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Full Length Research Paper

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

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Effects of different indole-3-butyric (IBA) concentrations (0, 0.5, 1.0, 2.0, 3.0 and 5.0 mg/l), two light regimes of *in vitro* rooting and acclimatization on *in vitro* teak (*Tectona grandis* L.f) were investigated. Shoots incubated in the light produced higher mean number of roots (2.0) and mean root length of 15.0 and 4.5 mm when supplemented with low IBA concentrations of 0.5 and 1.0 mg/l, respectively. Conversely, when shoots were incubated in the dark, higher mean number of roots (8.0 and 3.0) and mean root lengths (14.0 and 8.8 mm) were produced when supplemented with higher IBA concentrations of 2.0 and 3.0 mg/l, respectively. IBA was found necessary for root induction and growth in light and dark where 2.0 mg/l was optimum as it recorded high mean number of root (6.0 and 8.0) with mean root lengths of 11.8 and 14.0 mm, respectively. High survival percentage of 80% was recorded for those plantlets raised in "jiffy-7" but survival reduced to 40% when grown in the autoclaved soil after one week.

Key words: Teak (*Tectona grandis*) plantlets, indole-3-butyric acid, light regime, *in vitro* rooting, acclimatization.

INTRODUCTION

Tropical deforestation and degradation of forests in the world are negatively affecting the availability of forest goods and services (FAO, 2005). Recently, global trend towards greater reliance on plantation as a source of industrial wood has been adopted as an effort to reduce timber harvesting from natural forest. One of the current recommended high quality timber species, identified to be potential for this purpose in Malaysia is teak (*Tectona grandis*). Teak is listed as one of nine selected species for forest plantation in Malaysia (MTIB, 2007) and ranked among the top five tropical hardwood species in term of plantation area established worldwide (Dah and Baw, 2001). In addition, teak wood is considered to be one of the best general utility timber with worldwide reputation, being extensively used for shipbuilding, interior and exterior luxury furnishing, bridges and wharves, railway carriages and wagons, ordnance, shingles, wheels, carvings and general carpentry (Mendoza de Gyves et al., 2007; Appanah and Weinland, 1993).

Establishment of teak plantation programme is limited to its seed problems. Besides having low seed quality and late seed production, it also has hard seed coat which further contributes to poor germination rate (Bonal and Monteuuis, 1997). It also shows irregular seed bearing habit (Lee and Rao, 1981) and teak raised from seeds show wide phenotypic variation on its growth which becomes disadvantages to commercial propagation and plantation (Krishnapillay et al., 1998). Thus, vegetative propagation can facilitate the possibilities to have constant supply of quality seedlings or plantlets of teak with desired growth performance.

Tissue culture offers a practical afforestation program-

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Figure 1. The elongated shoots used for *in vitro* rooting experiment. Shoots used were healthy thick shoots, with average length of 70 mm (Note: white bar is equal to 1 cm).

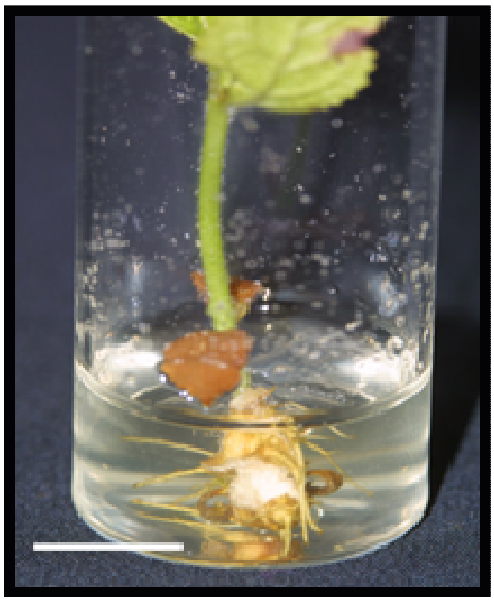

Figure 2. Good root growth with mean number = 8.0 when supplemented with 5.0 mg/l IBA (Note: White bar is equal to 1 cm).

mes by producing uniform plants on a large scale and in a short period of time (Pandey and Brown, 2000). Although, *in vitro* research has been conducted on *T. grandis*, little work has been reported on acclimatization of micro propagated plantlets onto soil or other suitable rooting media. The practical prospects of micro propagation are largely dependent on the success of the acclimatization process and the capability of the acclimatized plantlets to resume growth (Bonal and Monteuuis, 1997). Therefore, this study was carried out to investigate the effects of light and dark treatment on the rooting and different rooting media on the hardening of *in vitro* plantlets of *T. grandis*.

MATERIALS AND METHODS

In vitro rooting utilized elongated shoots which was initially cultured on MS medium supplemented with 0.2 mg/l of kinetin. They were raised for four weeks to allow the plantlets to produce thick shoots with an average length of 70 mm (Figure 1). The sources of the elongated shoots were provided by the Tropbio Research Sdn. Bhd., a private company specializing in plant tissue culture and molecular biology work.

The *in vitro* rooting experiments adopted a complete randomized design utilizing those elongated shoots cultured on white medium treated with six IBA concentrations; 0 mg/l (control), 0.5, 1.0, 2.0, 3.0 and 5.0 mg/l (15 replicates per treatment) for the dark and light conditions. Aluminum foils were cut in the size of 10 x 6 cm and were then wrapped around the borosilicate test tubes to create the dark effect. Observation was made for a period of six weeks and data on percentage of shoot rooted, mean number of root and mean length of root were evaluated at the end of the sixth week.

The *in vitro* grown plantlets with developed root system obtained from rooting stage were later transplanted into jiffy "7" and sterile medium of 3:3:1 sand, soil and compost mixture. Evaluation was based on the survival percentage on rooting media.

In addition, the effects of *in vivo* rooting were compared using T-test. Data was analyzed using the Statistical Analysis System (SAS) computer package. The Duncan's Multiple Range Test (DMRT) was used to determine the significant differences among treatment means only when the F test was significant. Differences at the probability level of $P>0.05$ was only considered significant.

RESULTS AND DISCUSSION

Result indicated that the *in vitro* rooting of teak was significantly affected by different concentration of IBA used. Despite no root growth in IBA free medium, there was sign of swollen callus at the cut end. Generally, the percentage of rooted shootlets was low when treated in different concentrations of IBA. Almost all those rooted shootlets showed callus growth when treated with different IBA. Gyves et al. (2007) also reported that callus would normally form at the cut end with an increase of IBA concentration.

Root growth in terms of mean number and length showed significantly differences in different IBA (Table 1). Those supplemented with 2.0 and 5.0 mg/l produced reasonably many roots with mean of 6.0 and 8.0 respectively (Figure 2).

Roots initiation and development were also found to be

Table 1. Effects of various concentration of IBA on root formation of *in vitro* shootlets.

IBA (mg/l)	Percentage of rooted shoots (%)	Mean number of roots	Mean length of roots (mm)
0	0	0^{e}	0^{e}
0.5	15	2.0^{cd}	15.5^{a}
1	20	2.0^{d}	4.5^{d}
2	22.5	6.0^{b}	11.8^{b}
3	20	3.0^{c}	3.8^{d}
5	22.5	8.0^{a}	8.0^{c}

*Values having the same superscripts are not significantly different at P<0.05 based on Duncan's Multiple Range Test.

Table 2. T-test of rootability of shoots incubated in the dark and light of different concentrations of IBA.

IBA concentrations		No of Root			Root Length		
		Dark		Light	Dark		Light
0.5 mg/l	Mean	1.5		2.3	3.0		15.5
	Std Deviation	1.0		0.9	0.9		0.8
	Observed T value		1.4^{ns}			1.5*	
1.0 mg/l	Mean	2.6		1.8	1.8		4.5
	Std Deviation	0.5		0.5	0.3		0.2
	Observed T value		2.7*			7.6*	
2.0 mg/l	Mean	7.5		6.3	14.0		11.8
	Std Deviation	1.2		0.8	2.4		1.9
	Observed T value		1.9^{ns}			0.9^{ns}	
3.0 mg/l	Mean	3.3		3.1	8.8		3.8
	Std Deviation	0.5		1.2	2.2		0.7
	Observed T value		0.3^{ns}			5.2*	
5.0 mg/l	Mean	3.8		7.6	4.5		8.0
	Std Deviation	1.0		1.2	1.0		0.9
	Observed T value		5.4*			6.2*	

^{ns}Non significant at p<0.05, *Significance different at P<0.05.

affected by light regime. Roots started to develop after 10 days in culture dark incubation. Though root formation initiated earlier when incubated in the dark (after 10 days in culture), most cultures showed sign of defoliation and browning effects. Almost all the leaves turned brown and were detached from the stem after 30 days in culture. Mean number of roots and root length were significantly different between light regime after 42 days (Table 2).

Generally, cultures which were supplemented with 2 mg/l IBA exhibited good root growth either when incubated in the light and dark treatments which were not significantly different (Table 2). In fact, higher mean number of roots of 8.0 and length of roots of 14.0 mm were obtained when incubated in the dark (Figure 3).

Result indicated that both IBA and light regime affected root formation of *in vitro* shootlets. This is in accordance with those reported by Druart et al. (1982), who stated that low level of light and low temperature were necessary to stimulate shoot proliferation and rooting. In fact, Durga and Metha (1993) and Rahman et al. (2004) recommended complete darkness to be given at the beginning of the rooting stage in *Cammiphora wightii* and *Elaeocarpus robustus*, respectively. The browning and the defoliation to the plantlets incubated in dark could be due to reaction of peroxidase and phenolic activities of adventitious shoots. This phenomenon was also shown in *Malus domestica* when they were kept in the dark (Druart et al., 1982). When light become the limiting factor, more auxin is required for rooting signified by shoots incubated in the dark gave better rooting especially for those being supplemented with higher auxins. Such responses were also shown by many woody species whereby at higher photon fluxed caused inhibition to rooting (Rugini et al., 1998). To date, the effect of dark treatment in rooting is

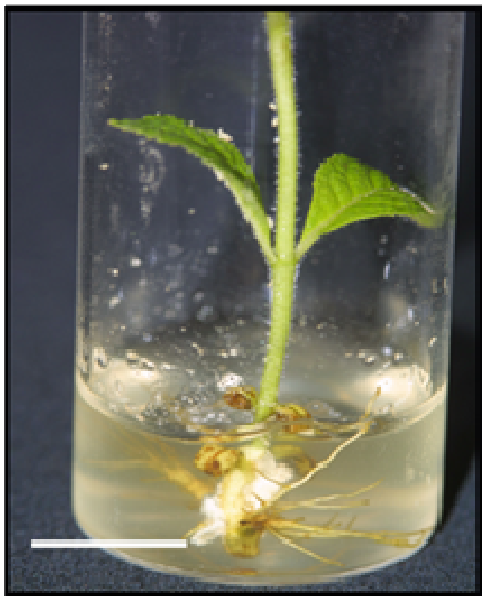

Figure 3. Good root growth (mean numbers) when supplemented with 2.0 mg/l IBA and incubated in the dark (Note: white bar is equal to 1 cm).

Table 3. T-test on the number of plantlets survival on "jiffy-7" and Unautoclave soil.

Parameter	''Jiffy-7		Autoclaved soil mixture
N	20		20
Std Deviation	0.41		0.50
Observed T value		2.75*	
Percentage of survival	80%		40%

[ns] Not significant at 0.05%, *Significant difference at P<0.05.

still not fully understood.

Acclimatization is the determining key for the survival of the *in vitro* raised plantlets since they are not suited for *in vivo* conditions (Baksha et al., 2007). The survival rate of teak plantlets transferred to *ex vitro* acclimatization treatments showed that there was significant between rooting media that is 80% for "jiffy 7" and 40% for autoclaved soil mixture after 35 days (Table 3). Jiffy 7 was found to be a better rooting medium and Chan et al. (2009) reported 100 % of survival rate for *Gynura procumbens* when raised on the same medium.

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