Mass production and quality control of teak clones for tropical plantations:

The Yayasan Sabah Group and CIRAD Joint Project as a case study

Doreen K. S. Goh[1]
Gilles Chaix[2]
Henri Baillères[3]
Olivier Monteuuis[2]

[1] Yayasan Sabah Group
Biotechnology & Horticulture Division
PO Box 11623
88817 Kota Kinabalu
Sabah
Malaysia

[2] CIRAD
UPR 39
TA A-39/C
34398 Montpellier Cedex 5
France

[3] CIRAD
UPR 40
TA B-40/16
34398 Montpellier Cedex 5
France

Photo 1.
Example of a teak Plus tree.
Photo O. Monteuuis.

Producing high yields of top-quality teak wood in short rotations is now becoming a priority for many landowners and investors. As the result of a fruitful collaborative work between the Sabah Foundation Group and CIRAD, such quality planting stock is now available.

Doreen K. S. Goh, Gilles Chaix,
Henri Baillères, Olivier Monteuuis

RÉSUMÉ

PRODUCTION INDUSTRIELLE ET CONTRÔLE QUALITÉ DE CLONES DE TECK POUR LES PLANTATIONS EN ZONE TROPICALE

L'attrait pour des clones de teck qui peuvent être plantés sous forme monospécifique ou en association avec d'autres espèces végétales (agroforesterie) devient de plus en plus évident dans de nombreux pays. Néanmoins, contrôler la qualité du matériel produit et planté est essentiel pour assurer la réussite des plantations clonales de teck. Cet aspect revêt une importance toute particulière au Sabah. La prise en compte précoce des caractéristiques du bois à fort impact économique associée à des tests clonaux rigoureux permet d'affiner la sélection initiale des têtes de clones potentielles, basée traditionnellement sur des critères phénotypiques. Conjointement, la mise au point de techniques d'analyse au niveau de l'Adn par des marqueurs adaptés permet une meilleure identification de l'origine génétique et des degrés d'apparentement éventuels des génotypes présélectionnés, pour une utilisation plus rationnelle sous forme de clones. Ces analyses moléculaires sont aussi mises à profit pour vérifier la conformité génotypique des clones propagés, et veiller au bon respect des droits de propriété intellectuelle dans le cadre de transactions commerciales. Le contrôle de la qualité concerne également les différentes phases de propagation clonale, de l'introduction en conditions de culture *in vitro*, où l'utilisation de méristèmes apicaux comme explants primaires est bien préférable aux segments nodaux, jusqu'à l'enracinement et l'acclimatisation *ex vitro* des microboutures produites. Les étapes ultérieures d'élevage en pépinière et de comportement en plantation sont également prises en considération dans cette démarche de contrôle de la qualité afin de satisfaire au mieux les desiderata des clients. Enfin, une attention toute particulière est accordée au conditionnement des plants destinés à l'exportation par les voies aériennes les plus rapides et les plus sûres.

Mots-clés : clone, marqueur Adn, contrôle qualité, *Tectona grandis*, caractéristique du bois.

ABSTRACT

MASS PRODUCTION AND QUALITY CONTROL OF TEAK CLONES FOR TROPICAL PLANTATIONS

The attractiveness of teak clones that can be planted either as monocultures or in combination with other crops is becoming more and more obvious in various countries. However, quality control of the planting material is essential for ensuring the reliability and future of teak clonal forestry. Development of quality control on the various aspects of teak clone production is being undertaken in Sabah. Refining the initial phenotypic selection of the candidate plus trees by taking into account economically-important wood characteristics, combined with appropriate clone testing are aimed at identifying superior clones. Conjointly, the development of reliable DNA markers can be used for identifying the genetic background and the possible relatedness of the candidate genotypes for wise clonal deployment. It is also a means of controlling the genotypic conformity of the mass-produced clones while at the same time, ascertaining property rights associated with their involvement in commercial transactions. Quality control is also applied to the successive steps of mass clonal propagation, from the introduction phase to in vitro conditions where meristem culture is preferable to nodal culture, up to the ex-vitro rooting and acclimatization of the microshoots. The further steps of nursery cultivation and field behavior of clones are then further monitored in order to optimize the quality of the plant material offered to clients. Proper packing and conditioning of microshoots for overseas shipment and delivery in the shortest delays to foreign countries are also crucial issues for preserving the quality of the plants.

Keywords: clone, DNA marker, quality control, selection, *Tectona grandis*, wood characteristic.

RESUMEN

PRODUCCIÓN INDUSTRIAL Y CONTROL DE CALIDAD DE CLONES DE TECA PARA LAS PLANTACIONES EN ÁREAS TROPICALES

El atractivo por los clones de teca que pueden sembrarse en cultivo monoespecífico, o en asociación con otras especies vegetales (agroforestería) es cada vez más patente en muchos países. Sin embargo, es fundamental controlar la calidad del material producido y plantado para garantizar el éxito de las plantaciones clonales de teca. Este aspecto tiene una especial importancia en Sabah. La consideración temprana de las características de la madera con alto impacto económico, asociada a pruebas clonales rigurosas, permite determinar la selección inicial de las cabezas de clones potenciales, basada tradicionalmente en criterios fenotípicos. Al mismo tiempo, la puesta a punto de técnicas de análisis de ADN mediante marcadores adaptados permite una mejor identificación del origen genético y de los posibles grados de emparentamiento de los genotipos preseleccionados para una utilización más racional en forma de clones. Estos análisis moleculares se aprovechan también para comprobar la conformidad genotípica de los clones propagados y velar por el buen respeto de los derechos de propiedad intelectual en el marco de transacciones comerciales. El control de la calidad concierne también las distintas fases de propagación clonal, desde la introducción en condiciones de cultivo *in vitro*, donde la utilización de meristemas apicales como explantes primarios es mucho mejor que los segmentos nodales, hasta el enraizamiento y aclimatación *ex-vitro* de los microinjertos producidos. Para satisfacer al máximo los deseos de los clientes, las posteriores etapas de cría en vivero y comportamiento en plantación también se tienen en cuenta en este enfoque cualitativo. Por último, se presta especial atención al acondicionamiento de las plantas destinadas a la exportación a través de las vías aéreas más rápidas y seguras.

Palabras clave: clon, marcador ADN, control de calidad, *Tectona grandis*, característica de la madera.

The increasing attractiveness of teak clones

As predicted (Monteuuis, Goh, 1999; Monteuuis *et al.*, 2004; Goh, Monteuuis 2005) teak clonal plantations are rapidly expanding under the strong demand of private investors eager to maximize their return on investments in the shortest delays. In addition to enabling superior volume yield and enhanced wood quality in both traditional plantation systems and agroforestry applications, accession of teak clones offers an opportunity to enrich the teak genetic resources available locally. Using the best clones in optimal silvicultural management should be a priority in plantation establishment, as it can be assumed that yield and, predominantly, the quality of the timber produced, will be the two overriding commercial factors for the near future, especially when considering the reduction of the natural sources due to depletion or international bans. This is particularly true for the highly prized Myanmar or Burma teak. Selecting, testing and providing outstanding clones therefore deserve strong emphasis and particular attention.

Selection of superior clones

To enhance the value of future teak plantations, genetic improvement of growth rate and wood quality must be pursued. This paper gives special attention to outlining means for maximizing marketable wood recovery rate per hectare in plantations. So, in addition to classical growth rate, the following traits must be taken into consideration in field selection of the Plus trees from which clones are to be produced:

- Clear bole diameter and length, the greater the length, the higher the yield.
- Bole shape (straightness, circularity, taper) with minimal knots, buttresses or flutes at the bottom of the tree.
- Frequency and size of lateral branches responsible for nodes affecting wood quality for veneer end-use especially.

Clonal tests properly established are needed to identify genetically superior Plus trees (photo 1) among those initially selected on phenotype. The superior genotypes are then clonally mass-produced to be used for developing industrial clonal plantations, in accordance with the strategies described in previous papers (Goh *et al.*, 2005; Goh, Monteuuis, 2005). This part of the program needs to be backed up by an efficient genetic improvement program so that better clones may be selected in the future.

Photo 2.
Board destructive sampling using a chainsaw.
Photo O. Monteuuis.

Refined selection using wood analysis criteria

The selection criteria mentioned so far relate only to external features of the trees. The importance of also considering intrinsic wood qualities of the candidate Plus trees from which the teak clones are going to be produced is obvious owing to the great value of teak timber, which is liable to vary a lot from one individual to another, even when closely related genetically (ZOBEL, JETT, 1995).

"Wood quality" is classically defined by the combination of five main groups of factors detailed in Table I.

However, in practice, teak buyers and end-users give more emphasis to the characteristics listed in Table II according to the final utilization of the timber (BAILLÈRES, DURAND, 2000).

Also, it is desirable that teak wood be as homogeneous as possible with minimal within-tree variations, such as differences between juvenile and mature wood, notwithstanding site effects, liable to induce differences between individuals issued from the same clone.

Two sampling methods, destructive or not, depending on plant material availability, can be considered for analyzing wood characteristics of individual trees.

Destructive sampling

Destructive sampling involves securing a diametrical plank 1m in length and 3 to 5 cm in width from the lower portion of felled trees. The good correlation between results from such a sample and 'whole tree' have been demonstrated already (ZOBEL, JETT, 1995).This approach can be used for genotypes represented by several trees, in other words, from clonal tests or plantations (photos 2 and 3). The information that can be obtained from such samples includes:

- Ring analysis (when the limit between rings is clear enough).
- Heartwood proportion out of the total wood without bark.

Table I.
List of the main factors determining general wood quality (drawn from BAILLÈRES, DURAND, 2000).

Wood quality factors	Wood properties	Remarks
1. Mechanical factors	1a. Modulus of elasticity 1b. Modulus of rupture 1c. Maximum crushing strength 1d. Hardness (for flooring uses) 1.e. Growth stresses	1a, 1b, 1c and 1d are correlated to specific gravity in the early stage of tree development. For young trees.
2. Physical factors	2a. Shrinkage 2b. Tangential / Radial shrinkage 2c. Sorption properties such as fiber saturation point	Assessed in the three different directions of wood structure. Anisotropic ratio indicating dimensional stability. Key impact on dimensional stability.
3. Biological factors	3a. Decay resistance 3b. Termite resistance 3c. Weather resistance	Reflect the natural durability.
4. Aesthetic factors	4a. Color and veining 4b. Grain 4c. Texture	Can be referred to under the same generic term of "figure".
5. Structural factors	5a. Heartwood / sapwood ratio 5b. Bole shape (straightness, taper, buttressing, fluting) 5c. Knots size and frequency 5d. Grain angle	Important for sawing as directly related to timber yield (i.e.: sawn timber grade and recovery).

- Basic density, i.e. the ratio of dry weight per unit volume to that of saturated wood weight.
- Figure including grain, texture and color – the most important, market-wise is measured with a Datacolor Microflash 200d spectrocolorimeter.
- Modulus of elasticity determined by the non-destructive "Bing" method (Baillères *et al.*, 1998).
- Strength, defined as the maximum stress that can be applied to a beam in pure bending before permanent deformation occurs. It is most commonly used to describe flexure properties of wood products.
- Radial and tangential shrinkages measured at green state, 6% and 0% moisture content.
- Natural durability along with extractives content (standard measurement and NIRS prediction); standard measurements usually take 5 to 6 months due to the time needed for assessing fungal attack (16 weeks minimum).
- Dimensional stability assessed as the ratio of tangential to radial shrinkage.
- Wood uniformity (with minimal within-tree variation in anatomical, physical and mechanical properties between juvenile and mature wood).
- Figure (color, grain, texture)/appearance) usually scored.

This set of wood characteristics is more than what is usually needed for up-grading the initial phenotypic selection to superior wood quality plant material with higher market value, either in the form of raw materials, or more or less processed end products (Bath, 2000).

Table II.
Main end-use-related teak wood technological characteristics.

End-uses	Relevant wood characteristics
Joinery	Natural durability Shrinkage and movement in use or dimensional stability ("nervosity") Strength Sapwood percentage
Flooring and parquets	Figure: color, grain and texture Shrinkage and movement in use or dimensional stability ("nervosity") Natural durability Hardness Sapwood percentage
Furniture	Figure: color, grain and texture Knot occurrence Shrinkage and movement in use or dimensional stability ("nervosity") Strength Sapwood percentage
Sliced veneer	Figure: color, grain and texture

Non-destructive methods

Similar information or knowledge can be obtained using non-destructive methods, e.g. core sampling (photos 4). These approaches are appropriate in cases where genotypes are represented by one tree only that must be kept alive to be potentially used as a mother tree for seed or clone production, or some other use requiring the intact tree.

By attaching the wood corer specially developed for teak to a hand-held motor driven drill (photos 4), a suitable core of 15mm in diameter and 40 to 60 cm in length can be easily extracted from any teak tree within 2 to 5 minutes, depending on the size of the tree.

Using the near infra red spectroscopy technology (NIRS) will help for assessing from such core samples the basic density, the modulus of elasticity and strength, the radial and tangential shrinkages, the natural durability as well as the extractive content.

Once properly calibrated, the NIRS is a fast, low-cost, easy-to-use, non-destructive, reliable and versatile analytical method which can accommodate heterogeneous wood samples and point out slight chemically- induced wood variations (*cf.* annex for more information).

Photo 3.
Freshly collected board samples.
Photo O. Monteuuis.

Usefulness of adapted molecular markers

The development of "clonal forestry" with teak can be supported by DNA molecular markers in four different ways.

- Through determining the primary origin of the various teak populations available locally, i.e. whether they were initially imported from India, Myanmar (ex Burma), Thailand or Laos, which are the four native countries for teak. The usefulness of such an indication is obvious for basic research i.e. range of adaptability of the native teak provenances to other environments in various countries, as well as for operational and commercial activities. As an example, the highly prized Burma teak origin may now exist in several other countries. In fact, it had been speculated that the Latin America teak introduced via Trinidad and Tobago was originally imported from Tenasserim (Burma), like the Solomon island teak sources.
- Through assessing the genetic diversity and levels of co-ancestry in the teak germplasm that exists locally for optimal management and utilization within sound tree improvement programs. Knowledge of the genetic background of the seed producers will enable tactics to reduce risks of inbreeding to be employed, for instance by limiting the numbers of close relatives included in seed orchards. Information on the genetic relatedness of candidate clones for wood production plantations will also enable implementation of tactics to control the level of genetic diversity in such plantations and possibly render them better buffered in the presence of pest and diseases, should they arise.
- Through phenology and gene flow studies aimed at understanding then taking actions to maximize gene exchanges and perhaps achieve panmixia within seed stands and seed orchards, thus limiting inbreeding and while enhancing genetic recombination.
- Through clonal identification by DNA fingerprinting with application to property rights or genetic fidelity of the mass-propagated clones.

Choice of the most appropriate DNA marker technologies among all those available and detailed by Mueller and Wolfenbarger (1999) is a crucial issue. Respective efficiencies of technologies such as the Amplified Fragment Length Polymorphism or AFLP, and the Simple Sequence Repeat or SSR, also known as microsatellites, were therefore assessed in our laboratory facilities on several teak origins.

Notwithstanding the advantages of AFLP, especially for rapidly saturating the teak genome with limited polymerase chain reactions, or PCR for short, the SSR technology was found to be more reliable and adapted to our current objectives as mentioned above, which is in accordance with findings with other species (Rahman, Rajora, 2002; Kirst *et al.*, 2005). Further, the availability of a teak microsatellite bank developed previously by our team under another teak project (Verhaegen *et al.*, 2005), had a determining influence on this choice (photo 5).

Photos 4.
Non-destructive core sampling method using a hand-held motor driven drill (a) and a core sample (b).
Photos H. Baillères.

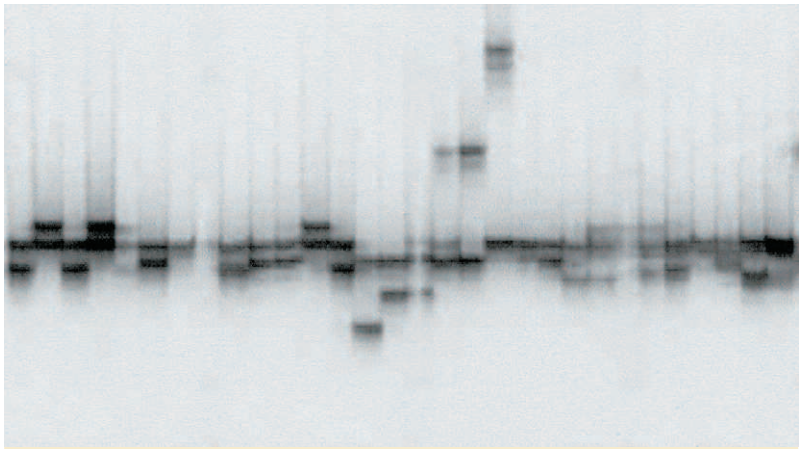


Photo 5.
Teak origin screening using DNA microsatellite profiles.
Photo G. Chaix.

Mass clonal propagation process

Most of the teak planting materials developed by ICSB/CIRAD are clones that are mass produced by tissue culture and introduced as nodal explants collected from mature selected Plus trees. The methodology used has already been described (Monteuuis *et al.*, 1998; Monteuuis, 2000; Goh, Monteuuis, 2001). Although requiring more skillfulness and expertise than for nodal explants, teak meristem – 0.1 mm as overall size – culture (photos 6) is more efficient for rejuvenating mature geno-

Photos 6.
Clonal propagation of mature selected teak using shoot apical meristem (0.1 mm as overall size) *in vitro* culture three (a) and eight weeks (b) after introduction, then five years later in outdoor conditions (c) in absence of any pruning.
Photos O. Monteuuis.

Photos 7.
Container-grown ready for planting clonal offspring (a) and packed microshoots ready for international dispatch (b).
Photo (a) O. Monteuuis ; photo (b) D. Goh.

types while overcoming the contamination problems that commonly affect the primary cultures. This latter technique is therefore becoming more frequently used for our new introductions of mature genotypes resulting from field selection.

Regardless of the technique used, the target is to rapidly mass produce actively growing 4 – 6 cm long micropropagated shoots with high adventitious rooting capacity from any teak genotype irrespective of its age. These shoots can be easily transferred to *ex-vitro* conditions for rooting under appropriate mist-system conditions, then raised to a suitable developmental stage for field planting.

The behavior of the tissue-cultured shoots is assessed regularly during the *ex-vitro* rooting and acclimatization phases, as well as under different field conditions.

Assistance can be provided upon request to help ensure the best acclimatization success of the tissue-cultured shoots dispatched to various destinations overseas.

DNA fingerprinting can be used at any step of the process for certifying the genetic identity of the micropropagated plant material.

Plant material for sale

Ready-for-planting teak plants from different clones or genetic background cultivated in 6 x 15 cm cylindrical containers are available for local uses from nurseries located in three different sites in Sabah (photo 7 a).

Due to phytosanitary restrictions, tissue-cultured plantlets are the only means currently available for successfully exporting teak clones to oversea destinations (photo 7 b). The 4 to 6 cm long microshoots are packed in sterilized lightweight polyethylene containers, each with 150 to 200 microshoots, then placed in Styrofoam boxes lined with newspaper to be delivered by air to overseas clients. A shipment should not exceed 10 days in transportation. Thus, reducing the delay between the time of packing the microshoots and of their setting in an appropriate misting propagation system at the final destination is quite critical.

Clone identification form

Under the present commercial production system, in order to ensure efficient and reliable mass micropropagation, only the best 12 to 15 clones are kept in intensive production for sale. However, the current list of clones can be extended at any time by reactivating the clones stored in our *in vitro* clonal bank, or by introducing additional clones resulting from new selection work.

Information on each clone is readily available upon request. Potential clients are welcome to visit both the micropropagation laboratory and the field trials of different clones, as well as other local stands of teak to which we have access. The main characteristics of individual clones are described in a comprehensive leaflet as illustrated in Figure 1.

ICSB/CIRAD Teak Clone Characteristics

Species: *Tectona grandis*

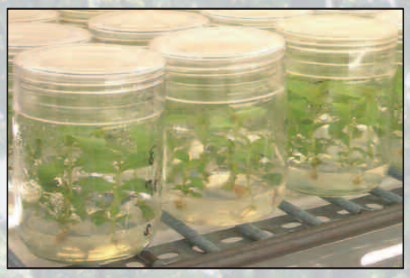
Origin: Solomon Island

Identity: ICSB/CIRAD Clone TG2

Available in the form of:

Ready for planting cuttings (for local market)

or ***In vitro*-derived microcuttings** (for international market)



Packed and delivered under contamination-free conditions to meet foreign country phytosanitary requirements

4 yr-old Teak clones in Sabah (East Malaysia)

For more information:

Dr Doreen KS Goh

YSG BIOTECH SDN BHD
Yayasan Sabah Group
P.O. Box 11623
8817 Kota Kinabalu,
Sabah, Malaysia
Tel: 60-88-263-185;
Fax: 60-88-263-424;
Email: dorngoh@pc.jaring.my
Web site: ysnet.org.my

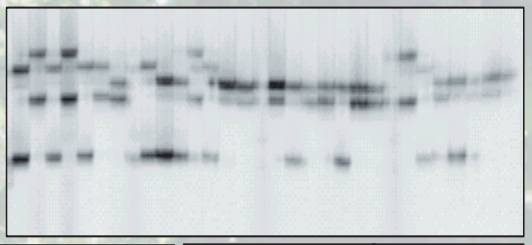
Narrow crown and clear bole clones suitable for intercropping with cash crops such as oil palm

Figure 1.
The clone identification form: clone TG2 as an example.
Source: ICSB-CIRAD, 2006.

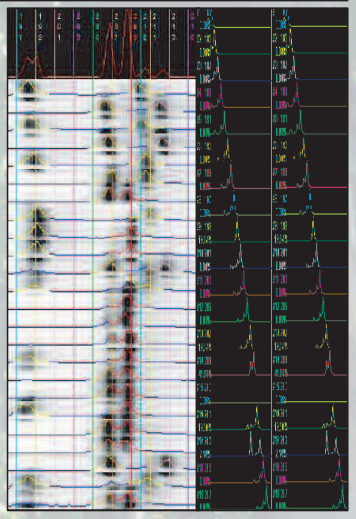
ICSB/CIRAD CloneTG2

DNA fingerprinting - Wood characteristics

DNA Fingerprinting



Microsatellite locus name	Accession EMBL Database	Alleles
CIRAD1TeakA06	AJ968929	205 221
CIRAD1TeakB03	AJ968930	248 250
CIRAD1TeakF05	AJ968931	268 276
CIRAD1TeakG02	AJ968932	168 170
CIRAD1TeakH10	AJ968933	228 242
CIRAD2TeakB07	AJ968934	143 145
CIRAD2TeakC03	AJ968935	280 280
CIRAD3TeakA11	AJ968936	274 280
CIRAD3TeakB02	AJ968937	237 251
CIRAD3TeakD09	AJ968938	207 213
CIRAD3TeakF01	AJ968940	219 229
CIRAD4TeakD12	AJ968941	139 151
CIRAD4TeakH09	AJ968943	226 228



Wood characteristics after 10 years of growth in Sabah conditions		Tropix reference [2]
Heartwood proportion	61 %	-
Basic density	590 kgm^{-3}	670 kgm^{-3}
Total shrinkage	2.6 %	-
Radial shrinkage	1.3 %	2.6 %
Tangential shrinkage	1.2 %	4.7 %
T/R Ratio (Nervosity)	1.0	1.8
Modulus of Elasticity	1413 Mpa	-
Modulus of Rupture	123 Mpa	98 MPa
Natural Durability [1]	Very durable	Very durable

[1] Durability towards Basidiomycete fungi,[2] http://tropix.cirad.fr/asia/teck.pdf

Data produced by: **UR 39 Genetic Diversity and Breeding of Forest Species** laboratory (http://www.cirad.fr/en/pg_recherche/ur.php?id=101) with the help of M Poitel, G Chaix and **UR 40 Production and Processing of Tropical Woods** laboratory (http://www.cirad.fr/en/pg_recherche/ur.php?id=102) with the help of N Boutahar, MF Thévenon and H Baillères, both research units of the Forestry Department of CIRAD

Figure 1.
The clone identification form: clone TG2 as an example.
Source: ICSB-CIRAD, 2006.

The first page of this leaflet illustrates the two methods of propagating and supplying the clones, distinguishing between rooted cuttings for the local market and tissue-cultured microshoots ready for export in well-insulated styrofoam boxes. This latter method must satisfy the phytosanitary conditions specified by the destination country where the plantlets will be set for rooting, then acclimatized before planting out in the field. The clones can be deployed either in the form of monoclonal blocks, as clone mixtures or in some association with annual or perennial crops within agroforestry systems.

The second page Figure 1 shows the growth potential, DNA profile for genetic background identification and wood characteristics as detailed previously. It also includes photos of the wood produced by the given clone when grown under Sabah conditions.

Complementary information from clone performance in the different sites where the same plant material has been planted, for assessing its adaptability to various environments, can also be provided in some cases.

Conclusion

The dramatic reduction of high-grade teak timber supplies from natural stands and an increasing worldwide demand have accounted for the greater interest in teak plantation establishment. Production of high yields of top quality teak wood in short rotations is now becoming a priority for a lot of landowners and investors. This presents a challenge requiring early delivery and on-going improvement of planting stock capable of providing high yields of logs with the most prized wood properties.

Results from fifteen years of collaborative research work between the Sabah Foundation Group and CIRAD on genetic improvement, clonal selection, development of efficient nursery and *in vitro* propagation protocols, and on wood property determinations have led to the availability of such quality planting stock. The sustained focus on the development of this "package" of technologies and genetic material is now paying off, as evidenced by the widespread interest and demands for our clonal materials from buyers in Malaysia and around the world.

The application of DNA fingerprinting and wood analyses lend emphasis not only to our seriousness in developing the best quality planting materials, but also on the usefulness of having such information. Interested parties are now able to access this information and make wiser decision on the selection of clones according to their own planting needs and conditions.

With such strong interest, the need to continuously upgrade the performance and quality of new "generations" of clones remains our priority target. As the interest in establishing quality teak plantations continues, so shall the research within our collaborative program attempt to keep pace, if not to lead into the production of planting material that will enhance plantation profitability.

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ANNEX.

Near infrared spectroscopy on wood

NIR spectroscopy is a fast, low-cost, easy-to-use, non-destructive, reliable and versatile analytical method. Moisture is a relatively low absorber in NIR, which is also important when measuring biological products. NIR is widely used in determining a variety of quality parameters in a large number of biological materials, such as grains, feeds, meat and meat products, vegetables and fruits, both in the laboratory and as an on-line analysis technique (Burns *et al.,* 2001; Gindl *et al.,* 2001; Kelley *et al.,* 2004; So *et al.,* 2004).

The NIR spectra are results of many overlapping and often broad peaks, which are difficult or impossible to interpret using univariate methods unlike mid- and far-infrared (MIR and FIR) regions where the spectroscopic peaks are resolved and can be interpreted directly. A NIR spectrum is a result of overtone absorbances and combinations of absorbances from several functional groups, such as C-H, N-H and O-H.

In addition, a diffuse reflectance or transmittance NIR spectrum is a result of the physical conditions of both the instrument and the sample: instrument geometry; sample particle and droplet size, form and distributions; refractive indices, etc. The resulting NIR spectrum from a diffuse measurement is therefore a mixture of several physical and chemical effects. This is the reason why extensive use of multivariate data analytical methods is necessary to reveal specific and useful information from such NIR spectra (chemometrics tools are usually applied to NIR spectra).

NIR spectra are usually presented in "absorbance" units defined by either $A = \log (1/R)$ or $A = \log (1/T)$, depending on whether the data are based on reflectance (R) or transmission (T).

The NIR region of the electromagnetic spectrum extends from 780 to 2 500 nm (wavelength) or 12 800 to 4 000 cm^{-1} if measuring in wave numbers (the number of waves per cm). NIR spectroscopy is concerned with absorptions of NIR energy by molecules within a sample. The absorption of energy increases the energy of vibration in a molecule.

Absorptions in this spectral region are caused by three different mechanisms. These are the following.

- Overtones of fundamental vibrations which occur in the infrared region (4 000-300 cm^{-1}).
- Combinations of fundamental vibrations which occur in the infrared region.
- Electronic absorptions.

Overtones are approximate multiples of the fundamental vibrations. A fundamental vibration, "f", will give rise to a series of absorptions 2f, 3f, 4f... which are known as the first, second, third overtone. The intensity of these successive absorptions will decrease by a factor between 10 and 100. Moreover, absorption of NIR energy can be shared between two (or more) vibrations which would be individually observed as fundamental then these would give rise to a combination band. Combinations can be multiples of one or more of the vibrations and there is no theoretical limit to the number of absorptions that can be involved. Combination bands have two effects on NIR spectra:

- Absorptions occur at unexpected positions in the NIR spectrum.
- Regions of absorption occur as broad peaks caused by the overlapping of a multitude of different absorptions.

The consequence of these different mechanisms makes an NIR spectrum very complex but often this complexity is hidden as a few rather broad areas of absorption.

Hydrogen bonding is caused by the tendency of hydrogen to form weak bonds with electron donating atoms, especially oxygen and nitrogen. The formation of a weak bond will affect all the vibrations with which that hydrogen and its electron-donating partner are associated. This results in peak shifts (to longer, less energetic wavelengths) which are often observed as peak broadening. The hydrogen bonding structure of water is very complex and depends on temperature, pH, ionic concentration etc., so whenever water is present in a sample there will a complex, dynamic interaction between water and the sample, which may be unique for that sample.

Although NIR spectra are very complex, the fact that the same atoms are involved in many different absorptions means that these absorptions can be utilized, via complex mathematical analyze which belong to the domain of chemometrics, to provide analytical information on specific functional groups. Chemometrics is the application of mathematical or statistical methods to chemical data. The International Chemometrics Society (ICS) offers the following definition: "chemometrics is the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods."

In the fields of foods, medicines, papers, and so forth, intense interest has been directed towards NIRS because of its accuracy and rapidity. Many researchers have also reported the application of NIRS to wood science and technology. For example, NIR measurements of moisture content in wood under unstable temperature conditions. Hoffmeyer and Pedersen (1995) reported the usefulness of this technique as non destructive measurement of the density and strength of Norway spruce. Schimleck *et al.* (1997) applied NIR spectra to the evaluation of the chemical components. Baillères *et al.* (2002) determined on a narrow genetic base, quantitative relations between NIR spectral bands and extractive content, lignin composition, surface longitudinal growth strain and shrinkage relative

to prediction accuracy. The results revealed that NIRS can be used effectively to predict characteristics linked closely with the chemical composition of wood. GIERLINGER *et al.* (2002) reported that NIRS could be an easy approach to facilitate, reliable, accurate and fast method for non-destructive wood extractive determination. BAILLÈRES *et al.* (2003) evaluated the ability of NIRS to assess the resistance of teak wood of different origins to fungi, in comparison with standard test methods. The results prove that NIRS can predict resistance to different species of fungi, which use different schemes of attack.

Thus, NIRS has been generally characterized as a non-destructive quantitative measurement, but there are other options for qualitative analyses based on a mathematical-statistical method, which would be preferable for wood. For example, it is important for timber marketing or warehouse management to discriminate the wood species and their quality correctly. This would allow correlating the species and the quality to a wide range of pertinent characteristics link to forestry management. An effective method for improving these circumstances is therefore required world-wide in regions where unfamiliar wood species are utilized.

Advantages

Unlike most conventional analytical methods, NIRS is non-destructive, requires little or no sample preparation, does not use chemicals, or generate chemical wastes requiring disposal. The technique is rapid, can be portable, and simultaneously determines numerous constituents or parameters. NIRS instrumentation is simple to operate by non-chemists, and operates without fume hoods, drains, or other installations.

Drawbacks

A primary drawback of NIRS is that it is not a stand-alone technology. Its accuracy is dependent on the accuracy of the reference method, although its predictions can be more reproducible than the reference method. Separate calibrations are required for each constituent or parameter. A portion of unknown samples must routinely be analyzed by the reference method to ensure that calibrations remain reliable. It may be necessary to update calibrations several times during the initial phases of use to incorporate “outlying” samples, until the calibrations become highly robust.

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