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TEAK PROPAGATION BY *IN VITRO* CULTURE

Translated from the article published in BFT n° 255

Photo 1. Pilot industrial production of teak tissue-cultured plants.

Propagation of teak by rooted cuttings in nursery conditions was already presented*. This paper deals with the propagation of the same species by *in vitro* culture, within the same ICSB/CIRAD-Forêt joint project.

Teak (*Tectona grandis*) is a high value timber species originating from South East Asia and much prized for the technological characteristics as well as the aesthetic value of its wood ; it is also well-known for its durability. It is therefore very much utilized in shipbuilding and high quality woodworking (CTFT, 1990). Worldwide demand is much greater than the resources available (DUPUY, 1990), and reafforestation operations require the production of large quantities of good quality planting stock. The traditional means of propagating this species is through seeds, with the possibility of storing the seedlings in the form of « stumps » when necessary : transport, wait for suitable planting conditions (KAOSA-ARD, 1986). This mode of sexual propagation is however associated with serious handicaps. The number of seeds produced per tree is limited and the germination capacity remains low overall, despite the fact that some seed lots may germinate better than others depending on the origin, storage conditions and seed treatments prior to sowing (KAOSA-ARD, 1986 ; WHITE, 1991). In addition, plants issuing from seeds look variable from one another, even within progenies. This variability can be related to growth parameters, tree form as well as technological and aesthetic characteristics (BEDEL, 1989 ; DUPUY, VERHAEGEN, 1993). These are the problems associated with tree improvement through sexual reproduction of this species, in addition to the incertitude related to the heritability of traits of major economical importance and to the duration of the reproduction cycles, bearing in mind that the higher the bole, the longer the cycles.

Theoretically, vegetative propagation allows for the unlimited reproduction of any individual while preserving its genotype and consequently its whole characteristics (HARTMANN *et al.*, 1990). This is essential to ensure the transfer of traits which are under non-additive control, especially those that have a great economical impact. This is most likely the case for a lot of arborescent forest species (CHELIAK, ROGERS, 1990). Vegetative propagation, and more particularly true-to-type cloning of selected individuals, should hence result in a substantial increase of the market value of teak plantations by gaining in overall quality and uniformity (MASCARENHAS, MURALIDHARAN, 1993).

A vegetative propagation technique has been developed to fulfill this purpose through the collaboration between CIRAD-Forêt and Innoprise Corporation Sdn Bhd in Sabah, East Malaysia, on northern Borneo island (MONTEUUIS *et al.*, 1995). Within this collaborative framework, the establishment of a biotechnology laboratory (MONTEUUIS, 1993) allowed us to study the responsiveness of teak to *in vitro* culture conditions, already tested elsewhere (KAOSA-ARD *et al.*, 1987 ; MASCARENHAS, MURALIDHARAN, 1993 ; SUNITIBALA DEVI *et al.*, 1994). The aim of this paper is to report on this subject, and to consider the prospective applications that can be useful for development.

IN VITRO PROPAGATION FROM SEEDS

In vitro culture conditions can be very useful for rapidly increasing the number of individuals from seeds of presumed high genetic value but available only in restricted number or with low germination capacity. This may be the case of provenances or progenies deriving for instance from controlled pollination.

* See B.F.T. 243, 1995.

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 » which can be applied for various lengths of time, depending on the needs. 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 capacity than others, should not be underestimated. 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. Practically however, this option remains far heavier to handle than « Bulk Propagation ».

METHOD

The procedure consisted first in breaking the mature fruits, or more precisely the drupes, without damaging the seeds they contained, which ranged from one to two on average. This can be done using a hammer, the impact being absorbed by the thickness of the fibrous mesocarp and the utilization of a folded towel underneath the fruit. Extraction of large quantities of seeds may warrant using an alveolar receptacle, the depth of which should be slightly inferior to that of the diameter of the fruit in order to protect the seeds from any damage that may result from hammer strokes. Just after extraction as delicately as possible, the seeds were placed in humid conditions, for instance in contact with a water-moistened piece of fibrous paper in order to prevent them from any desiccation stress. After soaking in an aqueous solution of 0,1 % $HgCl_2$ for 10 min., followed by three abundant rinses in pure water sterilized by autoclaving, the seeds were individually inoculated in sterile conditions into glass test tubes (21 × 150 mm) covered with polypropylene caps ; each contains 10 ml of gelled culture medium adapted to germination and to early development stages. Inoculation of only one seed per tube restricts the spread of contaminations to healthy seeds. However, in case of reduced risks of contamination of seeds and when the germination capacity is low, it is more advantageous to inoculate two seeds per culture tube. Each seedling can be subsequently transferred individually into separate test tubes when they both germinate.

The resulting cultures were placed first in darkness at 24 ± 2 °C for two weeks, then transferred under a 16/8 h photoperiod, with 50-60 µmol m^{-2} s^{-1} as light intensity and 28/22 ± 2 °C day/night temperature. These were the standard conditions. About two to three months later, depending on the seed lot, most of the seedlings which germinated under these *in vitro* conditions were 5 to 6 cm tall. This stage was suitable for nursery acclimatization with a view to field planting.

The *in vitro* germinated seedlings can also be micropropagated as soon as their epicotyl is long enough to be sectioned, being aware that the original seedling root system will then be replaced by adventitious roots. The procedure carried out under laminar flow hood in sterile conditions consisted in cutting transversally the seedling stems into 1 to 2 cm long nodal segments. These segments must include at least one node, with the underneath portion longer than the portion above the node. Foliar surface can be reduced by half or even by two thirds. The resulting microcuttings can then be introduced for further culture either in glass jars, each containing six explants when there is enough microcuttings per origin, or in a test tube. Each 10 cm in height and 6 cm in diameter glass jar contained 50 ml of culture medium and was covered with a polycarbonate cap. The culture medium was conceived in order to favour shoot growth and multiplication by axillary budding. Addition of only one growth regulator at low concentration should reduce the possible risks of somaclonal variations, as well as the risk of phytotoxicity due to unexpected accumulation of these substances in plant tissues ultimately. The cultures were maintained under the same standard environmental conditions as previously mentioned.

Sub-culture duration was two months, at the end of which the explants, after they had been sectioned as previously described, were transferred onto a fresh culture medium or were sent for the acclimatization/*ex vitro* rooting phase. This consisted in soaking the microcuttings from the glass jars, regardless of the adventitious roots that they might have produced spontaneously *in vitro,* in an aqueous solution of 5 g/l Thiram, prior to insertion into the rooting beds filled with sand under « mist system » and 50 % shade. No root promoting substance – « exogenous auxin » – was applied. Mist system frequency was automatically controlled by an « electronic leaf » (HARTMANN *et al.*, 1990). The environmental conditions were as described for propagation by rooted cuttings (MONTEUUIS *et al.*, 1995). Three

weeks later, the *ex vitro* rooted microcuttings were potted in 10 × 15 cm black polypropylene containers filled with cultivation substrate consisting of local clayish top soil. 50 % shade was maintained, but the mist-system frequency was halved. The potted plants were kept three weeks under this regime, then removed to the field nursery where, after three months of intensive cultivation, they reached a suitable development – around 30 to 40 cm in height – to be field planted (photos 2 to 6).

RESULTS

□ Production of *in vitro* seedlings

Around 50,000 teak seeds from various origins were inoculated *in vitro* according to the procedure described. A comparative study involving a total of 24,000 seeds of presumed low germination capacity, corresponding to 10 different geographic origins and 57 progenies from La Sangoué teak seed orchard in Côte d'Ivoire, gave rise to the following observations two months after inoculation :

- The success rate, corresponding to the number of suitably developed seedlings to be acclimatized out of the number of seeds initially introduced *in vitro* N_i, ranged from 2 to 57 % depending on to the geographic origins, with 30 % as average value. This rate varied from 0 to 64 % for the progenies, with 19 % as average value.

- The contamination rate, established by dividing the number of seeds plus seedlings contaminated by N_i – varied from 5 to 76 % according to the geographic origins, with 26 % being the average value. For the progenies, this rate ranged from 2 to 73 %, with 21 % as average value.

- The rate of morphologically abnormal seedlings, calculated based on N_i, averaged 17 % for the geographic origins, with 4 % and 32 % as limits. It fluctuated between 0 % (seed lot that did not germinate at all) and 53 % for the progenies, with an average value of 12 %.

- The rate of non-contaminated seeds that did not germinate at the end of the two months of incubation, established with reference to N_i, ranged from 3 to 47 % according to the different geographic origins, with 28 % as average value. Considering the progenies, this rate varied from 0 to 98 %, with 47 % as average value.

□ *In vitro* micropropagation of germinated seedlings

Records have established a multiplication rate of three at the end of every two month duration subcultures. This corresponds to an exponential increment of the number of explants in culture during time course of the form of 3^n, with n as the number of sub-cultures. This is an average and realistic multiplication rate based on a 4 year-long experience, and on several thousand microcuttings from various geographic and genetic origins, with possible losses included. Spontaneous rooting rate on the basal elongation/ multiplication medium averaged 80 % for the first culture cycles, and stabilized subsequently to about 60-70 %.

Regardless of whether *in vitro* roots were produced or not, the microcuttings were acclimatized to *ex vitro* conditions with a success rate of more than 90 %. This is again an average figure corresponding to several thousands of microcuttings acclimatized on different dates over the past few years.

MICROPROPAGATION OF GENOTYPES SELECTED *IN VIVO*

The genotypes selected *in vivo* to be micropropagated *in vitro* can be of different ages, and can consist of *in situ* individuals as well as of nursery stock plants, providing vegetative buds can be collected.

Mononodal – single node – and terminal portions from vegetative shoots and shoot apical meristems are the two types of primary explants utilized for micropropagating teak with the maximum of guarantees as far as genotypic fidelity is concerned (HAINES, 1994).

FROM MONONODAL AND TERMINAL PORTIONS FROM VEGETATIVE SHOOTS

□ Method

Mononodal and terminal portions were collected preferably from herbaceous or semi-hardwood shoots with a diameter of less than 1.5 cm. These can be produced from sticks forced under proper conditions (as detailed in MONTEUUIS *et al.*, 1995). Hardwood plant material is more difficult to disinfect. Once collected from the donor plant, leaves can be removed from the shoots to make the transportation easier, kept under shade and in humid conditions created for instance by opaque plastic bags containing a piece of water moistened toilet paper. Once in the laboratory, remaining leaves were removed by cutting off the petiole at an optimal distance from the stem in order to prevent the axillary buds from any damage, then sectioned in 10 cm long stem segments. After immersion for 30 min. in a 0,25 % $HgCl_2$ aqueous solution followed by three abundant rinses in pure water sterilized by autoclaving, these segments were cut with scalpels into 1 to 2 cm

SUCCESSIVE STEPS OF PILOT INDUSTRIAL PRODUCTION OF *IN VITRO* PRODUCED TEAK PLANTS : FROM LABORATORY TO PLANTATIONS :

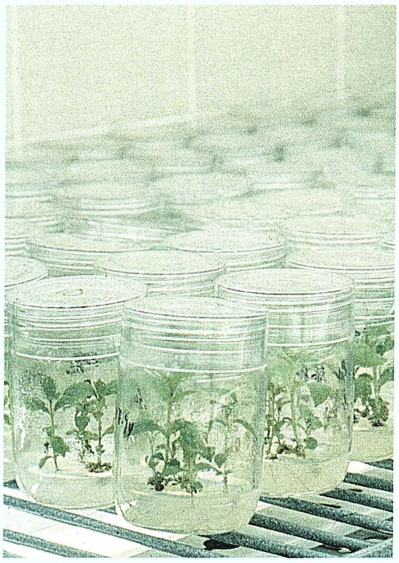

Photo 2

Photo 3

Photo 4

Photo 5

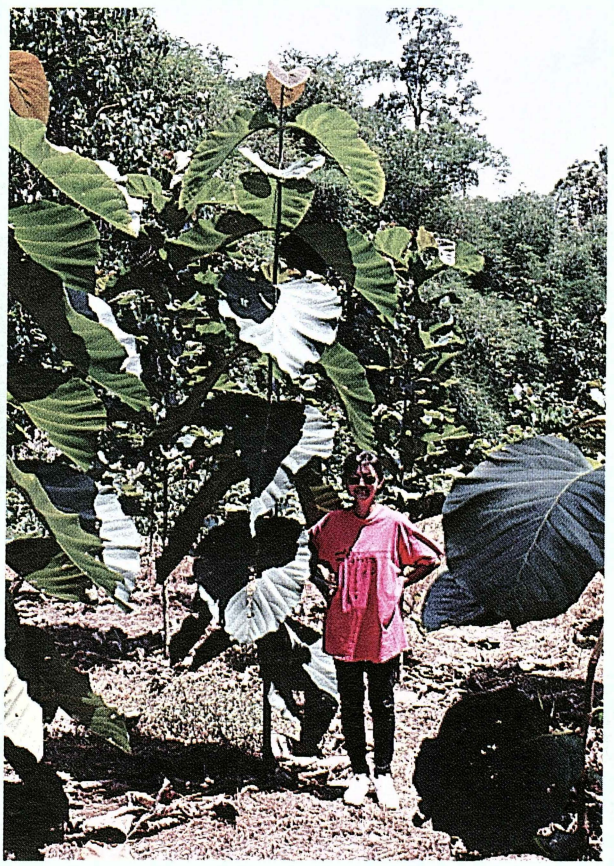

Photo 6

Photo 2. Intensive production of microcuttings in culture glass jars.
Photo 3. Rooting-acclimatization phase under mist-system in *ex vitro* nursery conditions.
Photo 4. Hardening phase after potting in containers filled with suitable cultivation substrate ; mist-system frequency has been halved compared to the previous rooting-acclimatization phase.
Photo 5. Raising phase in nursery.
Photo 6. *In vitro*-issued plant 4 months after planting.

long mononodal and terminal portions under laminar flow hood in sterile conditions. These were individually introduced into test tubes and maintained in the same total darkness conditions as previously mentioned for seed germination. Nodal segments with 0.5 to 1.5 cm diameter can be divided longitudinally into two primary explants ; each is bearing one bud, then is inoculated individually into two separate tubes in order to avoid the loss of two potential sources of new shoots in case of explant contaminations. The tubes contained 10 ml of gelled culture medium conceived to stimulate onset and rapid elongation of the new shoots (photo 7). The shoots which did not show any sign of contamination and attained 1 cm in length (photo 8) were excised to be transferred into culture tubes first for one to two sub-cultures to check the risks of contamination ; then they are placed in glass jars in the same conditions as previously described. Acclimatization and nursery raising conditions were identical to those described for the *in vitro* germination-derived microcuttings.

□ Results

Data recorded for several genotypes introduced into *in vitro* conditions on different dates established that 5 to 30 %, depending on the manipulator, of the primary explants inoculated gave rise to shoots suitable for initiating *in vitro* culture. The reactivation phase involving physiological rejuvenation (MONTEUUIS, 1989) can last from 4 to 8 months, depending on the genotypes and on their maturation stage. At the end of this period, the physiologically reactivated plant material can be further micropropagated by axillary budding with similar multiplication rates as young seedlings : three or even more at the end of every 2-month sub-culture. 50 to 60 % of the microcuttings rooted spontaneously

IN VITRO INTRODUCTION OF PLANT MATERIAL COLLECTED *IN VIVO*

□ Through 1 to 2 cm long mononodal portions

Photo 7. Early stages of axillary shoot development.

Photo 8. More advanced development stage making possible the removal of the new shoot from the primary explant to start the sub-culture process.

□ Through vegetative shoot meristems

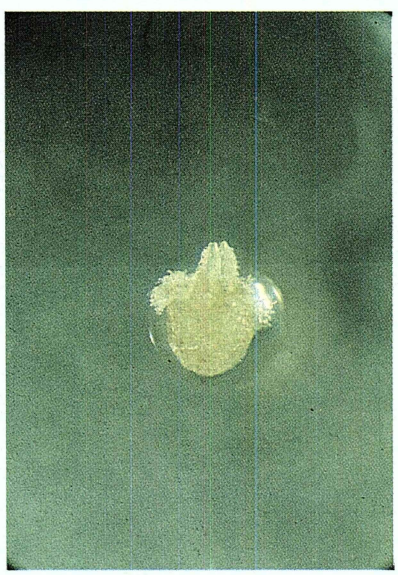

Photo 9. Meristem after two weeks of culture on the initiation medium.

Photo 10. Meristem culture-issued explant after four weeks of culture on the initiation medium.

which further attested the physiological rejuvenation of the mature selected genotypes.

These observations correspond to a three year period of *in vitro* culture of several genotypes from 6 to 15 years old which had already entered the flowering stage in natural conditions, and thus could be considered as mature from a physiological point of view (HACKETT, 1985 ; WAREING, 1987).

Investigations were further conducted by comparing a 15-year-old genotype to its progeny in *in vitro* conditions. Several thousands of microcuttings have thus been produced up to now, and then acclimatized, without any auxin treatment, in the same conditions as their homologs from seedlings, with more than 90 % success.

FROM SHOOT APICAL MERISTEMS

□ Method

After superficial bud disinfection using 70 % ethanol sprays, young leaves and leaflets were removed using fragments of razor blade. This could be done utilizing a binocular microscope until the meristematic dome, and possibly the foliar primordia depending on the plastochrone, in opposite decussate position, had been well cleared. The whole, whose overall size did not exceed 0.3 mm, was then carefully excised and inoculated in 3.5 cm diameter polystyrene Petri dishes containing 4 ml of appropriate culture medium onto which 5 meristems per Petri dish were placed.

All these manipulations were carried out as carefully and rapidly as possible in sterile conditions under laminar flow hood. Every two weeks, the explants were transferred onto a fresh medium. After two to three months, the 5 to 10 mm tall microshoots regenerated from the meristems initially set in culture were transferred into test tubes in the same conditions as the cultures initiated from mononodal and terminal shoot portions.

□ Results

About 7,000 to 8,000 shoot apical meristems collected from mature teak genotypes – 6 to 50 years old – were inoculated with the aim of developing protocols suitable for regenerating teak from *in vitro* meristem culture. The technology established allowed the production of « mericlonal lines » deriving from one single meristem for 60 % of the total number of meristems excised and inoculated initially as primary explants (photos 9 and 10). Six months later, the meristem-derived microcuttings demonstrated the same *in vitro* organogenic capacities as those issued from *in vitro* seedlings. Similarly also, acclimatization success rates averaged 95 %. Several "mericlones", some of them consisting of thousands of microcuttings, have thus been produced during the past three years within the ongoing project, some of these « mericlones » being set up in experimental plots (photo 11).

DISCUSSION-PROSPECTS FOR DEVELOPMENT

Observations over a four year period from the protocols developed within the common CIRAD-Forêt/ICSB biotechnology laboratory in Tawau have established that teak demonstrates a good capacity for *in vitro* culture, contrary to many other arborescent forest species (HAINES, 1994).

Seeds, when exposed to the *in vitro* experimental conditions stated, germinated with noticeably higher rates, that is to say 21 % on average, than in nursery. The average germination rate for the same plant material origin was in nursery only 4 % for seeds extracted from the fruits and 11 % for fruits exposed al-

Photo 11. "Mericlonal" – derived from *in vitro* culture of a single meristem – teak plot recently established and growing well despite serious weed competition.

ternately to water immersion and sun drying (PISP activity report 1996). In situations of low germination capacity, *in vitro* germination can be useful for rescuing certain seed lots by stimulating the germination process and enriching thereby the gene pool available. The advantage is therefore obvious for *ex situ* conservation and genetic improvement programs for this species, at different levels.

In vitro conditions can also be useful for investigating the germination capacity according to seed origin, as well as abnormal development of the germinating embryos that may affect certain seed lots as reported above.

The disinfection protocol developed and currently used makes it possible to initiate routinely contamination-free and responsive *in vitro* cultures from vegetative shoot mononodal and terminal portions collected from different genotypes growing outdoors, regardless of their age. This can be objectively considered as the requisite step for micropropagating any *in situ* selected teak individual. Nevertheless, efforts aiming at improving the success rates of primary culture initiation are being pursued each time new plant material from outdoors is introduced *in vitro*.

Initiation of *in vitro* cultures from shoot apical meristems requires greater skilfullness, dexterity and concentration from the manipulator. As an indicative figure, a competent person can excise and introduce 25 teak meristems in *in vitro* conditions within one hour. Providing there are enough shoot tips available, the efficiency in terms of primary cultures successfully initiated can be noticeably superior to what can be expected from introduction through vegetative shoot portions. Meristem culture can be useful for introducing *in vitro* and initiating cultures free of pathogenic organisms, more particularly those of endogenous origin which are very difficult to eradicate by other means (HARTMANN *et al.*, 1990). Meristem culture can be appropriate also for the *in vitro* introduction of plant material straight on the *in situ* collection zones. It is due to the small size of the Petri dishes, the proximity of a strong flame thereby reducing the risks of atmospheric contaminations. Lastly, meristem culture appears to be a suitable *in vitro* method for developing cryopreservation protocols adapted to teak, for which the advantages of such *ex situ* germplasm storage of the species are obvious (HAINES, 1994).

Irrespective of the origin and the introduction procedure of the outdoor plant material, the micropropagation protocol has been conceived to be adapted to an intensive and cost efficient production of true-to-type teak plants. This protocol consists of a sole elongation-multiplication basal culture medium, complemented with only one growth regulator at a very low concentration. Simple in composition, the most economical solutions were adopted for choosing certain medium components and other consumables.

The possibility of rooting and acclimatizing the microcuttings directly in nursery conditions (KAOSA-ARD *et al.*, 1987 ; KAOSA-ARD, APAVATJRUT, 1988) also results in a noticeable reduction of the *in vitro* production cost (MONTEUUIS, BON, 1987) while ensuring success rates of more than 90 %. Several year-long experience established that such tasks can be successfully handled by staff without any special qualification at the origin, providing the manipulators are serious, careful and motivated.

The procedure developed, applicable to any teak genotype regardless of its age, is objectively simpler than other protocols published previously on the same topic (MASCARENHAS, MURALIDHARAN, 1993 ; SUNITIBALA DEVI *et al.*, 1994). It resulted so far in the production at an experimental scale of some 50,000 teak microcuttings in satisfactory economical conditions. This pilot production allows foreseeable bright prospects for *in vitro* micropropagation of teak.

The plant material germinated *in vitro*, or introduced into tissue culture from outdoor genotypes of varying ages, can be micropropagated to fulfill different purposes :

- Establishment of « gene banks » (CHELIAK, ROGERS, 1990 ; HAINES, 1994), synonymous with germplasm or genotype collections. These maintain the satisfactory organogenic responsiveness of the selected materials during a time course in much more restricted space than the traditional outdoor conservation plots, which are constraining in terms of proper management.

- Production of (micro)cuttings from selected genotypes with a view to setting up seed orchards while avoiding problems of illegitimates which may occur with grafted plants when the stock supplants the scion.

- Mass and intensive production of genetically superior quality (micro)cuttings for forest plantations. These propagules can be micropropagated either as a mixture of genotypes, deriving from seeds as described previously or else from polyclonal varieties, or as clones (photo 12). The *in vitro* production cost and the adaptability of the plantlets to acclimatization and then to the subsequent steps of nursery and plantation conditions (photos 13 et 14) are supportive arguments in favour of this option. It seems in fact that for teak the micropropagation method developed can be more efficient than *in vivo* propagation by rooted cuttings. This is in contrast with a lot of other forest tree species for which *in vitro* culture may be recommended only to reactivate once the organogenic capacities of certain genotypes. Subsequently, mass propagation by

Photo 12. Monoclonal plot four months after planting of *in vitro*-produced teak plants derived from a mature genotype (minimum 15 year-old).

Photo 14. *In vitro*-issued teak plants one year after planting.

Photo 13. Morphological appearance of the adventitious root system of an *in vitro*-issued teak plant one year after planting.

cuttings of the responsive *in vitro*-derived plant material used as stock plants can proceed in the nursery at lower costs (MONTEUUIS, BON, 1987).

The development of adapted protocols for propagating teak both by *in vitro* culture and by stem cuttings within the same project allows a better comparison on a realistic basis of these two vegetative propagation methods, which can profitably complement each other. A comparative study, mainly from an economical standpoint, is in progress and this remains the determining factor for industrial development prospects. Although the *in vitro* option must take into account the amortization of the laboratory, it does not require, contrary to the nursery option, a stock plant orchard which needs intensive management by a sufficient number of competent people when good results are expected (MONTEUUIS *et al.*, 1995). In addition, another advantage connected with *in vitro* micropropagation lies in the possibility of sending the tissue-cultured plants off to different destinations, at various distances ; meanwhile the benefit will be a phytosanitation immunity in the case of international dispatches. This should induce large market prospects, particularly needed and beneficial for teak plantations in case of improved quality planting material. ❐

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ABSTRACT

PROPAGATION OF TEAK BY *IN VITRO* CULTURE

Possibilities of propagating teak by *in vitro* culture were assessed from a realistic standpoint of production within the framework of a Research and Development project in Sabah (East Malaysia). The tissue culture conditions developed noticeably improved the germination rate of poor germination capacity seed lots. Those obtained *in vitro* could be further micropropagated. The micropropagation protocol was conceived as simply as possible to multiply vegetatively, through axillary budding-derived shoots, teak genotypes issuing from *in vitro* germination or of different ages growing outdoors. For this latter type of plant material, the cultures were initiated using 1 to 2 cm long mononodal portions collected from preferably actively growing shoots. Cultures could also be initiated from shoot apical meristems with a success rate of 60 %. Regardless of their origin, the genotypes were micropropagated according to an exponential multiplication rate of 3^n, with n as the number of the 2-month-duration subcultures. Acclimatization was carried out under mist-system in nursery conditions with more than 90 % success. The tissue-culture produced plants developed vigorously during 3 to 4 months of culture in nursery until they reached a suitable size for planting out.

To date, 50,000 *in vitro* plants have been produced on an experimental scale and at low cost applying this protocol that is suitable for intensive production of superior quality teak planting stock.Considering these achievements, the prospects of *in vitro* culture for propagating selected teak genotypes compared to what can be expected from propagation by rooted cuttings are discussed, mainly from a development point of view.

Key words : *Tectona grandis*. Adaptation. Environmental factors. Cloning. Genotypes. Germination. *In vitro* culture. Micropropagation. Vitroplants.

RÉSUMÉ

PROPAGATION DU TECK PAR CULTURE *IN VITRO*

Les possibilités de propager le teck par culture *in vitro* ont été analysées sur des bases réalistes de production dans le cadre d'un projet de Recherche-Développement au Sabah (Malaisie orientale). Les conditions *in vitro* définies permettent d'améliorer sensiblement la germination de lots de graines à faible faculté germinative. Les semis obtenus *in vitro* peuvent être ultérieurement micropropapagés. Le protocole de micropropagation, simple de conception, permet de multiplier végétativement, par microbouturage de pousses issues de bourgeonnement axillaire, des génotypes de tecks germés *in vitro*, ou d'âges variables poussant en conditions naturelles. Dans ce dernier cas, l'introduction se fait sous forme de portions mononodales ou terminales, de 1 à 2 cm de long, d'axes végétatifs préférentiellement en croissance active. L'initiation des cultures peut également se faire sous forme de méristèmes primaires caulinaires avec 60 % de succès. Quelle que soit leur origine, les génotypes se multiplient de façon exponentielle à raison de 3^n, n étant le nombre de subcultures de deux mois. L'acclimatation sous « mist-system » en conditions de pépinière s'effectue avec plus de 90 % de succès. Les plants se développent de façon tout à fait satisfaisante durant 3-4 mois de culture en pépinière avant de pouvoir être plantés.

A ce jour, 50 000 vitroplants ont été produits à titre expérimental dans des conditions économiques très avantageuses en appliquant ce protocole conçu pour favoriser une production intensive de plants de tecks de qualité supérieure. Sur ces bases, l'intérêt de la culture *in vitro* pour propager des génotypes sélectionnés de teck par rapport au bouturage horticole est analysé, essentiellement dans une perspective de développement.

Mots-clés : *Tectona grandis*. Adaptation. Facteurs du milieu. Clonage. Génotypes. Germination. Culture *in vitro*. Micropropagation. Vitroplants.

RESUMEN

PROPAGACIÓN DE LA TECA POR CULTIVO *IN VITRO*

Se ha procedido al análisis de las posibilidades de propagar la teca por cultivo *in vitro* fundándose en bases realistas de producción en el marco de un proyecto de Investigación y Desarrollo (I&D) en Sabah (Malasia oriental). Las condiciones *in vitro* definidas permiten mejorar de forma apreciable la germinación de lotes de semillas de reducida facultad germinativa. Las siembras obtenidas *in vitro* se pueden micropropagar ulteriormente. El protocolo de micropropagación, que obedece a un concepto sencillo, permite multiplicar, vegetativamente, por microreproducción mediante estacas de vástagos procedentes de brotes axilares de genotipos de tecas germinadas *in vitro*, o de edades variables que se desarrollan en condiciones naturales. En este último caso, la introducción se efectúa en forma de porciones mononodales o terminales de 1 a 2 cm de longitud de ejes vegetativos en crecimiento activo, preferentemente. La iniciación de los cultivos se puede efectuar, asimismo, en forma de merisistemas primarios caulinarios con la probabilidad de un 60 % de logros. Sea cual fuere su origen, los genotipos se multiplican de forma exponencial a razón de 3^n, siendo n el número de subcultivos de dos meses. La aclimatación en forma de mist-system en condiciones de vivero se efectúa con más de un 90 % de logros. Las plantas se desarrollan de forma perfectamente substancial durante 3 a 4 meses de cultivo en vivero antes de poder ser trasplantadas.

Hasta la fecha, se han producido 50 000 vitroplantas a título experimental en condiciones económicas sumamente ventajosas, aplicando este protocolo diseñado para propiciar una producción intensiva de plantas de teca de calidad superior. Con arreglo a estas bases, se analiza el interés del cultivo *in vitro* para la propagación de genotipos seleccionados de teca, por comparación con el procedimiento hortícola por estacas, y principalmente, situándose en una perspectiva de desarrollo.

Palabras clave : *Tectona grandis*. Adaptación. Factores ambientales. Clonación. Genotipos. Germinación. Cultivo *in vitro*. Micropropagación. Vitroplantas.