UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF DIFFERENT IBA CONCENTRATIONS, LIGHT REGIMES OF IN VITRO ROOTING AND ACCLIMATIZATION OF INVITRO TEAK (TECTONA GRANDIS L.F) PLANTLETS

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The effect of different IBA concentrations, two light regimes of *in vitro* rooting and acclimatization on *in vitro* teak (*Tectona grandis* L.f) was investigated. The experiments followed a Completely Randomized Design (CRD) involving six concentrations of IBA (0mg/l, 0.5mg/, 1.0mg/l, 2.0mg/l, 3.0mg/l and 5.0mg/l) and two light regimes with 15 replicates each. The *in vitro* rooting experiments showed that shoots cultures in White medium supplemented with 2.0mg/l and 5.0mg/l IBA produced the best results in terms of mean number of root (6.33≈6 and 7.66≈8) and mean root length (11.82mm and 8mm) respectively. *In vitro* shootlets incubated in the dark exhibited earlier root initiation by five days compared to those incubated in the light. Generally, shoots incubated in the light produced significantly higher mean number of roots (2.3) and mean root length (15.5 mm and 4.5 mm) when supplemented with low concentrations of 0.5mg/l and 1.0mg/l IBA. Conversely when shoots were incubated in the dark, higher mean root length of (14.0mm and 8.8mm) and mean number of root (7.5≈8 and 3.3≈3) were produced when being supplemented with higher concentrations of 2.0mg/l and 3.0mg/l respectively. Chiefly 2.0mg/l IBA was found to be optimum for rooting of teak as it recorded high mean number of root (6.3≈6 and 7.5≈8) and mean root length (11.8mm and 14.0mm) regardless of light regime. The higher survival percentage of 80% was recorded for plantlet grown in "jiffy-7" but only 40 % survived in the autoclaved soil after one week. However the plantlets failed to continue to survive after the second week due to unforeseen technical error such as a faulty growth chamber. The present result substantiates the requirements of IBA to promote the *in vitro* rooting of teak shootlets and dark incubation to accelerate rhizogenesis.

KESAN BEBERAPA KONSENTRASI IBA, KEADAAN CAHAYA DALAM PENGAKARAN IN *VITRO* DAN AKLIMITASI ANAK POKOK *IN VITRO* JATI (*Tectona grandis* L.f)

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Kesan beberapa kepekatan IBA, dua keadaan cahaya dalam pengakaran *in vitro* and aklimitasi anak pokok *in vitro* Jati (*Tectona grandis*) telah dikaji. Ujikaji menggunakan rekabentuk rawak lengkap yang melibatkan enam kepekatan IBA (0mg/l, 0.5mg/, 1.0mg/l, 2.0mg/l, 3.0mg/l and 5.0mg/l) dan dua keadaan cahaya yang mana masing-masing mempunyai 15 replikasi setiap satu. Hasil kajian pengakaran *in vitro* telah menunjukkan bahawa pucuk di dalam media yang ditambah dengan 2.0mg/l dan 5.0mg/l menghasilkan keputusan yang baik dari segi purata bilangan akar (6.33≈6 and 7.66≈8) dan purata panjang akar (11.82mm and 8mm). Anak pokok *in vitro* yang diinkubasi dalam gelap menunjukkan pembentukan akar yang lebih cepat iaitu lima hari lebih awal berbanding proses pengakaran dalam cahaya. Secara amnya pucuk yang terdedah kepada cahaya meghasilkan purata bilangan akar (2.3) dan purata panjang akar (15.5 mm and 4.5 mm) yang lebih tinggi apabila media dibekalkan dengan IBA yang mempunyai kepekatan yang rendah iaitu 0.5mg/l dan 1.0mg/l IBA. Tetapi apabila pucuk diinkubasi dalam gelap, pengakaran yang lebih baik ditunjukkan oleh media pada kepekatan IBA yang tinggi iaitu 2.0mg/l dan 3.0mg/l yang mana menghasilkan purata bilangan akar (7.5≈8 and 3.3≈3) serta purata panjang akar (14.0mm and 8.8mm). Walaubagaimanapun didapati kepekatan optimum untuk pengakaran *in vitro* adalah 2.0mg/l kerana menghasilkan purata bilangan akar (6.3≈6 and 7.5≈8) serta purata panjang akar (11.8mm and 14.0mm) yang baik dalam kedua-dua kesan cahaya. Peratus kemandirian anak pokok dalam "Jiffy-7" adalah 80% berbanding campuran tanah steril yang hanya mencatatkan 40% selepas seminggu dalam proses aklimitasi. Namun kesemua anak pokok ini mati pada minggu kedua disebabkan oleh kerosakan teknikal yang berlaku pada peti pertumbuhan. Hasil kajian ini membuktikan peranan IBA dalam pengakaran serta keperluan inkubasi gelap dalam meningkatkan inisiasi akar.

APPROVAL SHEET

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Title: The Effects of Different IBA Concentrations, Light Regimes of *In Vitro* Rooting and Acclimatization of *In vitro* Teak (*Tectona grandis* L.f) Plantlets.

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Father, Mother and Brother

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
^{0}C	degree centigrade
cm	centimeter
Df	degree of freedom
g	gramme
IBA	Indole-butyric-acid
L	liter
mg	milligramme
ml	milliliter
m	meter
mg/l	milligramme
M	molar
µM	micro molar
NaOH	sodium hydroxide
pH	negative logarithm of the hydrogen concentration
SS	sum of square

TERMINOLOGY

Acclimatization	Show change in the physiology of an organism/plant, as a result of its exposure to change environment.
Adventitious	Developing from unusual point of origin, such as shoots or root arising from a leaf or stem tissue other than the axils or apex, often dependent on close physical or temporal association with organized or semiorganized tissue or cell.
Auxins	Plant hormone naturally synthesized (Indole-3-Acetic Acid-IAA) in the apex and transported downwards the stem. Also occurred in synthetic form (Naphthalene Acetic Acid-NAA and Indole-3-butyric Acid-IBA), auxins influence cell elongation, cell division, induction of primary vascular tissue, adventitious root formation senescence, fruit growth, out growth of axially buds and sex expression.
Callus/Callous	Actively growing relatively undifferentiated tissue, devoid of macroscopic organized structure, normally produce in higher plants in response to wounding or infection but often formed *in vitro* during the artificial culture of plant tissue.
Culture medium	A mixture of organic and inorganic nutrients used for the cultivation of cells.
In vitro	A sterile artificial environment typically in glass vessels, which cultured cells, tissue, organ or whole plant may reside.

In vivo	Literally "in life" applied to any process occurring in a living whole organism.
Micropropagation	Rapid vegetative propagation of a plant via small pieces of tissue and usually beyond that obtained in nature. The process includes many steps-stock plant cares, explant selection and sterilization, media manipulation to obtain proliferation, rooting, acclimatization, and growing of liners.
Plantlet	A tiny plant with a distinct root and shoot system formed *via* tissue culture either by embryogenesis or organogenesis.
Rhizogenesis	Initiation of root-like structure from an organ or callus.
Tissue culture	A general term used to describe the development of tissue in culture under sterile conditions.
Vegetative propagation	Somatic non-sexual propagation of plant parts without fertilization.

CHAPTER 1

INTRODUCTION

There are about 3870 million ha of forest worldwide, of which almost 95% are natural forest and five percent are forest plantations. Tropical deforestation and degradation of forests in many parts of the world are negatively affecting the availability of forest goods and services (FAO, 2001). This has lead to numerous efforts to reduce timber harvesting in natural forest and develop alternative source of industrial wood. Therefore a global trend towards greater reliance on plantation as a source of industrial wood has been adopted recently. The development of significant global plantation estate is quite recent and half of all plantations in the world are less than 15 years old (FAO, 2001). Asia has lead plantation establishment globally and at 2000 about 62 % of all forest plantation are located in Asia (FAO, 2001).

In Malaysia both private and public sectors are involved in tree planting because of its high economic returns from the investment in forest plantations and the short rotation cycle for wood production (Ab. Rasip *et al*., 1995). One of the current high quality timber species, which is greatly appreciated by the Malaysians, is teak (*Tectona grandis* L.f). Teak occupies a dominant position as the highly sought after timber in the world. The species originates from countries with tropical monsoon climates, chiefly India, Myanmar, Thailand and Cambodia. The annual pronounced dry season followed by wet weather in these countries concerned imparts a beautiful grain structure teak (Borata, 1991). At the same time its wood is characteristically strong, durable and easily workable. Teak wood is considered to be the best general utility timber with worldwide reputation, being extensively used for shipbuilding, bridges and wharves, railway carriages and wagons, ordnance, shingles, wheels, carving and general carpentry (Appanah and Weinland, 1993). As a result, it is much sought after throughout the world and fetches a high price as compared to other species of wood.

In response to that Malaysia saw the potential of teak as a plantation crop. However teak cultivation in Malaysia is relatively new when compared to neighboring countries like India, Myanmar, Thailand and Indonesia. Even though the first teak plantation in Peninsular Malaysia was established in 1909, the total acreage of teak plantation is still relatively very small. Various small trials in Perak (Lenggong), Selangor (Kuala Selangor) and Johore (Yong Peng) have shown teak can thrive equally well if not better on wet and hotter regions around the country. Furthermore the early growth performance of the trees in these regions was also remarkable. This has sparked off the interest to promote teak on a larger scale within Malaysia (Krishnapillay *et al*, 1998).

To ensure teak plantation on a large scale can be materialised, there must be a constant supply of quality seedling or plantlets with good growth performance and desired characteristic. The conventional method of vegetative propagation has limitations. They are slow and time consuming. Teak being a cross-pollinated species, progenies raised from seeds show wide variations. Teak tree also has an irregular seed bearing habit and the production of seed is very low. (Lee and Rao, 1981). Teak raised from seeds collected at random tend to show fairly wide variability in its growth (Krishnapillay *et al*., 1998). Planting stock is still produced from seeds despite certain handicaps associated with this, such as quantitatively limited and late seed production, low germination rates, substantial variability in growth and wood quality among individuals within progenies and a lack of accurate knowledge about the inheritance of economically significant traits (White, 1991). Thus vegetative propagation by cuttings and tissue culture techniques has provided promising inroads and possibility for the production of uniform teak. Historically clonal propagation of woody perennial has focused on those species raised to produce food or seed, though there are one or two ancient examples of mass propagation for timber e.g *Cyptomerias sp* in Japan since the beginning of the fifteenth century. It was soon realized, however that this vegetative propagation technique could also be used for large-scale production of improved materials for commercial planting especially when selection of genotype has been incorporated into the programme. With such flexibilities to modify and improve genotypes besides mass producing, thus the potential of using the micropropagation was then realized and emphasized.

Micropropagation of tree species is being increasingly recognized as a tool with much potential for application in the field of forestry. It offers a rapid means of forestation multiplying woody biomass, conserving elite and rare germplasm, the production of virus free stocks and many other advantages. The ultimate success of micropropagation on commercial scale depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rate. Tissue culture conditions that promote rapid growth and multiplication of shoots often result in the formation of structurally and physiologically abnormal plants. The heterotrophic mode of nutrition and poor mechanism to control water loss render micropropagated plants vulnerable to the transplantation shocks. Although considerable efforts have been directed to optimize the conditions for the *in vitro* stage of micropropagation, scanty attention has been paid to understand the process of acclimatization of micropropagated plants to the soil or other suitable *in vivo* rooting media. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants (Earl and Langhans, 1975; Brome and Zimmerman, 1978; Corner and Thomas, 1981; Ziv, 1986).

This study attempts to study the most crucial process in micropropagating *in vitro* plantlets of *Tectona grandis* which is the rooting, and hardening of the plantlets. This is to ensure that production of a complete stock plantlets are with well develop root system. It is hoped that tissue culture can be an alternative method of propagating selected genotypes of forest trees, obviously this could be used to compensate for timber shortage as well as to maintain future domestic timber requirement.

The main objective of this project is to determine the possibility of rooting and acclimatizing of *in vitro* plantlets of teak (*Tectona grandis* L.f). In connection with the above, the following specific objectives were undertaken.

1. To study the effects of different concentration of IBA on *in vitro* rooting of teak plantlets.

2. To observe the effects of light and dark treatment on *in vitro* rooting of teak plantlets

3. To investigate the effects of different rooting media for acclimatizing these plantlets.

CHAPTER 2

LITERATURE REVIEW

Taxonomy of *Tectona grandis*

Tectona grandis L.f belongs to the family Verbenaceae. It is marketed as plantation-grown teak, or as teak, teck (French) or teca (Spanish) (Timber of the world, 1980). Its local names are Jati (Malaysia and Indonesia), Shegun, Sagun (India), and Kyun (Burma).

Botany of *Tectona grandis*

Teak is a deciduous tree, reaching a height of 25-30m and a diameter of 50-60cm in 60 years (Borota, 1991). The different races or provenances differ from one another with regard to the form and colour of the leaves, colour and structure of bark, and stem form. The provenances from northern Thailand and Myanmar are known for their straight boles; short clear boles usually characterize teak from India and Indonesia. The bole is generally cylindrical, and quite frequently forked. The soft bark is up to 15.0 mm thick and usually has deep longitudinal cracks; it peels off and has a bitter taste. The root system of teak remains shallow and responds very sensitively to oxygen deprivation. The leaves are large (35 X 60 cm^2) and leathery. They are elliptical and grown in whorls of 2 or 3. The upper surface of the leaf is rough like sandpaper, while the lower surface is densely pubescent. Its flowers are small and whitish, and are often visited by bees. The fruit is a spherical brown stone, about 1.0 cm in diameter, and contains between 1 to 3 seeds (Appanah and Weinland, 1993)

Ecology of of *Tectona grandis*

Teak is native to the Indian subcontinent and Southeast Asia, especially Myanmar, Thailand, Laos, Cambodia and Vietnam (Patterson, 1988). It is an exotic species to Malaysia. It is the most cultivated species in Southern Asia. The origins of teak planted in Peninsular Malaysia were from Sumatera and Java (Darus and Zakaria, 1983). Elsewhere in Asia, teak has been established in Bangladesh (~73 000 ha), Sri Lanka (~38 000 ha), China (~9 000 ha), the Philippines (~8 000 ha), the Lao People's Democratic Republic (~3 000 ha), Nepal (~2 000 ha) and Vietnam (~1 500 ha) (Krishnapillay, 2000). Its altitudinal distribution in Java is 0-700m above sea level. A number of geographical provinces have been identified (e.g Myanmar, Rangoon, Siam and Java teak); and they were found to differ greatly from one another in terms of yield, growth and wood quality.

Teak occurs naturally in monsoon climates but under an extreme variety of growth conditions. The most favorable growth conditions for teak exist in those tropical climates, which have an annual precipitation of 1,250-1,800 mm and a more or less uniform temperature with a minimum of 12 ^{0}C and a maximum of 38 ^{0}C (Borota, 1991). Dry deciduous forest containing teak (eg. In India at annual rainfall of about 800mm) can often be found adjoining arid thorn scrub belts, while deciduous moist forest. (at annual rainfall of around 1500mm) with teak can be found near evergreen rainforests. *Tectona* consistently grows best in well-drained, well-aerated soils with high oxygen content and a neutral pH. The best sites are slopes loams and not light alluvial clays. Teak does not tolerate waterlogged ground or sites subject to prolonged inundation, laterite stiff clay, black cotton soil and deep dry sand (Evans, 1981). It is light demanding and requires plenty of growing spaces.

Growth Performance of *Tectona grandis*

Modest growth rates are reported for teak plantations. Under favourable conditions in early life, a plantation may exhibit growth rates of between 10 and 20 m^3 per hectare per year. However, growth falls to the general reported level of 4 to 8 m^3 per hectare per year as the plantation ages (Htwe, 1999; Cao, 1999). On the best sites in Myanmar and India, 50-year-old plantations exhibit heights of 30 m and diameter at breast height (DBH) of 60 cm. Rotations are between 70-80 years for high production of high-grade timber in plantation. However the quality of growth depends on depth, structure, porosity, drainage and moisture holding capacity of the soils (Krishnapillay *et al*, 1998).

Properties and Uses of *Tectona grandis*

The narrow, light-colored sapwood zone of teak is clearly distinguished from the deep brown, shiny heartwood. The odor of the fresh wood is reminiscent of leather. Teak yields one of the worlds most beautiful and best timbers, which is of excellent quality in virtually every aspect. The high commercial value of teak timber has prompted its introduction as a plantation tree in a number of countries (Tho, 1981). Price of sawn teak on the world market fluctuates between U$1500 and U$2000 per tonne (Krishnapillay *et al*, 1998).

It is one of the two species that dominate the industrial plantation in the world. Teak alone makes up 14% of all tropical plantations (Evans, 1992). Teakwood is used for a wide variety of applications. It is rightfully considered to be second to none for ship construction, is excellent for structure of all kinds, both in and out of water, as well as luxurious interior finishing and expensive furniture. The wood contains oil, which prevents nails from rusting. It is highly valued raw material for the veneer and plywood industry. Teak is also a source of high-quality firewood and charcoal wherever it is grown.

Micropropagation in Forest Species.

Micropropagation is the true-to-type propagation of a selected genotype using *in vitro* culture techniques. Most often micropropagation is also associated with mass production at a comparative price (Debergh and Read, 1991). One of the aims of