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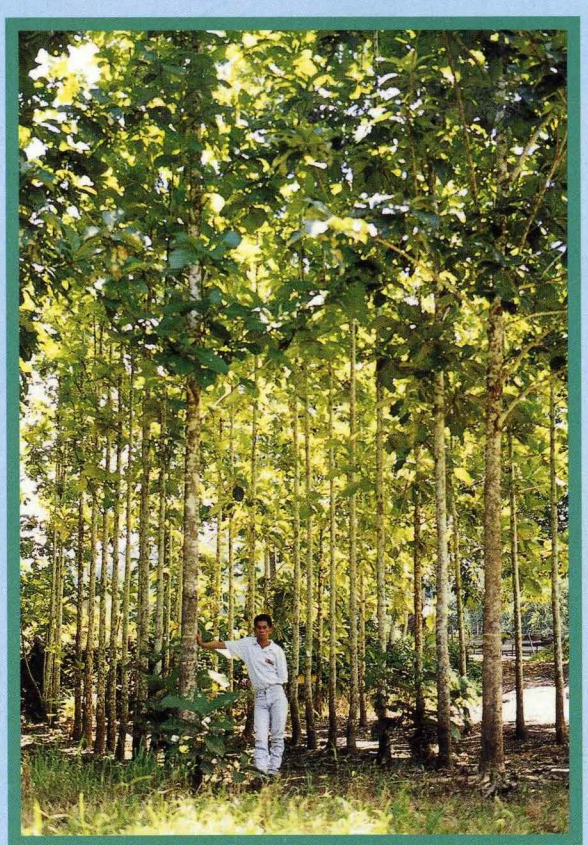

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VEGETATIVE PROPAGATION OF TEAK

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ABSTRACT

Tectona grandis, commonly known as Teak, is a high value hardwood species prized for its attractiveness and durability. Owing to the depletion of natural forests as demand for teak wood increases worldwide, alternative means for producing planting stock beside the use of seeds are sought. This is particularly necessary when considering factors such as the poor production of seeds per tree and an overall low germination rate of most seed lots. Further, the use of seeds is confounded by the uncertainty of the growth performance of the plants issued from such seeds especially if they are from unknown sources.

As a result of these limitations, the vegetative propagation of teak provides a useful means to increase the planting stock of teak, particularly those with known attractive genetic traits. The collaboration between Innoprise Corporation and Cirad-Foret provides the opportunity to embark upon such a program for the selection and mass propagation of good quality teak material using both nursery cuttings and tissue culture methods. The research undertaken on these aspects over the past 4 years have yielded useful results and are now applicable on a routine basis.

At the nursery level, the rooting of young stem cuttings from stock plants with the help of a rooting hormone and efficient misting system demonstrated a success rate of about 50-70%. On the average, about 40 rooted cuttings per stock plant can be produced annually, corresponding to about 600 cuttings per square meter. At the micro-level, the development and improvement of the tissue culture protocol over the past few years have resulted in a highly workable and efficient method for the mass propagation of teak, regardless of the age of the selected genotypes. Under the given *in vitro* conditions, plantlets could be produced at a multiplication rate of 3 cuttings per plant, coupled to a spontaneous rooting rate of 85% of these plantlets. Further, under optimal nursery conditions, more than 95% of these plants rooted and survived the *ex-vitro* acclimitization process.

To date, more than 70,000 micro-shoots and *in vitro* germinated seedlings have been transferred

to the nursery and then to the field for various studies. Results from these field trials, though still young, are promising. About 30,000 tissue cultured-plantlets have also been produced to meet demands from planters in Peninsular Malaysia in our first few commercial ventures. These successful transactions with the benefit of a phytosanitation immunity for international dispatches, creates the possibility to send teak plantlets to any destinations, at any distances.

Regardless of the methods used, the ability to attain this true-to-type cloning of selected teak genotypes of any age, through the use of either or both tissue culture or nursery cuttings methods, is certainly feasible for carrying out large-scaled plantation or reforestation programs.

INTRODUCTION

Tectona grandis, commonly known as teak, is a high quality timber valued for its attractiveness and durability of the wood it produces. Worldwide demand is greater than the resources available. Using teak as an indicator of hardwood trends, natural forests are observed to be depleted at a substantially rapid rate. In terms of the total area of tropical forest plantations, teak had decreased from 11% in 1980 to 5% in 1990 (Keogh 1997). To prevent the further depletion of the supply of teak in the face of an ever increasing demand in the world market, reafforestation operations on teak are quite critical.

Practically, these would require the availability of large quantities of good quality planting stock for plantation developments. The conventional method of propagation of teak is through seeds, particularly when these are in restricted quantity and presumably, have a high genetic value such as from controlled-pollination orchards. This means of sexual propagation is however, generally handicapped by the limited number of seeds produced per tree coupled to low germination rates in some seed lots, depending on the origin, storing conditions and seed treatments prior to sowing (Kaosa-Ard 1986). In addition, plants issued from seeds are not homogenous in growth and performance due to the uncertainty related to the inheritance of important and economical traits (White, 1991, Dupuy and Verhaegen, 1993).

In view of these limitations and in contrast to sexual propagation by seeds, vegetative propagation offers an attractive alternative. This allows the potentially unlimited reproduction of any individual while preserving its genotype and consequently, its whole characteristics (Hartmann et al., 1990). This is of utmost importance considering that some traits of economic importance, such as tree growth, are under the control of non-additive genes, which cannot be reproduced through seed propagation (Cheliak and Rogers, 1990). Essentially, vegetative propagation or true-to-type cloning of selected individuals should therefore result in a substantial gain in the overall value of teak plantations through gains in uniformity and quality (Mascarenhas and Muralidharan, 1993).

Techniques on the vegetative propagation of teak at the macro- (nursery) and the micro- (*in vitro*) levels have been developed to fulfil the above purpose in the framework of a collaboration between CIRAD-Foret and Innoprise Corporation Sdn. Bhd. (ICSB, the investment subsidiary of the Sabah Foundation). This paper will focus on the subject and the prospective applications of the technologies for vegetative propagation of selected teak for research trials and commercial plantings.

VEGETATIVE PROPAGATION METHODS

In general, several methods of vegetative propagation have been developed and are available for use in forestry (Zobel and Talbert, 1984). Grafting is usually employed to preserve trees in clone banks or for seed orchards in which the objective is for large-scale seed production. However, a frequent and major problem with grafting is incompatibility between the stock and the scion which makes it unattractive in a tree improvement program through the loss of clones (Duffield and Wheat, 1964; Hong, 1975). Another method is by air-layering in which roots are generated on an intact branch by girdling followed by hormone application (Kadambi and Dabral, 1954; Hoekstra, 1957). Air layering has several uses, one of which is to produce propagules directly to establish seed orchards, thus avoiding graft incompatibility (Barnes, 1969).

In addition, vegetative propagation through rooted cuttings has been in use for a long time and much progress has been made for some species (Rauter and Hood, 1980; Hartney, 1980). In contrast, the newest and presently the most publicised of the vegetative propagation method is by tissue culture. This method is developing very rapidly and has great potential if pursued realistically (Bonga 1980). It is through the use of these two latter methods that mass propagation of teak is carried out in our project.

In our situation, vegetative propagation of teak can be done on a group of specimens with different genetic make-up (genotypes) without having to maintain the identification of the individual. This is referred to as 'bulk propagation'. This mode of propagation allows the mass multiplication of a number of juvenile genotypes of high but similar genetic value such as from controlled pollination. Otherwise, clones of superior genotypes selected based on their morphological features (phenotype) can be propagated and later mixed for sale or field trials, or their identity preserved for their subsequent use in more rigorous tree improvement programs.

Using these options, based on our research and experience in the nursery and laboratory, the two propagation methods of teak are described in the following sections.

Nursery propagation of rooted cuttings

From our joint effort within the collaboration, the techniques developed under the nursery conditions in the Luasong Forestry Centre (LFC) and later applied in the Forestry Rehabilitation and Regeneration Unit (FRR), both ICSB units, have established the prospects for propagation of teak via rooted cuttings (Monteuuis 1995; Monteuuis et al., 1995). In general, a major deterrent to using rooted cuttings is their dependency on age. Young trees may root easily but when older, may be quite impossible to root (Zobel and Talbert, 1984). In our situation, this is not the case. About 50 -70% of the cuttings corresponding to several mature genotypes (four to six-year-old) can be rooted and subsequently developed into superior quality cloned offsprings.

Fig1. Rooting of macro-cuttings in the sand bed under proper misting.

Rooted cuttings arise as newly developed shoots from either stock plants derived from ortets which have been cut at about 20 -30 cm above ground level or plants kept under 50 to 70% shade in polybags in the nursery and watered as needed. The plastic bags (25 × 37 cm) contain a mixture of local clay:loam top soil supplemented with a monthly application of 1.5 to 2 g of complete NPK fertiliser (Agroblen). Management of these stock plants in polybags is quite intensive in order to allow the production of certain types of shoots with a high potential for adventitious rooting.

The growth of these stock plants is also limited to about 20 to 30 cm in height by frequent hedging. A pinching operation helps to further stimulate the production of many expanding axillary shoots for use as cuttings. The length of the cuttings and the morphological characteristics of the cuttings appear to be important for rooting response. The criteria for the selection of these cuttings based on distinctive traits, referred to as Type 1 and Type 2, have been described (Monteuuis,1995). However, it should be noted that the selection of such cuttings to improve the rooting percentage will decrease the total number of cuttings per stock plant. On the average, about 40 rooted cuttings can be produced annually per plant, corresponding to about 600 rooted cuttings per square metre.

The bases of selected cuttings, with the surface area of large leaves reduced by 50% to lower the evapo-transpiration rate (Hartmann et al.,1990), are dipped into a commercial rooting powder such as Seradix 3 (with 0.8% indole-3-butyric acid; Fig.1). The cuttings are then rooted in sand beds which are placed in 50% shade and equipped with an intermittent mist water system. Weekly sprays of an aqueous fungicide solution (5 g/l Thiram-80) are undertaken to reduce the number of diseased cuttings. The cuttings are checked for rooting over a period of 3 to 8 weeks. Rooted cuttings are then transferred to polybags (15 × 23 cm) filled with topsoil supplemented with 5 to 10 g of fertiliser (Agroblen) and 0.1 g of fungicide (Thiram) and maintained under the mist system for two more weeks.

After this weaning process, the plants are transferred from the mist chamber and maintained under 50% shade for about 2 to 3 months. During this hardening process, the plants are watered daily, sprayed with fungicide every two weeks, and manually weeded as needed. Spacing of the plants is carried out gradually depending on the growth and development of the plants. Different sizes of plants are selected to get batches which are more homogenous. A two-week exposure to direct sunlight is sometime carried out before the plants are ready for the field, converted to stock plants for future cuttings, or for sale.

Fig2. New sprouts arising from branches collected from selected mature trees placed under a proper misting system; these shoots could be introduced for micro-propagation or as rooted cuttings.

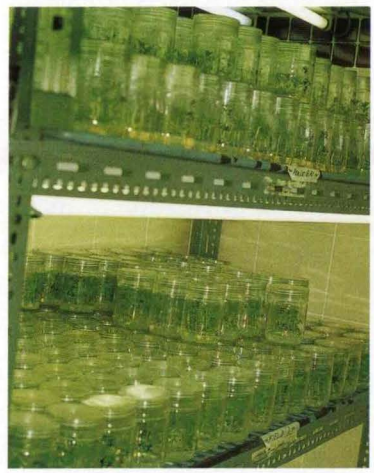

Fig3. Mass micro-propagation of selected teak genotypes in flakes in the Plant Biotechnology Laboratory, Tawau.

Tissue culture propagation of micro-shoots

The protocols for the tissue culture of teak were conceived in such a manner that the constraints of large-scale application related to costs and a high productivity rate were manageable. The technology developed and subsequently improved is well-established and now routinely carried out in the Plant Biotechnology Laboratory (PBL) in Tawau, Sabah (Monteuuis et al., 1998).

1. From *in vitro*-germinated seedlings
   The plant material used for tissue culture arises from a bulk or mixture of clones, or from individuals with their identity strictly maintained. The bulked-up materials can be obtained through the germination of seeds of presumed high genetic value but which are available in restricted numbers or with low germination capacity; these seeds can be from controlled pollination orchards. From seed source, the benefits of tissue culture can be noted through the improvement of the germinating capacity (21% in the laboratory in contrast to 4% in the nursery) as well as the possibility to propagate the juvenile plant material in vitro, thereby enriching the available gene pool. As there is a lack of reliable information to select, at such an early stage, a particular genotype over others, it is therefore necessary to maintain their propagation as a mixture rather than individually.
   The extraction of seeds from the mature fruits is quite laborious and time consuming as it involves care in avoiding damage to the delicate seeds. This is followed by a crucial disinfection and rinsing of the seeds prior to their individual inoculation onto a suitable germinating medium in glass test tubes under sterile conditions. The samples are then placed in total darkness at 27±2 ℃ for two weeks after which the germinants are transferred to a room equipped with a 16:8 h photo-period. Two to three months later, depending on their development, the seedlings can be multiplied in vitro or else transferred to the nursery for acclimatisation and eventually, for field planting. To date, more than 50,000 teak seeds from 10 different geographic origins were inoculated under the given tissue culture conditions in the PBL. A detailed report on the procedure for introduction of the seeds for in vitro germination has been published (Monteuuis et al.,1998).
2. From nodal portions of vegetative shoots
   Tissue culture propagation of selected genotypes of different ages (7 to 35 years old) as well as nursery stock plants, introduced either

Fig4. Acclimatisation of tissue-cultured plantlets in the misting nursery.

Fig5. Hardening of tissue-cultured plants under partial shade in the nursery. These plants could further be converted into stock plants or are ready for field-planting.

as a bulk or with the identity of the individuals preserved, is also undertaken out in the lab (Fig.2). Mono-nodal or single node segments particularly from the terminal portion of vegetative shoots are the type of explants utilised. Nodal segments (< 1.5 cm in diameter) are also derived from new growths which are produced from sticks or young branches placed under proper mist system conditions (Monteuuis et al., 1995).

After collecting from the donor plant, excess leaves are trimmed from the shoots to make transportation easier, and then placed in humid conditions created by using plastic bags containing a piece of moistened tissue paper. In the laboratory, remaining leaves are removed by cutting the petiole at a safe distance from the axillary or terminal bud. Sections of about 10 to 15 cm in length are made and washed thoroughly. These are then immersed in a 0.25% HgCl solution followed by three abundant rinses in sterile distilled water. These and further manipulations such as trimming and inoculation of the explants are all done under sterile conditions in the laminar flow hood. Explants are individually introduced into test tubes containing suitable culture medium and kept in darkness under conditions similar to those for seed germination.

The reactivation phase involving physiological rejuvenation, as described by Monteuuis (1989), can last 4 to 8 months, depending on the genotypes and maturity of the arborescent species concerned. For teak, we observed that the response of explants under the given tissue culture conditions varied differently according to the genotypes introduced. The production of axillary buds occurred on some clones within two weeks after inoculation whereas some took longer than six months. Maturity, however, did not appear to be a limiting factor as explants from some ortets older than 15 years were observed to be equally, if not more responsive than those from trees as young as 4 years old. This indicates that the rejuvenation of teak may not be difficult as it is for some other species. Further, it should be noted that beside physiological aspects, the overall success of introduction using in vivo materials can be deterred by contamination from pathogens such as fungi and bacteria. This can be objectively considered as the requisite step to the micro-propagation of any in situ selected teak individual. Efforts aimed at improving the success rate of primary culture initiation are being pursued each time outside plant materials are introduced for tissue culture in the laboratory.

To date, data from different dates of introduction have established that 5 to 30% success can be attained depending on all these factors (Monteuuis et al., 1998). Once physiologically activated, new shoots which attained 1 cm in height and did not show any sign of contamination were excised and transferred to fresh culture medium for one or two subcultures to ascertain if contamination exists. These materials can then be used to initiate the micro-propagation process with multiplication rates similar to young seedlings, that of 3 or 4 cuttings per plantlet every 6 to 8 week-subcultures. Our observations from more than three years of in vitro culture have established that 80 to 85% of the micro-cuttings rooted spontaneously in the early culture cycles which finally stabilised to about 60-70%. The ability to root spontaneously further attests the physiological rejuvenation of the mature genotypes of this species.

3. From shoot apical meristems

Introduction of shoot apical meristems was undertaken with the aim of developing protocols for the in vitro meristem culture of teak genotypes, whether collected from ortets or nursery stock plants. This procedure is especially useful for introducing pathogenically free cultures, particularly of endogenous ori-

gin which are difficult to eradicate by other means. The introduction of shoot meristems (< 0.3 mm in size) requires greater skill and concentration from the manipulator, and with specific tools such as a light microscope and fragments from a razor blade. As a result of the introduction of several thousand apical shoot meristems from mature genotypes, this technology is well-worked out (Monteuuis et al. 1998).

Similarly to micro-shoots introduced from seedlings or nodal segments, the meristem-derived micro-cuttings developed the same organogenic capacities to *in vitro* culture. Spontaneous rooting and a multiplication rate of 3 to 4 cuttings per plantlet are observed. Several ''mericlones'', that is, of different genotypes, have been introduced and are now undergoing various experiments in the laboratory. In addition, a few thousands plantlets derived from shoot meristems of a genotype have been put out in field trials in a comparative study with rooted cuttings from the same clone.

The simplicity and efficiency of these procedures in contrast to previously published protocols (Mascarenhas et al., 1987; Sunitibala-Devi et al., 1994) on the same topic make the micro-propagation of teak highly attractive (Fig.3). In particular, the formulation of a sole elongation-multiplication basal culture medium, complemented with a very low concentration of a growth regulator, allows for an intensive and cost-efficient production procedure.

4. *Ex-vitro* acclimatisation

The acclimatisation success rate of tissue-cultured plants averaged at about 95%, regardless of whether they were rooted or not at the time of transfer (Fig.4). At the nursery, these plants are soaked in a mild fungicide solution (Thiram-80) and then inserted into the sand bed. Definitely, the benefits of a mist system cannot be over-emphasised especially in countries with a dry season (Kaosa-ard and Apavatjrut, 1988). In the first week, a constantly humid environment is quite crucial to these micro-shoots in the prevention of stress from dehydration. The appearance of newly produced roots usually signal their successful adaptation to *ex-vitro* conditions. After two to four weeks in the sand beds under the mist system, rooted plantlets reaching an appropriate height (about 10 cm) are transferred to polybags and then maintained as described for rooted cuttings (Fig.5).

Fig6. About five-month old teak plants from tissue-culture, macro-cuttings and seedlings of several clones in a comparison trial on vegetative propagation methods, Taliwas, Lahad Datu.

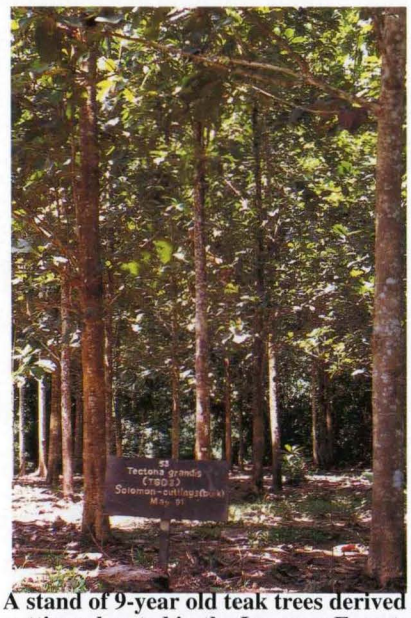

Fig7. A stand of 9-year old teak trees derived from cuttings, located in the Luasong Forestry Centre, Tawau, Sabah.

CONCLUSIONS AND PROSPECTS

The protocols developed jointly within this collaboration between CIRAD-Forêt and ICSB have ascertained that teak demonstrates a good capacity for rejuvenation through vegetative propagation methods by rooted cuttings and *in vitro* culture.

The efficiency of both the nursery and tissue culture methods for mass propagating selected genotypes has been assessed in a comparative study. The gain in time obtained from using tissue culture for the propagation of selected materials, particularly from mature genotypes (15-30 years), is quite significant (about half of that required in the nursery). This appears to be one of the most important results from this assessment. The ability to increase the number of micro-shoots at an exponential rate of 3^n, in most cases every 6-8 weeks, also provides strong evidence for the usefulness of the tissue culture technique.

The preference of either option or a combination of both will be determined to a large extent, on economic considerations. As emphasised by Haines (1994), to be worth applying, micro-propagation must have the capacity to deliver, to the commercial planting program at a particular time, genotypes which are genetically superior to those which could be delivered as seedlings or cuttings. An estimation then of the cost incurred in the production of a plantlet to that of the price per seedling or rooted cutting will have to be made in order to get the most optimal returns from tissue culture. For overseas market, the costs of transportation will definitely be an important factor to consider. In this case, micro-shoots will be the cheaper and more convenient option in contrast to transporting rooted cuttings in polybags.

Regardless of the preference of one or the other, both methods have been extremely useful in our project for the extensive production of plant materials from various sources and origins for both field trials and commercial sales. From the LFC and FRR research nurseries, several thousands rooted cuttings were used in comparative studies with seedlings and micro-shoots as well as in sales to local buyers. From the Plant Biotechnology Laboratory, the development and subsequent improvements to the tissue culture of teak during this 5-year period have resulted in the transfer of more than 70,000 micro-shoots and in vitro-germinated seedlings to the nurseries. Similarly, these materials were used in setting up trials based on origins, progenies and provenances, demonstration plots, and comparison of planting design (line versus open planting) and propagation methods (macro-cuttings versus micro-cutting versus seedlings). In addition, about 30,000 micro-shoots were successfully sent overseas to Peninsular Malaysia in our first few commercial transactions. This success, coupled to the benefit of a phytosanitation immunity in the case of international dispatches, creates the possibility to send teak plantlets to any destinations, at any distances.

Although our field studies are relatively young (Fig.6 and 7), we are nonetheless confident that these vegetative methods, in particular tissue culture, can now be carried out on a routine basis for the introduction of any teak genotype regardless of its age. To date, growth data of three to four year-old teak trees are quite impressive. With the surge in interest in both overseas and local markets for teak, the possibility to provide improved clonal material in conjunction with plantation or reafforestation programmes is highly promising.

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