Potentials and Opportunities in Marketing and Trade of Plantation Teak: Challenge for the New Millenium

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PRODUCTION OF TISSUE CULTURED TEAK: THE PLANT BIOTECHNOLOGY LABORATORY EXPERIENCE

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Abstract

The Plant Biotechnology Laboratory (PBL) set up in Sabah, Malaysia, originated from a collaborative project between Innoprise Corporation Sdn Bhd (ICSB), a Malaysian company, and CIRAD-Forêt, a French Research and Development organization. One of the major achievements of the PBL to date has been to establish adapted protocols for *in vitro* mass propagation of superior teak (*Tectona grandis*) genotypes, taking advantage of the tree improvement activities developed within the same joint project. Research undertaken over the past few years in close relationship with field and nursery experiments has resulted in the development of a simple, efficient and cost-effective *in vitro* method for mass producing superior teaks, regardless of the age of the ortet. The technology is applicable for the introduction and multiplication of plant materials from seeds, nodal segments or meristematic tissues derived from selected genotypes. In the field, these tissue culture-issued materials have demonstrated true-to-type and vigorous development. With this capability to introduce and multiply any selected genotype, the establishment of large-scaled clonal plantations of teak is now highly feasible. The various origins of teaks produced by the PBL can be commercialized locally as ready for field planting material, or to oversea markets. Contamination-free tissue culture conditions further facilitate, in the absence of sanitation restrictions, cost efficient international dispatches in adapted airfreight containers of large numbers of good quality plants that can be easily acclimatized. Specific origins that are more suitable to the environmental conditions of distant countries can now be

delivered safely in a very short time. These materials, encompassing different teak sources are undergoing various field tests. They have also been maintained in the laboratory with the intention of future deployment to suitable planting areas upon demand by interested planters.

Keywords: clonal plantations, commercialization, *in vitro* culture, micropropagation, teak (*Tectona grandis*)

Introduction

The Plant Biotechnology Laboratory (PBL) is located in Tawau, on the eastern part of Sabah, Malaysia. The PBL was established in 1992 within the framework of a collaborative project between Innoprise Corporation Sdn Bhd (ICSB), a semi-governmental subsidiary which owns the biggest forest concession in Sabah, and CIRAD-Forêt, a French Research and Development Organization. The mandate of the PBL was to come up with suitable techniques for supporting the Plant Improvement and Seed Production unit (PISP), set up previously within the same collaboration. Since inception and in line with this, the PBL has been developing its expertise in two fields of biotechnology activities *i.e.* the use of molecular markers for genetic investigations of various plants (Bon 1996, Bon and Monteuuis 1996, Goh *et al.* 1997), and the *in vitro* production of forest plantation species. These activities have been carried out synergically with field and nursery operations undertaken within the same collaboration.

The species to be micropropagated were selected according to the priorities of the project and their economical importance. They encompassed the large caned rattans (*Calamus spp.*) and various forest tree species, with emphasis on *Acacias spp.* for pulp and paper production and *Tectona grandis*, commonly known as teak, for high value timber plantations. Teak is indeed famous all around the world as a high quality timber species owing to the durability and the attractiveness of its wood (Baillères and Durand 2000, Bhat 2000). Although originating from India, Myanmar, Thailand and Laos, it has been observed to be quite adaptable and thrives in many other tropical countries such as Indonesia, Vietnam, Sri Lanka, Malaysia, Ivory Coast, Costa Rica, and Solomon Islands (White 1991). It can grow on a variety of soils of different geological formations; however, it performs best on deep alluvial soils with good drainage (Kaosa-Ard 1995). The huge and still increasing market demand for this valuable timber has however resulted in a rapidly depleting supply of the natural resources (Dupuy 1990). As such, much effort presently is aimed at the production of sufficient planting materials, either through seeds or vegetative propagules for the replenishment of natural forests or the establishment of teak plantations in adapted situations. In this respect, micropropagation appears to be worth particular attention.

Rationale for Micropropagation of Teak

The traditional means of propagating teak is through seeds, as has been practiced for centuries, with the possibility of storing the seedlings in the form of "stumps" when necessary, followed by transportation and awaiting suitable planting conditions (Kaosa-ard 1986). This mode of sexual propagation is however associated with serious handicaps such as:

- Quantitatively limited seed production (Wellendorf and Kaosa-ard 1988, White 1991);
- Late flowering stage: in teak, straight bole length, which affects directly its market value, is strictly dependent upon the capacity of the terminal meristem to remain vegetative as long as possible (White 1991). Its conversion into the flowering stage induces a fork formation, the result of a true dichotomy process;
- Low germination rates (Kaosa-ard 1986, Mascarenhas *et al.* 1987, White 1991);
- Substantial variability among individuals, even among half-sibs, within progenies, with regard to traits of major economical importance such as growth, form, technological and aesthetic characteristics like wood pattern (Dupuy and

Verhaegen 1993, Baillères and Durand 2000);

- Limited accurate genetic knowledge about the inheritance of such economically significant traits, and consequently, some uncertainty for the ultimate gain, notwithstanding the time constraints associated with sound breeding programmes (Wellendorf and Kaosa-ard 1988).

From Kjaer and Foster's assumptions (1996) it will take at least 50 to 70 years before genetically improved teak plantations established from seeds can be harvested; the relevant genetic gain however remains uncertain. Recently, Kjaer *et al.* (2000) further stressed the fact that such breeding strategies will remain greatly penalized by low seedling productivity. These aspects have been recently developed by White and Gavinlertvatana (1999) who concluded that the "seedling route is outdated and actually represents a deterrent to wide scale increased productivity in teak plantations", and as such to commercial teak plantation investment. According to these authors, the magnitude of the real genetic gain associated with the seedling route has yet to be clearly demonstrated, and the basic question to know whether all the efforts invested during the past decades are worthwhile remains to be seen. From practical and theoretical information, it can be objectively assumed that for teak greater genetic gain can be expected from the (micro)cutting forestry option - particularly if clonal - than that from the seedling forestry, as demonstrated for other forest tree species (Zobel and Talbert 1984, Ahuja and Libby 1993a and b).

The rationale of opting for vegetative propagation for teak has already been argued (Monteuuis 2000, Monteuuis and Goh 1999). More practically, our collaborative project prompted us to develop protocols for mass propagating teak by cuttings in nursery conditions with success scores high enough to be economically viable (Monteuuis 1995, Monteuuis *et al.* 1995). However, as already highlighted (Monteuuis 2000), the efficiency of this technique remains strongly dependent upon factors such as suitable nursery facilities, proper stock plant management and mastery of adapted rejuvenation techniques for mature selected genotypes, staff workmanship, space, manpower, and also perhaps, climatic conditions.

In contrast, as demonstrated for different species, tissue culture can be more efficient to mass propagate any genotypes in shorter delays and with much higher multiplication rates (Timmis 1985, Cheliak and Rogers 1990). This is especially true when selecting from mature trees, due to possible limitations of efficient rejuvenation treatments in nursery conditions (Monteuuis 1989). The resulting gain in time has to be considered as a real advantage of tissue culture compared to propagation by rooted cuttings (Timmis 1985). In addition, adapted micropropagation protocols permit the mass production of *in vitro* plants year-round, irrespective of the climatic conditions, and in a more restricted environment. These same selected plant materials can be stored also as germplasm or in clone banks, or even used for more advanced biotechnology like genetic engineering (Cheliak and Rogers 1990). Another advantage is the possibility to send the *in vitro* plants off to different destinations, even far away, in the absence of phytosanitary restrictions. This increases considerably the market prospects compared to more conventional vegetative propagation techniques. All these reasons thereby prompted us to explore the prospects of the micropropagation of teak, taking advantage of the PBL facilities, to come up with the following technology.

The Tissue Culture Technique

The adopted tissue culture technique presents basic similarities to propagation by rooted cuttings in the

nursery. In both environments, the shoots are regenerated via axillary budding to ensure the required genotypic fidelity and are ultimately producing adventitious roots in horticultural conditions, at the end of the process chronologically detailed hereinafter.

Selection and Initiation of Explants into Tissue Culture Conditions

The plant materials used for the initiation of the propagation process can be either seeds of presumed high genetic value but limited in numbers, or nodal segments from young shoots produced by stock plants or from *in situ* donor trees in the field called "ortets"

From Seeds

The option of using seeds could be particularly helpful to increase the number of individuals of presumably high genetic value deriving from "Elite" trees or from controlled pollination. The beneficial effect of tissue culture can be seen firstly through the improvement of the germination capacity, and secondly, through the possibility of vegetatively propagating the newly germinated plant material. The lack of reliable information to select, at such an early stage, a particular genotype over others warrants their propagation as a mixture, without maintaining any individual identity. This is referred to as "Bulk Propagation". However, while proceeding in successive propagation cycles, the risks of narrowing the original genetic base owing to the fact that certain genotypes may exhibit a greater propagation response than others, should not be overlooked. Alternatively, as soon as *in vitro* germinated seedlings are developed enough to be cut into microcuttings, clonal propagation can be applied depending on the objectives in mind, for instance, among and within clone variability assessment. However, from a practical standpoint, this option will be heavier to handle than "Bulk Propagation".

The seedling procedure has been described in detail by Monteuuis *et al.* (1998). Briefly, it consists first in breaking the mature fruits, or more precisely the drupes without damaging the seeds they contain, which range from one to two on average. Just after extraction, the seeds are properly disinfected, rinsed thoroughly, then individually inoculated in sterile conditions into glass culture tubes that are covered with polypropylene caps, each containing 10 ml of gelled culture medium adapted to germination and early developmental stages. Inoculation of only one seed per tube restricts the spread of contamination to healthy seeds. However, in the case of reduced risks of contamination of seeds and when the germination capacity is low, it is more practical to inoculate two seeds per culture tube. Each seedling can be subsequently transferred into separate test tubes when they both germinate. The resulting cultures are placed first in darkness at 24 ± 2°C for 2 weeks, then transferred under the standard culture conditions.

About 50,000 teak seeds from various origins have been inoculated *in vitro* so far according to the procedure described. Generally, in the laboratory, the potential hurdle to getting a good supply of seedlings for micro-propagation is the risk of contamination from fungal or bacterial infection due to inefficient disinfection treatments or simply from reduced viability of the seeds. A comparative study involving *in vitro* seed germination and conventional nursery sowing, corresponding to 10 different geographic origins and 57 progenies from a teak seed orchard in Ivory Coast, was undertaken within the project. Out of a total of 24,000 seeds of presumed low germination capacity, the germination rates were observed to be significantly enhanced using the laboratory protocol than that in the nursery, notwithstanding noticeable differences according to the origins, both at the provenance and progeny level (Monteuuis *et al.* 1998).

From Shoots of Outdoor Plants

Conversely, the introduction of nodal or terminal segments from stock plants in the nursery or from 5 to 50 year-old ortets from field trials or plantations, is routinely carried out within the PBL project. The collection of shoots from the older ortets generally requires the assistance of a good climber as the trees are at times more than 30 m in height. Once collected, proper handling of the plant material include trimming of the shoots into suitable sizes and conditioning to prevent hydric stress followed by transportation to the laboratory for *in vitro* introduction in the shortest delays. Some semi-hardwood shoots or sticks are also collected and forced to produce young shoots when placed under proper misting conditions in the nursery (Monteuuis *et al.* 1995). The critical step of disinfection and further manipulation of these segments were described previously (Monteuuis *et al.* 1998). The time for the disinfecting treatments can vary depending upon the maturity and thus, texture of the selected segments.

Using a suitable culture medium in the sterile environment of a laminar flow hood, the success rate of introducing contamination-free explants of teak is quite variable ranging from 5-30%, depending on the manipulator. Both fungal and bacterial contaminations are observed although the primary deterrent to the successful introduction of this species is generally due to bacteria. To overcome the problem of endogenous contamination of explants which is difficult to eradicate by other means, the introduction of shoot apical meristems has been undertaken (Monteuuis *et al.* 1998). Another benefit associated with meristem culture is the possibility to radically rejuvenate the mature genotypes (Monteuuis 1989). The excision of meristems, less than 0.3 mm in size, requires greater skill and concentration from the manipulator. Having introduced successfully more than 7,000 thousand apical shoot meristems from various clones, with the production of mericlonal lines each deriving from a single shoot apical meristem that did not exceed 0.2mm in overall size, the technology is now well established and applicable in the project. Several field trials comprising meristem-issued plants have already been set up as further demonstration of the application of this technique (Monteuuis *et al.* 1998, Monteuuis and Goh 1999).

Stabilization Phase

The stabilization phase for teak in our culture conditions varies between 4 and 8 months according to the samples introduced. As for many tree species, explants responsiveness is very much dependent upon the physiological stage of the tissue at the time of removal from the outdoor plants. This may explain why explants from superior-looking ortets older than 15 years were observed to be as equally responsive in tissue culture as those taken from 4 years old trees (Goh and Galiana 1998). However, in general, the physiological stage is strongly influenced by physiological ageing (Monteuuis 1989), and consistently with many species, the prevailing statement is that the stabilization process takes longer for shoots collected from mature or old trees than from younger ones, or even from intensively managed stock plants. Usually explants collected from old trees produced very stunted axillary shoots that do not elongate much over a period of several transfers. The multiplication rate at this time is seldom more than one microcutting per explant. Then progressively, from one sub-culture to another, the microshoots starts elongating more readily, ultimately demonstrating similar organogenic potential as explants originating from juvenile sources. The cultures are then considered to have reached a rejuvenation level suitable for the production phase.

Production Phase

Based on our *ex-vitro* experience, the tissue culture protocol was conceived with simplicity, cost efficiency and high productivity in mind, with emphasis on sustaining the production of *in vitro* microshoots while minimizing the risks of somaclonal variation that can result from excessive use of growth regulators. In this respect, the methodology developed in the PBL lies in the use of a sole multiplication-elongation culture medium with minimal hormone concentration. Data recorded over more than 6 years have established an exponential multiplication rate of about 3-4 microcuttings per explant at the end of every 6 or 8 weeks culture. No aberrations in the performance of these plantlets, in relation to the development and multiplication rate, have been noted during this period. Observations of these materials will however continue to fully ascertain the long-term effects, if any, on the growth of the plants at both tissue culture and field levels. 50 to 60% of the microshoots in the production phase root spontaneously on the multiplication-elongation medium conceived to produce good quality and genetically true-to-type shoots without callus to be rooted ex-vitro. In fact, callus appeared to inhibit the production of adventitious roots, and particularly so in the misting sand beds during the acclimatization phase.

Ex-vitro Acclimatization

During the acclimatization phase, under favourable misting and light conditions in the nursery, plantlets are usually rooted within 3 weeks following their transfer to the sand beds. A good indication of new roots being produced is the appearance of new leaves or an increase in the height of the plantlet. In contrast, unrooted plantlets with callus can be sustained in these misting conditions for 6 weeks or more, and with a healthy appearance even in the absence of any new roots. Once new roots are produced, the plantlets are transferred to the polybag for further weaning and hardening for up to 3 months after which the plants are ready for either field planting or for sales at the local level. Gradual weaning can be done by keeping the potted plants within the misting area for 3 to 4 more weeks prior to their transfer to the nursery area under 50% shade for the hardening phase. Average scores of around 95% success in the acclimatization phase are obtained based on observations of more than 150,000 plants.

Discussion and Prospects for the Developed Technique

Teak is traditionally known to be a long rotation crop, with average harvest age ranging between 50-90 years in its natural range while outside its range, it varies between 40-60 years (Ball *et al.* 2000). The productivity of most teak stands, natural or planted, remains low, despite the increasing market demand. This situation leads to a progressive depletion of teakwood unless special measures can be taken, especially aiming at increasing the productivity of teak plantations. This is of overriding importance for private investors who are willing to consider investing in teak plantations only if they can benefit returns on capital investment much sooner than usually expected from traditional teak plantations.

Another aspect to take into consideration is the shift from large-scale to numerous small-scale plantations by an increasing number of smallholders who are mixed planting teak with other species such as oil palms, rubber and cocoa in Malaysia. Intercropping of teak with these cash crops is becoming an attractive option due in part to labor shortage and variable market returns, particularly, for rubber and cocoa. This option also offers early fast returns from cash crops that in turn will help to offset the initial investment costs during the waiting period. This situation

is getting more and more common in many countries suitable for teak plantations.

Teak adaptability to local site conditions remains indeed a major requisite for such ventures and one of the goals within our project has been to establish sufficiently rich base populations, being aware that teak may behave quite differently in a given environment according to its geographical origin (Kaosa-ard 2000). Resorting to molecular markers in this case can be very helpful for investigating the genetic aspects of our teak germplasm. Our initial attempts in this field have been very promising, warranting further development of this technology for supporting our propagation programs. It is a fact that shortage of planting stock can be a serious impediment for plantations programs. The situation becomes even worse for genetically superior planting stock with a view to ensure within shorter rotations high yield of prime value teakwood (Bhat 2000). Recent reviews of the current situation have reported the limitations of the traditional breeding strategies to meet the objectives and the expectations of private teak planters. This is undoubtedly a major concern for potential investors, for which rapid pay-off is a decisive argument.

With this and the success of the cutting forestry option in hand, the vegetative propagation techniques for teak as an alternative to propagation by seeds were therefore emphasized within our project. The respective pros and cons of bulk versus clonal propagation (Monteuuis and Goh 1998), as well as propagation by cuttings versus by microcuttings (Monteuuis 2000) were already highlighted. More specifically, a feasibility study undertaken within the project to compare between tissue culture and nursery production established that with a large volume of *in vitro* production, the fixed cost components could be offset by the factors influencing the economies of scale, thereby reducing the cost of producing each plantlet. The study also indicated a definite gain in time for the establishment of the *in vitro* method, that is, 6 months versus one to three years for the nursery method due to the numerous factors involved in the maintenance of stock plants (Monteuuis *et al.* 1995). Thus, with further improvement in various efficiencies of the developed *in vitro* technique, the benefits of using tissue culture for production can be tremendously increased.

Essentially, several early publications from various countries have reported on the possibility to clone both juvenile and mature teak genotypes using the tissue culture technique, at least at the experimental scale (Gupta *et al.* 1980, Mascarenhas *et al.* 1987, Kaosa-ard *et al.* 1987). Pioneering work on this method of teak production has been carried out in Thailand in the 1980s, however, production on a large scale is reported only recently (Gavinlertvatana 1998). Within the PBL project, although mass propagation by cuttings was developed in 1992, tissue culture became operational at a pilot scale since 1994 with more than 150,000 microcuttings produced using the developed protocol. It has to be stressed that most of these plant materials came from selected mature trees, taking advantage of the field and nursery research and development activities undertaken conjointly within the same project. Preliminary observations of these planted clonal materials in field trials indicate a fast and vigorous growth in the first two to three years with the superior characteristics of the mother "Plus" trees initially selected, such as an excellent increment in height and diameter, clear bole, and minimal branching. The most striking illustration of the benefits that can result from teak tissue culture is given by the performance of plants issued from meristematic tissues. Within a methodology-comparison field trial between this technique

and that of other propagation methods such as by cuttings or by micro-cuttings, the meristem-derived trees exhibit the most vigorous growth, probably as a consequence of a radical rejuvenation. As an indication, the mean height and diameter at breast height (DBH) of 2.5 year-old trees were, on the average, 11.5 m and 10.2 cm, respectively.

In the absence of reliable concrete data, there is a lot to expect that wood characteristics, which have made teak so popular world wide, can also be greatly improved by cloning. This aspect of overriding importance for teak is going to be further investigated in the near future within our project taking advantage of Cirad-Forêt expertise in the field of wood technology (Baillères and Durand 2000). This will allow us to refine our ortet selection combining various traits of major economical importance with special mention for wood characteristics which is one of the factors to consider for "Plus" trees selection (Bhat 2000).

As developed previously, it has become highly challenging to successfully micro-propagate selected clones regardless of the age of the ortets. This is also crucial in terms of costs and efficiency of production, to be able to deliver at a particular time and to various destinations, both locally and overseas, site-adapted genotypes which are genetically superior to those produced from seeds. In contrast to other vegetative propagation techniques, tissue culture allows overseas shipments and thus can be considered as a real asset for various purposes such as international scientific exchange of germplasm, or commercial operations. For instance, results from our experience have shown that the transportation of tissue-cultured plantlets in lightweight containers to Peninsular Malaysia could be easily achieved within 48 hours (Goh and Galiana 1998). These consignments, with the benefit of a phytosanitary certification, have certainly opened up a wide array of possibilities for the deployment of clonal teak to distant locations, be it for field trials or large-scale plantation establishments. One of these would be an assessment of genotypic adaptability of these tissue cultured-clones in different environments for ensuring the success of highly productive and superior quality teak plantations.

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