propagators’ society, volume 53.
Slator, N.J., Callister, A.N. and Nichols, J.D. (2013). Mechanical but not physical dormancy is a cause of poor germination in teak (*Tectona grandis L.f.*). New Forest, 44(1):39-49.

Tambarusi, E.V., Rogalski, M., Galeano, E., Brondani, G.E., Martin, V.D.F.D., Silva, L.A.D. and Carrer, H. (2017). Efficient and new method for *tectona grandis* in vitro regeneration. Crop Breeding And Applied Biotechnology 17: 124-132.
Tomas, W., Vaclav, M., Miroslav, S. and Thomas, S. (2001). Regulation of plant growth by cytokinin. Proceeding of the National Academy of Sciences of The United States Of America. 98(18):10487-10492
Umachandran, K. and Sawicka, B. (2019). Study of timber market of malaysia and its impact on the economy and employment. Journal of advance in agriculture. Vol 7(3):1109-1116.
Yasodha, R., Sumathi, R. and Gurumurthi, K. (2005). Improved micropropagation methods for teak. Journal of Tropical Forest Sciences. 17(1): 63-75