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Status of the "YSG Biotech" program of building teak genetic resources in Sabah

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Photo 1. Converting the "Provenance-progeny" trial of Taliwas into a seed orchard. Photo V. Naudet. BOIS ET FORÊTS DES TROPIQUES, 2009, N° 301 (3) FOCUS / GENETIC IMPROVEMENT

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RÉSUMÉ

INVENTAIRE DES RESSOURCES GÉNÉTIQUES DE TECK DE LA FILIALE « YSG BIOTECH » AU SABAH

Depuis le début des années 1990, le Plant Biotechnology Laboratory, projet de collaboration en recherche et développement entre la Division Forestière du Yayasan Sabah Group et le Centre de coopération internationale en recherche agronomique pour le développement (Cirad), a œuvré à regrouper au Sabah, en Malaisie orientale, des origines génétiques de teck (Tectona grandis) aussi diverses que possible. Outre son intérêt scientifique, le but de cette démarche était d'établir au champ des populations de base suffisamment riches génétiquement pour l'amélioration de l'espèce et la sélection de clones en vue de plantations industrielles ou de leur diffusion à des fins commerciales. Durant cette même période, Plant Biotechnology Laboratory est devenu une filiale commerciale à part entière du Yayasan Sabah Group, sous l'appellation de « YSG Biotech Sdn Bhd ». Un inventaire des ressources génétiques de teck rassemblées dans ce cadre à l'issue de deux décennies est présenté dans cet article. Les têtes de clone originelles ainsi que les parcelles de démonstrations mises en place pour examiner le comportement et la conformité au champ des plants issus de bouturage, de microbouturage et de culture de méristèmes in vitro sont décrites. Des semis d'origines génétiques très diverses ont également servi à établir des essais de provenancesdescendances, convertis par la suite en peuplements semenciers et en vergers à graines. L'article étaye l'intérêt d'utiliser, dans le cadre du projet, la culture *in vitro* et des biotechnologies connexes pour gérer, micropropager à l'échelle industrielle, et expédier au niveau international, la ressource génétique. L'ensemble de ces atouts devrait faciliter le développement, dans différentes régions du monde, de plantations de teck de qualité hautement productives et ainsi permettre de faire face aux difficultés croissantes d'approvisionnement en germplasme à partir de pays appliquant une réglementation stricte de protection de leurs ressources génétiques.

Mots-clés : *Tectona grandis*, amélioration, caractéristiques du bois, clones, *in vitro*, marqueurs ADN, peuplement semencier, ressources génétiques, sélection, vergers à graines.

ABSTRACT

STATUS OF THE "YSG BIOTECH" PROGRAM OF BUILDING TEAK GENETIC RESOURCES IN SABAH

Since the early 1990's, the Plant Biotechnology Laboratory, a collaborative project between the Forestry Division of the Yayasan Sabah Group (YSG) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), has been gathering as many different teak (Tectona grandis) genetic resources as possible in Sabah, East Malaysia. Beside genetic assessments, the objective was to establish diverse base populations that can be used for genetic improvement of the species including identification and commercial distribution of superior clones for establishment of plantations. Within that same period, the Plant Biotechnology Laboratory evolved from a full Research and Development unit into a commercial subsidiary of Yayasan Sabah Group, and is now known as YSG Biotech Sdn Bhd. A review of the status of the teak genetic resources gathered since the beginning of this project, two decades ago, is detailed in this paper. The teak "plus trees" from which the first clonal rooted cuttings and in vitro plants originated, as well as the demonstration plots established for assessing the field conformity of the resulting offspring propagated by different nursery and in vitro vegetative propagation methods are described. Seedlings from highly diverse genetic origins were also used for setting up provenance-progeny trials which were subsequently converted into seed stands and seed orchards. The usefulness of the tissue culture facilities and related biotechnologies available within the project for efficiently managing and mass propagating these field resources, or for international dispatch are presented. All these assets are appealing for establishing high yielding and quality teak plantations locally and in various parts of the world, especially with the increasing difficulty to access teak germplasm from many countries applying strict genetic resource conservation policies.

Keywords: *Tectona grandis*, clones, DNA markers, genetic resources, improvement, *in vitro*, seed orchard, seed stand, selection, wood characteristics.

RESUMEN

INVENTARIO DE LOS RECURSOS GENÉTICOS DE TECA DE LA FILIAL "YSG BIOTECH" EN SABAH

Desde principios de los 90, el "Plant Biotechnology Laboratory", proyecto de colaboración de investigación y desarrollo entre la División forestal del Yayasan Sabah Group y (YSG) y el Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), ha trabajado para agrupar en Sabah, Malasia oriental, tantos orígenes genéticos de teca (Tectona grandis) como fuera posible. Además de su interés científico, el objetivo de esta iniciativa consistía en establecer poblaciones de base suficientemente ricas genéticamente para poder aprovecharlas en la mejora de esta especie y la selección de clones para su uso en plantaciones industriales o para su difusión comercial. Entre tanto Biotechnology Laboratory ha ido evolucionando para convertirse en una filial al 100% de Yayasan Sabah Group, con la denominación de "YSG Biotech Sdn Bhd". Se presenta un inventario de los recursos genéticos de la teca reunidos en el ámbito de este proyecto en sus dos décadas de existencia. Se describen los árboles élite que sirvieron para obtener los primeros esquejes y plantas in vitro de teca clonados así como las parcelas de demostración establecidas para examinar el comportamiento y conformidad de estas plantas procedentes de distintos métodos de propagación vegetativa o in vitro. Se usaron también plantones de orígenes genéticos muy diversos para establecer ensayos de procedencia-progenie, que posteriormente fueron convertidos en rodales y huertos semilleros. Se expone el interés de haber utilizado, durante el provecto, el cultivo in vitro y las biotecnologías relacionadas para manejar, micropropagar a escala industrial, e incluso enviar a nivel internacional el conjunto de estos recursos genéticos. Estas ventajas deberán facilitar el desarrollo de plantaciones de teca de alta calidad y productividad a nivel local y en distintas partes del mundo y así solucionar la creciente dificultad de suministro de germoplasma procedente de países que aplican una estricta política de protección de sus propios recursos genéticos.

Palabras claves: Tectona grandis, mejora genética, características de la madera, clones, *in vitro*, marcadores ADN, rodal semillero, recursos genéticos, selección, huerto semillero.

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Introduction

Since the early 1970's, the increasing worldwide demand for teak (Tectona grandis) wood on the one hand and the alarming diminution of the resources available from natural forests of the species on the other hand have resulted in an intensification of teak planting. The aims of most plantings are to produce within the shortest delays as much as possible teak wood of the highest value and at the lowest cost (BALL et al., 2000: Кеодн 2000, 2001). Opting for the most suitable sources of teak planting stock remains a crucial requisite to reach this goal, bearing in mind the prominent effects of genetic origin X site interactions on teak timber yield and guality (BALL et al., 2000; KEOGH, 2001). Opting for the most suitable sources of teak planting stock remains a crucial requisite to reach this goal, bearing in mind the prominent effects of genetic origin X site interactions on teak timber yield and quality (KAOSA-ARD, 1999, 2000; KEOGH, 2001).

Being aware of this situation, the Plant Biotechnology Laboratory, a collaborative project between the Forestry Division of the Yayasan Sabah Group and CIRAD¹, has been gathering as many different teak (Tectona grandis) genetic resources as possible in Sabah, East Malaysia. Beside genetic assessments, the objective was to establish diverse base populations that can be used for genetic improvement of the species including identification and commercial distribution of superior clones for establishment of plantations. Within that same period, the Plant Biotechnology Laboratory evolved from a full R & D unit into a commercial subsidiary of the Yayasan Sabah Group, and is now known as YSG Biotech Sdn Bhd.

Two decades since the inception of the project, the status of the teak genetic resources that have been gathered are described and beneficial results arising from the efforts made to date are highlighted.

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Project background

Teak resource development in Yavasan Sabah Group first started in 1991 in LFC² (figure 1), under the PISP³ collaborative program between ICSB⁴ and CTFT⁵, which later became the Forestry Department of CIRAD.

The occurrence of a senescent teak tree (ortet) adjacent to LFC nurserv prompted us to test the efficiency of vegetative propagation methods that had been previously developed and applied for the propagation of true-to- type mature selected Plus trees of different broadleaved as well as coniferous species under temperate and tropical environments (FRANCLET, 1981; MONTEUUIS, 1989, 1993). Adapting this cloning technology to this old teak tree unexpectedly yielded successful results (MONTEUUIS, 1995; MONTEUUIS et al., 1995).). In 1992, the first rooted cuttings issued from this tree and referred to as clone 9 were field planted to assess their phenotypic conformity and soil stability. The observations were quite positive and

thereafter mass production of clone 9 was pursued. Subsequent rooted cuttings of clone 9 were used to establish the first monoclonal block in 1995 on a slope in compartment 311 (C311), 13 kilometers north of LFC (photo 2). To our knowledge, this is the oldest monoclonal block ever established from rooted cuttings of an old teak tree. As expected, all clone 9 trees of this block have been displaying the same phenotypic features and flowering synchronicity. Owing to the availability of tissue culture-issued plants, this clone was also used as buffer trees in other field experiments. The old ortet had since died in 1996, but its genotype has been preserved as rooted cuttings, and the clone is still propagated in nursery and tissue culture conditions, thanks to the suitable cloning techniques developed.

The cloning procedure was applied to several mature selected teak trees chosen within our local stands, starting in LFC, that were derived from seeds brought back from the Solomon Islands (photo 3). The first rooted cuttings, obtained from these Solomon Islands teaks



Figure 1.

Annual rainfall distribution map of Sabah, with LFC, Taliwas and Kota Marudu teak stand locations, as well as Kota Kinabalu, where YGS Biotech main office and biotechnology laboratory are situated.



Photo 2.

The oldest monoclonal block (clone 9 or MBo) from rooted cuttings of an old teak ortet in LFC, 7 years after establishment in 1995. Photo O. Monteuuis.



Photo 3. The few remaining ortets from Solomon Islands seed after seventeen years in LFC demoplot, from which the commercial clones TG1 to TG8 originated. Photo V. Naudet.

were planted in 1994 in the LFC demonstration plot (demoplot) (photo 4). As far as we are aware, together with clone 9 rooted cuttings, these are the oldest rooted cuttings produced from mature teak trees in the world. These materials gave rise to the commercial clones TG1 to TG8.

The selection and cloning procedures were then extended to every teak Plus tree chosen in different locations of Malaysia, such as the 70 yr-old Kota Marudu stand to the north of Sabah, and a 25-35 yr-old teak trial in Perlis, Peninsular Malaysia. The 'stick' method was used to "mobilize" vegetative material from these ortets in LFC nursery facilities for clonal propagation by rooted cuttings (MONTEUUIS, 1993; MONTEUUIS *et al.*, 1995).

The establishment in late 1992 of a tissue culture laboratory, the plant biotech laboratory (PBL) in Tawau within the same collaborative project allowed us to develop a more efficient mass-clonal micropropagation method that complemented the rooted cuttings technology (BON, MONTEUUIS, 1996; MONTEUUIS et al., 1998; Monteuuis, 2000; Goh, Mon-TEUUIS, 2001). The first tissue-cultured teak plants obtained from in vitro germinated seeds acquired from Mata Aver, Perlis, Peninsular Malaysia, were planted together as a mixture of genotypes or "bulk" in a block in the LFC demoplot in 1995. again, primarily for conformity and soil stability assessment.

From 1992 to 2004, the laboratory produced one to two million tissue-cultured teak clonal plantlets, mainly from clones TG1 to TG8 (Solomon Islands origin) and clone 9 (initially called "MBo", origin unknown) for local plantings. Following the first international dispatch (to Australia) in 2001 the laboratory began to focus even more strongly on the propagation of teak on a commercial scale (GOH, MONTEUUIS, 2001; GOH *et al.*, 2005; GOH *et al.*, 2007).



Luasong Forestry Center resources

In year 2003, in line with the achievements obtained from the works on teak as well as on other forestry and horticultural species, giving rise to the positive financial standing of the project, the Plant Biotechnology Laboratory was converted into a new entity known as the Biotechnology and Horticulture Division. Two years later, the business of tissue culture propagation was fully operational under the commercial subsidiary known as YSG Biotech Sdn Bhd. All the teak activities have been subsequently regrouped under the purview of the Company, and sited in a much bigger tissue culture facility in Kota Kinabalu in May 2005 (Gон et al., 2007).

Luasong Forestry Center (LFC) is located 120 km west of Tawau, Sabah, East Malaysia (lat 4°35'N, long117°40'E). Monthly temperatures are 26-28°C and annual rainfall averages 2500 mm without a distinct dry season (figure 1).

LFC teak genetic resources are located exclusively in two sites, the demo-plot and Compartment 311.

The demo plot

Located on a flat piece of land adjacent to the Luasong river, the demo plot land is 1 to 2 ha in area and prone to flooding. It encompasses the following teak genetic resources: • Seedling stands (from Solomon Islands seed) planted in 1989 and 1990 (photo 3). As mentioned, screening for superior trees gave selects named TG1 to TG8 from which commercial clones have been massproduced by rooted cuttings and micropropagation. Some of these ortets have attained heights of more than 30m and girths of around 150 cm at 14 years after planting.

• Rooted cuttings of TG1 to TG8 planted as mixed clones without retained identities ("bulk") in 1994. These trees exhibit striking phenotypic features, as expected from cloned plant materials, with the largest individuals having attained heights of around 26 m and girths of 120 cm at age 10 years (photos 4 and 5).







Trees from rooted cuttings from mature teak individuals (left) and from microcuttings from *in vitro* seedlings shown at 15 years and 14 years respectively after planting in LFC demoplot. Photo O. Monteuuis.

• Microcuttings derived from *in vitro* germinated seeds supplied from Mata-Ayer, Perlis, Peninsular Malaysia, also planted as "bulk" in a block in 1995. Despite some flood damages, some of these trees reached 29.5m in height and 110cm in girth at 9 yr-old (photos 5).

The distinctive feature of this demoplot is its inclusion of the oldest rooted cuttings and microcuttings of teak produced so far in Sabah. These trees have been very useful for assessing phenotypic variation of various traits, as well as root conformation, and for confirming that teak rooted cuttings and microcuttings are not prone to strong within clone variations or "C effects" (FRAMPTON, Foster, 1993; White et al., 2007), unlike many tree species propagated by cuttings. In all likelihood, this is highly possible if a suitable cloning technique such as the one developed in this project, is applied (GOH, MONTEUUIS, 2005).

The C311 seed orchard

C311, situated 13 km north of LFC, comprises several experimental plots of teak from rooted cuttings or microcuttings of clones TG1 to TG8 (Solomon Islands), of clone 9 (including the monoclonal block established in 1995 mentioned previously) and the seedling seed orchard. The seed orchard is derived from a "provenance/progeny" trial although according to ZOBEL and TALBERT (1984). seed-source would have been a more appropriate terminology than provenance. This trial was established towards the end of 1997 on a site, at 130-170 m above sea level, with a hilly slope of about 15° gradient, and mudstone-minor sandstone soils with pH ranging between 4.8 - 5.6. In all, 42 diverse seedlots mainly of Indian origin, are represented (Table I), including 15 bulks and 27 open-pollinated families from individual clones established in a seed orchard in Ivory Coast as described by DUPUY and VERHAEGEN (1993). The design consisted of contiguous randomized complete blocks with 3 replications (taking into account the variation of topography), each elementary plot comprising 3 rows of 5 trees, and each row being separated from the other by a buffer row that was also used to fill the ravines. The trees were planted at 4 m x 2 m and covered a total area of $45 \times 42 \times 2 \times 4 \times 2 = 30,240 \text{ m}^2$.

The buffers consisted of teak cuttings and microcuttings of the clone 9 (same as that in the monoclonal block MBo mentioned previously), and that of TG1 to TG8 Solomon Islands commercial clones. All the buffer trees were felled after 2 years, halving the overall density from 1250 trees/ha to 625/ha. Ten years after establishment, and from annual individual data and observations, the between-tree competition was becoming noticeably stronger. Thereafter, it was decided to convert this trial into a seed orchard by selective thinning (photo 6).

Table I.

Characteristics of the 42 teak seedlots and the associated number of trees remaining after thinning in the C111 seed orchard.

Seedlot N°	Origin	Composition of the seed acquired	Longitude	Latitude	Elevation (m above sea level)	Average Annual rainfall (mm)	Average annual temperature (°C)	No of trees left
1111	Ss ¹ Malaysia, Mata Ayer, Perlis	na ²	100°16'E	6°39'N	50-100	2 000-2 500	27	6
2222	Ss Malaysia Segama River, Sabah	na	118°18'E	5°6'N	300	2 500	27	6
4314	Ss Solomon Islands, Arara	na	156°30'E	6°40'S	80	3 000	27	6
5212	Ss Solomon Islands Viru	na	157°46 ' E	8°28'S	50-100	3 000	27	6
8367*	Prov India Chandrapur Maharastra	na	78°46'E	19°30'N 20°45'N	na	1 420	na	6
8668*	Prov ³ Thailand Mae Huat Lampang (natural stand)	na	99°54'E	18°39'N	350	900	27	6
8669	Prov Thailand Mae Huat Lampang (planted stand)	na	99°54'E	18°39'N	350	900	na	4
8822	Prov India Sakrebail Kamataka	Mixture from 100 OP ⁴ families	75°29'E	13°48'N	600	898	24	6
8823*	Prov. India Sakrebail Kamataka	Mixture from 100 OP families	75°29'E	13°48'N	600	1 000	24	6
8824*	Prov India Vimoli Vir. Kamataka	Mixture from 100 OP families	74°37'E	15°11'N	600	1 500	26	6
8831*	Prov India Karadibetta Kamataka	Mixture from 100 OP families	75°02'E	14°05'N	650	912	24	6
8832*	Prov India Gialegundi Kamataka	Mixture from 100 OP families	75°17'E	14°05'N	700	1 000	24	6
8833*	Prov India Vimoli Vir. Kamataka	Mixture from 100 OP families	74°37'E	15°11'N	600	1 500	26	6
8839	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	6
8844*	Prov India Maukal Kamataka	Mixture from 100 OP families	74°37'E	15°09'N	600	1 500	26	6
9411*	CSO ⁵ Ivory Coast Prov India Nilambur	A single OP family	5°03'W 76°21'E	6°06'N 11°21'N	200 49	1 470 2 565	26 na	6
9412*	CSO Ivory Coast Ss Tanzania Kihuhwi	A single OP family	5°03'W 38°39'E	6°16'N 5°12'S	200 260	1 470 1 880	26 na	6
9415	CSO Ivory Coast Ss Senegal Djbelor	A single OP family	5°03'W 12°35'N	6°16'N 16°6'W	200 10	1 470 1 640	26 na	6
9418*	CSO Ivory Coast Pro India Nilambur	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 2 900	26 na	6
9420	CSO Ivory Coast Prov India Nilambur	A single OP family	5°03 ' W na	6°16'N na	200 na	1470 na	26 na	5
9424	CSO Ivory Coast Ss Tanzania Mtibwa (Morogoro)	A single OP family	5°03'W 37°39'E	6°16'N 6°00'S	200 460	1 470 1 160	26 na	5

¹ Ss: seed source, in accordance with ZOBEL and TALBERT (1984). ² na: information not available. ³ Prov: provenance, in accordance with ZOBEL and TALBERT (1984). ⁴ OP: open pollinated. ⁵ CSO: clonal seed orchard. * Origins common to C111 and Taliwas seed orchards.

Table I (continued).

Seedlot N°	Origin	Composition of the seed acquired	Longitude	Latitude	Elevation (m above sea level)	Average Annual rainfall (mm)	Average annual temperature (°C)	No of trees left
9426*	CSO Ivory Coast Ss Tanzania Mtibwa (Morogoro)	A single OP family	5°03 ' W 37°39'E	6°16'N 6°00'S	200 460	1 470 1 160	26 na	6
9430*	CSO Ivory Coast Prov Thailand Mae Huat	A single OP family	5°03'W 99°00'E	6°16'N 18°06'N	200 350	1 470 1 300	26 na	6
9433	CSO Ivory Coast Ss Tanzania Kihuhwi	A single OP family	5°03'W 38°39'E	6°16'N 5°12'S	200 280	1 470 1 860	26 na	4
9435*	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 na	26 na	6
9436	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 na	26 na	6
9437*	CSO Ivory Coast Prov India Nilambur	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 na	26 na	6
9440*	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 na	26 na	6
9443*	CSO Ivory Coast Prov India Vernoli Range	A single OP family	5°03 ' W 74°35'E	6°16'N 15°10'N	200 573	1 470 2 032	26 na	6
9444	CSO Ivory Coast Prov Thailand Mae Huat	A single OP family	5°03'W 99°00'E	6°16'N 18°06'N	200 350	1 470 1 300	26 na	6
9446*	CSO Ivory Coast Prov India Vernoli Range	A single OP family	5°03 ' W 74°35 ' E	6°16'N 15°10'N	200 573	1 470 2 032	26 na	6
9447	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 na	26 na	6
9449	CSO Ivory Coast Prov Thailand Pong Salee	A single OP family	5°03'W 101°01'E	6°16'N 19°08'N	200 350	1 470 1 500	26 na	6
9450*	CSO Ivory Coast Prov India Vernoli Range	A single OP family	5°03'W 74°35'E	6°16'N 15°10'N	200 573	1 470 2 032	26 na	6
9451	CSO Ivory Coast Ss Tanzania Bigwa	A single OP family	5°03'W 38°39'E	6°16'N 6°50'S	200 580	1 470 900	26 na	6
9452*	CSO Ivory Coast Prov India Masale Valley	A single OP family	5°03 ' W 76°10'E	6°16'N 11°55'N	200 820	1 470 1 270	26 na	6
9454*	CSO Ivory Coast Prov Laos Paklay	A single OP family	5°03 ' W 106°00'E	6°16'N 15°00'N	200 120	1 470 200	26 na	5
9456	CSO Ivory Coast Prov.India Purunakote	A single OP family	5°03 ' W 84°00'E	6°16'N 20°00'N	200 133	1 470 1 200-1 500	26 na	6
9457*	CSO Ivory Coast Prov India Purunakote	A single OP family	5°03 ' W 84°00'E	6°16'N 20°00'N	200 133	1 470 1 200-1 500	26 na	6
9459*	CSO Ivory Coast Prov India Masale Valley	A single OP family	5°03 ' W 76°10'E	6°16'N 11°55'N	200 820	1 470 1 270	26 na	6
9463*	CSO Ivory Coast Ss source Ivory Coast Bamoro	A single OP family	5°03 ' W 5°07 ' W	6°16'N 7°48'N	200 330	1 470 1 100	26 26	6
9999*	Ss Papua New Guinea (PNG) Ex Brown River	na	147°14'E	9°20'S	400	2 100	26	6

* Origins common to C111 and Taliwas seed orchards.

Thinning was done based on the selection criteria traditionally used for teak, *i.e.* clear bole diameter and length, bole shape (straightness, circularity, taper, with minimal knots, buttresses or flutes at the bottom of the tree), and, frequency and size of lateral branches that are responsible for nodes affecting wood quality especially for veneer end-use.

The following factors were also taken into consideration:

the initial density of 625 trees/ha;
the number of trees of the same genetic origin – half sib for some of them (see table I) that must remain out of the 15 (3 x 5) trees per elementary plot, in order to avoid possible risks of inbreeding depression;
the respective location of these trees, avoiding those that are planted too close from the others, which may hamper good pollen exchange while limiting crown development, and hence, the number of seeds produced;

• the benefits of maintaining an overall high genetic diversity within the stand, keeping at least one or two best representatives for the worst origins.

At the end of the thinning exercise, a total of 249 trees were left, corresponding to a selection ratio of 1 in 7.6, with numbers per origin ranging between 4 and 6, resulting in a stocking of 82.3 trees/ha (table I for detailed information by seed source). Overall, the trees retained, some reaching 31 m in height and 34.5 cm in DBHOB at less than 9 years after planting, are evenly distributed. Hence, the need to consider infilling using genotypes of different genetic origins as practiced for the Perlis seed production area and the Taliwas seed orchard described hereinafter is not as strong.



Photo 6. Converting the "Provenance-progeny" trial of LFC C311 into a seed orchard. Photo O. Monteuuis.

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Photos 7.

Tests of teak clones produced from rooted cuttings of mature selected ortets in Taliwas. Photo O. Monteuuis.

Taliwas genetic resources

The place called Taliwas is located inland at 18 km on the main road from Silam to Danum Valley. Sabah, East Malaysia (latitude 4°58' N, longitude 118°13' E). The climatic conditions are similar to LFC (figure 1). As in C311, several teak stands have been planted in Taliwas, mainly from cuttings and microcuttings derived from the Solomon Island selected commercial clones, TG1 to TG8, and the other Sabah clone 9 (origin unknown). Various clonal trials and demonstration plots were also established, as well as the two seedlingderived stands detailed below.

Clonal trials and demonstration plots

The purpose of these clonal trials and demonstration plots (photos 7) that were established in 1997 and 2000 was to assess between and within clone variation in field performances, phenotypic features and possibly wood properties (GOH *et al.*, 2007). Certain clones were produced by different vegetative propagation methods i.e rooted cuttings, microcuttings from nodal explants or from meristem culture (photo 8), distinguishing the lines according to the single meristem from which they derived from (MONTEUUIS et al., 1998: MONTEUUIS, 2000). This information was also taken into consideration in the assessments. A total of 25 clones, all obtained from mature selected Plus trees have been compared. These include the 8 Solomon Island clones TG1 to TG8, clone 9, five clones derived from Indian origin Plus trees planted between 1979 and 1982 in Mata Aver, Perlis, Peninsular Malaysia, 3 clones from the Kota Marudu, Sabah stand mentioned above, and others of more uncertain origins.

The Perlis seed production area

From seeds supplied from the Mata Ayer plantation of the Forest Research Institute of Malaysia (FRIM), in the state of Perlis, Peninsular Malaysia,1028 teak seedlings were produced and planted in a mixture with cocoa plants on a flat piece of land of about 4 ha on the YSG concession known as "km 13", Taliwas, in 1993. These seeds, presumably originating from Indian provenances, were received as a bulk. Unfortunately, it was not possible to obtain more accurate information from FRIM on the genetic pedigree of these seeds although various teak seed sources were known to be represented in Mata Aver (India, Vietnam, Thailand, Papua New Guinea, Trinidad, Myanmar, Indonesia). Selective thinning of this plot, based mainly on growth and form was carried out in 2008, leaving only the best 245 phenotypes, unevenly distributed over the site, corresponding to approximately 61 trees/ha (photo 9). Abundant natural regeneration has been observed, attesting that the trees can produce numerous and fertile seeds under the local conditions.

Infilling the empty spaces of this plot with teak trees of different origins such as those from the tissue culture gene pool is currently underway. The intention of using such infills is to increase seed yield of the stand while enhancing the genetic diversity and quality of the seeds produced and, at the same time, to facilitate the maintenance of this area.

The teak trees in the vicinity are from a very different genetic background, consisting mostly of the commercial clones TG1 to TG8, which display the superior phenotypic features generally observed for these clones. This is also the case in the Taliwas seed orchard described below.

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The Taliwas seed orchard

The Taliwas "provenance-progenv trial" was established in 1997 on flat land about 40m above sea level. This area was prone to short periods of flooding, which necessitated the creation of ditches within and in the periphery of the trial. The greyish soil was more fertile and less acidic (6.0< pH < 6.3) than that of C311. The trial consisted of seedlings from 41 different seedlots, including 10 bulks and 31 open pollinated families from individual clones in the lvory Coast seed orchard mentioned above (table II). It was established according to the same randomized complete block design as in C311. These seed orchards have 26 seedlots in common (tables I and II). The total area covered was 45 x 41 x 2 x 4 x2 = 29,520 m². The initial density was halved to 625 trees/ha two years after planting by removing all the buffer trees followed by a selective thinning in November 2006 (photo 10). The

selection was done according to the same rules as detailed for C311, although less conservatively. The lower maximal height of 22.3 m and DBH of 31.4 cm at age 8.5 years presumably reflects site differences between C311 and the Taliwas orchard. Only 200 selected trees out of the 1 845 initially planted, fillers excluded, were retained corresponding to a selection ratio of approximately 1 in 9, with representatives per seed lot varying from 1 to 10 (table II for more detailed information). This resulted in an average number of 4.9 trees kept by origin and in an average density of 66.6 trees/ha. Due to the uneven distribution of the remaining trees, this intensive selective thinning resulted in a few large empty places, which are now to be infilled with trees of different and presumably good genetic background. In the event that these infills do not perform according to expectation, they will be rogued before flowering.



Photo 8.

A 9 yr-old teak tree produced by meristem culture from a mature selected ortet in Taliwas demoplot; no pruning was undertaken. Photo O. Monteuuis.



Photo 9. The recently thinned 16 yr-old Perlis seed production area in Taliwas. Photo O. Monteuuis.

Table II.

Characteristics of the 41 teak seedlots and the associated number of trees remaining after thinning in Taliwas seed orchard.

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Seedlot N°	Origin	Composition of the seed acquired	Longitude	Latitude	Elevation (m above sea level)	Average Annual rainfall (mm)	Average annual temperature (°C)	No of trees left
8367*	Prov ¹ India Chandrapur Maharastra	na ²	78°46'E	19°30'N 20°45'N	na	1 420	na	1
8668*	Prov Thailand Mae Huat Lampang (natural stand)	na	99°54'E	18°39'N	350	900	27	3
8823*	Prov India Sakrebail Kamataka	Mixture from 100 OP ³ families	75°29'E	13°48'N	600	1 000	24	3
8824*	Prov India Vimoli Vir. Kamataka	Mixture from 100 OP families	74°37'E	15°11'N	600	1 500	26	3
8831*	Prov India Karadibetta Kamataka	Mixture from 100 OP families	75°02'E	14°05'N	650	912	24	4
8832*	Prov India Gialegundi Kamataka	Mixture from 100 OP families	75°17'E	14°05'N	700	1 000	24	4
8833*	Prov India Vimoli Vir. Kamataka	Mixture from 100 OP families	74°37'E	15°11'N	600	1 500	26	3
8835	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	3
8836	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	3
8838	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	4
8839	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	1
8841	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	2
8842	Prov India Maukal Kamataka	A single OP family	76°00'E	12°15'N	850	1 532	22	3
8844*	Prov India Maukal Kamataka	Mixture from 100 OP families	74°37'E	15°09'N	600	1 500	26	4
9411*	CSO ⁴ Ivory Coast Prov India Nilambur	Mixture from 100 OP families	5°03'W 76°21'E	6°06'N 11°21'N	200 49	1 470 2 565	26 na	5
9412*	CSO Ivory Coast Ss ⁵ Tanzania Kihuhwi	A single OP family	5°03'W 38°39'E	6°16'N 5°12'S	200 260	1 470 1 880	26 na	6
9416	CSO Ivory Coast Ss Ivory Coast Kokondekro	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	4
9417	CSO Ivory Coast Prov India Nilambur	A single OP family	5°03'W 76°21'E	6°06'N 11°21'N	200 49	1 470 2 900	26 na	6
9418*	CSO Ivory Coast Prov India Nilambur	A single OP family	5°03'W 76°21'E	6°16'N 11°21'N	200 49	1 470 2 900	26 na	8
9426*	CSO Ivory Coast Ss Tanzania Mtibwa (Morogoro)	A single OP family	5°03'W 37°39'E	6°16'N 6°00'S	200 460	1 470 1 160	26 na	7
9429	CSO Ivory Coast Prov. India Nellicutha	A single OP family	5°03'W na	6°16'N na	200	1 470	26	5
9430*	CSO Ivory Coast Prov Thailand Mae Huat	A single OP family	5°03'W 99°00'E	6°16'N 18°06'N	200 350	1 470 1 300	26 na	10
9431	CSO Ivory Coast Ss Tanzania Kihuhwi	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	7

¹ Prov: provenance, in accordance with ZOBEL and TALBERT (1984). ² na: information not available. ³ OP: open pollinated. ⁴ CSO: clonal seed orchard. ⁵ Ss: seed source, in accordance with ZOBEL and and TALBERT (1984).

* Origins common to C111 and Taliwas seed orchards.

Table II (continued).

Seedlot N°	Origin	Composition of the seed acquired	Longitude	Latitude	Elevation (m above sea level)	Average Annual rainfall (mm)	Average annual temperature (°C)	No of trees left
9432	CSO Ivory Coast Prov Thailand Pong Salee	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	5
9434	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	9
9435*	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	5
9437*	CSO Ivory Coast Prov India Nilambur	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	6
9439	CSO Ivory Coast Prov Thailand Huoi-Nam- Oon	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	3
9440*	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	7
9442	CSO Ivory Coast Prov India Nilambur	A single OP family	5°03'W na	6°16'N na	200 na	1 470 na	26 na	7
9443*	CSO Ivory Coast Prov India Vernoli Range	A single OP family	5°03'W 74°35'E	6°16'N 15°10'N	200 573	1 470 2 032	26 na	8
9445	CSO Ivory Coast Prov India Nellicutha	A single OP family	5°03 ' W na	6°16'N na	200 na	1 470 na	26 na	6
9446*	CSO Ivory Coast Prov India Vernoli Range	A single OP family	5°03'W 74°35'E	6°16'N 15°10'N	200 573	1 470 2 032	26 na	8
9450*	CSO Ivory Coast Prov India Vernoli Range	A single OP family	5°03'W 74°35'E	6°16'N 15°10'N	200 573	1 470 2 032	26 na	4
9452*	CSO Ivory Coast Prov India Masale Valley	A single OP family	5°03'W 76°10'E	6°16'N 11°55'N	200 820	1 470 1 270	26 na	2
9454*	CSO Ivory Coast Prov Laos Paklay	A single OP family	5°03'W 106°00'E	6°16'N 15°00'N	200 120	1 470 200	26 na	6
9457*	CSO Ivory Coast Prov India Purunakote	A single OP family	5°03'W 84°00'E	6°16'N 20°00'N	200 133	1 470 1 200-1 500	26 na	3
9458	CSO Ivory Coast Prov Thailand Ban Pha Lay	A single OP family	5°03'W 99°59'E	6°16'N 18°13'N	200 200	1 470 1 100	26 na	7
9459*	CSO Ivory Coast Prov India Masale Valley	A single OP family	5°03'W 76°10'E	6°16'N 11°55'N	200 820	1 470 1 270	26 na	4
9463*	CSO Ivory Coast Ss Ivory Coast Bamoro	A single OP family	5°03 ' W 5°07 ' W	6°16'N 7°48'N	200 330	1 470 1 100	26 26	6
9999*	Ss Papua New Guinea (PNG) Ex Brown River	na	na	na	na	2 100	26	5
	* Origins common to C11	1 and Taliwas seed	orchards.					



Photo 10. The Taliwas seed orchard two years after the selective thinning in 2006 of the initial "Provenance-progeny" trial. Photo D. K. S. Goh.



Photo 11.

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YSG Biotech laboratory in Kota Kinabalu: a valuable asset for *in vitro* conservation of teak genotypes and rapid mass production of superior clones for efficient transfer and safe entry to foreign countries. Photo D. K. S. Goh.

In vitro resources

In addition to the genetic resources in the field as described above, YSG Biotech has been introducing and maintaining under *in vitro* conditions several teak clones obtained from different sources: new mature selected Plus trees and also germinants from very restricted numbers of seeds of presumably high genetic value from various countries (photo 11). This has been possible thanks to the appropriate *in vitro* technologies developed, with special mention of meristem culture that allows the possibility to clone any teak Plus trees regardless of age, from a low number of available buds, while preserving its genotypic and phenotypic conformity (MON-TEUUIS *et al.*, 1998). To date, this *in* vitro clone bank has amounted to some 50 genotypes, some of which are being field tested or used for infilling some of the empty spaces left by the intensive thinning. Further enriching this gene pool has been and remains a major aim as part of the Company's endeavors to stay ahead of potential competitors.

Table III. Summary of the teak genetic resources in YSG Biotech, Sabah.

Year of plantation and type of stock	Luasong Forestry Center	Taliwas Remarks
1989, 1990, Seedlings (S)	Solomon Is seed source, bulk; gave some <i>Tectona grandis</i> "TG" selects.	Presumably from Myanmar, ex Burma, provenance. Thinned.
1993, Rooted cuttings (RCs)	Bulked RCs from the Solomon Islands TG selects planted for observations in PISP demoplot .	Bulked seedlings ex Perlis, Mata Ayer, Peninsular Malaysia seed source of unknown origin.
1995, Tissue-cultured-(TC)	Trees from micro cuttings ex Perlis seedlings germinated in vitro for field observation.	Thinned for the last time in 2006.
RCs		First monoclonal block MBo of RCs ex one very old teak tree no. 9 planted in LFC. Thinned for the last time in 2006.
1997, S	"Provenance-progeny" trial.	"Provenance-progeny" trial Converted after selective thinning into seed orchards. See text and tables I and II for details.
TC		Clonal tests and tissue culture propagation methods (microcuttings, meristem culture) field assessment
2000, TC		Clonal tests (25 clones) and tissue culture propagation methods (microcuttings, meristem culture) field assessment

Overview, Prospects and Conclusions

During a period of about 20 years. YSG Biotech has been gathering teak genetic resources in Sabah from a wide range of different origins. As far as we are aware, the Company currently owns the world's richest teak genetic base, as described in the previous sections and summarized in Table III, that is being managed for improvement and for international use. We are aware that the one surviving provenance trial in the international series with a large number of diverse sources (IP038 with 25 different sources from 7 of the 8 regions described) (KEIDING et al., 1986) may comprise a richer genetic base; however, to our knowledge it is not being managed for improvement and international use.

These genetic resources can be utilized according to two basic deployment methods: via seedlings or through the clonal option, as detailed in GOH and MONTEUUIS (2005). Grower's choice of option depends on many factors including facilities and expertise, with efficiency, cost-saving and short tem returns remaining the over-riding concerns in the case of commercial enterprises. Other factors must be considered when taking cognizance of the long-term future of teak germplasm. For example, the retention of a broad genetic base in gene pools such as those of the C311 and Taliwas seed orchards, and their proposed enhancement, is conducive to their utility for international use such as establishing breeding bases elsewhere.

In the cases of the seed orchards and seed production areas described here, a detailed report on the results obtained, including Genotype X Environment interactions across the LFC and Taliwas orchard sites, is in preparation. Substantial genetic variation has been found, giving confidence that genetic improvement of the original populations can be expected to result from the intensive selective thinnings made, aiming at promoting the open mating of the best trees that remain, for each of the highly diverse genetic resources within the LFC and Taliwas seed orchards. In the cases of the Taliwas seed orchard and Perlis seed production area, this might be further enhanced by the breeding contribution of the infills planted in the empty spaces created by thinning. Seed collected (separately or bulked) from the best trees retained in the seed production area or seed orchards can be used to establish the next breeding cycle or generation of seed orchard (Goн, Monteuuis, 2005).

Another option would be to establish progeny tests to evaluate combining abilities, and then to select the best combiners for further use in clonal seed orchard, notwithstanding the relevant time, space, manpower and of course, economical constraints.

As already developed (GOH, MONTEUUIS, 2005; GOH *et al.*, 2007; CHAIX *et al.*, 2008), , advanced technologies for characterizing wood properties with special mention for the non-destructive core sampling method can be used for refining the initial selection on external traits, bearing in mind the overriding importance of wood quality for teak.

Resorting to adapted DNA markers (GOH, MONTEUUIS, 2005; GOH et al., 2007) allows a better identification of the genotypes and of their original provenances, in particular, for trees derived from seed lots of uncertain origins. These DNA investigations can also provide information on the genetic relatedness of the selected trees for wiser clonal deployment and seed orchard establishment, thus reducing risks of inbreeding.

Another interest of DNA fingerprinting is the genotypic identification of clones which may enable assertion of property rights associated with commercial transactions of these clones.

To sum up, YSG Biotech Bhd Sdn, in maintaining and enhancing its teak genetic resources, is presently able to fulfill most international requests for samples of Sabah's teak genetic resources via seeds from selected trees. in mixture or separate progenies/families, or via in vitro clonal propagules. Another option is to seek YSG Biotech's expertise for cloning in in vitro or in nursery conditions any teak trees of noteworthy interest for local mass clonal propagation or international dispatch, taking advantage of the possibility to meet phytosanitary requirement specified by the destination country.

All these assets are obviously quite appealing for establishing high yielding and quality teak plantations in various parts of the world, by allowing the introduction of new genotypes via seeds or clones, either directly for operational planting or through local breeding programs. The increasing difficulty to access teak germplasm from many countries applying strict genetic resource conservation policies reinforces this statement.

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