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Tissue Culture Propagation and Dispatch of Quality Teak Clones

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Abstract. The Plant Biotechnology Laboratory, operating under the Biotechnology and Horticulture Division, Sabah Foundation Group, was set up in the early 1990s as part of the plant improvement program jointly undertaken by ICSB and CIRAD-Foret, a French R & D organization. Special efforts have been devoted to vegetative propagation strategies based on the development of efficient techniques at both the laboratory and nursery levels. The primary species of interest is on teak, the most valuable timber species in the world today and as such, very much sought after. The determining strategy was to develop efficient nursery and in vitro techniques for clonally mass-propagating true-to-type "Plus" trees, the selection of which is constantly upgraded from genetically highly diverse base populations. With the possibility to overcome the constraints of phytosanitary certification, tissue-culture issued clonal materials have been successfully dispatched to various oversea destinations. Data collected to date have shown that our selected Solomon Island teak origins are adaptable to a wide range of environmental conditions, demonstrating their superiority with respect to traits of major economical value over seed-issued plants from local origins. Our selected plant materials are also characterized by DNA markers and wood analyses for enhanced quality and property right control, these being taken into consideration in the early stage of the selection process.

Keywords: Tissue culture propagation; Teak clones; *Tectona grandis* (teak).

INTRODUCTION

Tectona grandis (family Verbenaceae), commonly known as teak, is valued as a prized timber due to its versatility, durability and attractiveness of its wood. Its uses included flooring and parquet, shipbuilding, furniture-making and in building construction. Teak is grown naturally in India, Myanmar, Thailand and Laos. However, owing to increasing market demands, its natural resources are fast depleting. In Thailand, natural teak forest has decreased from 2.3 million hectare in 1954 to about 150,000 hectares in 2000 (Simula *et al*., 2006). The ever increasing need for teak timber has therefore resulted in large scale plantations beyond its native countries in Asia (Indonesia, Malaysia) Africa (Ivory Coast, Congo, Nigeria), and Latin America (Brazil, Costa Rica, Panama and Honduras). It now constitutes an estimated 75% of the world's high-quality tropical hardwood plantations (Nakata and Isoda, 2005).

Teak plantation establishment using seeds is no longer an option due to a number of problems such as low germination rates and variability in growth among individuals (Kaosa-ard *et al*., 1998). Private investors eager to maximize their returns in the shortest delays are now opting for clonal planting materials selected for superior volume yield and enhanced wood quality (Monteuuis and Goh, 1999). The establishment of teak clones further offers an opportunity to enrich the locally available teak genetic resources (Goh *et al*., 2006).

This paper highlights the research and results from the collaboration between the Plant Biotechnology Laboratory, Innoprise Corporation Sdn Bhd (ICSB), a commercial subsidiary of Sabah Foundation Group and CIRAD, a French R & D organization, over a span of more than 15 years. Using both in vitro and nursery techniques for the propagation of selected teak materials of Solomon Island origin, the possibility to produce true-to-type "Plus' trees is demonstrated in numerous teak trials locally and overseas. Analyses on economically-important wood characteristics and the development of DNA markers for clones used in commercialization are also presented.

MATERIALS AND METHODS

Teak materials of Solomon Island origin was first introduced in 1989 in the form of seeds. Information on the accurate origin is however lacking although it was presumably introduced to Solomon Island from Tenasserim (Myanmar) via Papua New Guinea (Goh *et al*., 2006). The seedlings were planted by the ICSB's Plant Improvement and Seed Production (PISP) unit as a demonstrational plot in Lua-

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song Forestry Center, Tawau, Sabah. Nodal explants from eight clones were then collected from this batch of five years old trees for tissue culture initiation. Following their successful introduction, these materials were subsequently multiplied in several cycles under optimal conditions on a suitably developed culture medium (Goh and Monteuuis, 2001) until sufficient numbers of plants are available for trials and overseas dispatch.

For local trials, plantlets of these clones undergo a series of *ex-vitro* acclimatization steps before attaining the field planting stage about four months later. Plants were set out in clonal trials in Brumas and Taliwas research centers. For overseas export, bare-rooted plantlets are packed in lightweight plastic containers which are placed in Styrofoam boxes for dispatch to destination countries within a period of 3 to 7 days to minimize any undue stress on the plantlets. These are then acclimatized under optimal prevailing nursery conditions until the planting stage. Controls in all trials usually consist of clonal materials from other origins and/or from seedlings obtained locally.

Wood quality is based on the five main factors pertaining to mechanical, physical, biological, aesthetic and structural features. These in turn are related to wood characteristics based on the final utilization of the timber as described in the paper by Bailleres and Durand (2000). Sampling methods employed for wood analysis can be either destructive (plank samples) or non-destructive (core sampling), depending upon the availability of the samples of the eight clones from demo and test plots in Luasong and Taliwas (Goh *et al*., 2006). For molecular analyses, young leaves from trees and from tissue culture plantlets of these clones were collected and DNA was extracted from these samples. Molecular markers using simple sequence repeats (SSR) from a teak microsatellite library developed by Cirad scientists (Verhaegen *et al*., 2005) are used in assessing the microsatellite profiles of each of the clones in question.

RESULTS AND DISCUSSION

Using the developed tissue culture technology, we are able to successfully introduce and mass produce any selected Plus trees, irregardless of ortet age. This possibility has considerably shortened the time needed to come up with the number of plants for further uses, and in our case, initially for trials and later in sales to interested parties. Further, due to phytosanitary restrictions, tissue-cultured plantlets are obviously the only means currently available for successfully exporting teak clones to oversea destinations. Proper coordination in the timing of dispatch and arrival has resulted in successful deliveries worldwide. To date, more than half a million plantlets from the PBL have been exported, with the majority of supply going to Australia.

Preliminary observation from 5-year old clonal plots indicated that the Solomon Island clones are thriving under local conditions compared to clones from other sources. In Taliwas, average diameter and height of these trees are 16.8 cm and 13.8 m respectively, whereas in Brumas, average diameter and height are 14.1 cm and 12.7 m respectively. Although both areas have annual precipitation in the range of 2,200-2,500 mm, the difference in growth rates of the same clonal materials indicates that the trees grow better in Taliwas due to deeper and more fertile soil whereas in Brumas, the soil is more sandy and shallower. Despite overall homogenous growth, the different results further confirm the site-selective nature of teak in general. In Brazil, early results of trials comprising of our Solomon Island materials and seed-issued plants obtained locally showed a 30% increase in yield (personal comm.). Similar observation was made in Tanzania and Queensland, Australia, with the Solomon Island clones outperforming clones from other sources in comparative trials (personal comm.). A paper on these results is forthcoming once all data have been compiled and analyzed.

The usefulness of either or both destructive and non-destructive methods to analyze wood quality of selected genotypes is obvious as demonstrated in our investigation. Genotypes represented by only one tree that must be kept alive for further uses, such as for seed or clone production, can be preserved using core sampling. Conversely, wood characteristics from plank samples analysis of 10-year old clones, though derived based on Sabah conditions, are useful for determining overall timber quality, particularly where the homogeneity of wood of the same clone on different sites is concerned.

Microsatellite patterns of the commercial clones are obtained using DNA isolated from the leaf samples collected. Through clonal identification by DNA fingerprinting, it is now possible to determine the genetic fidelity of the mass-propagated clones. This is highly applicable as one of the quality control steps in our commercial activities. Further, this technology is useful for ascertaining the genetic diversity of our teak germplasm collection for a sound tree improvement program as well as for property rights control of our clones. Results on wood and DNA analyses for each of the eight commercial clones are now available in the form of leaflet.

CONCLUSIONS

Collaborative research undertaken by the Sabah Foundation Group and Cirad for over fifteen years has resulted in the development of efficient nursery and tissue culture propagation protocols, as well as wood property determinations and DNA fingerprinting application, which in turn have led to the availability of high 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. With the continuous strong interest for superior teak

planting materials around the world, 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. Quality control of our planting materials in every step of the selective and propagation process therefore remains a key area in our endeavors to stay ahead of competitors.

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