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Key genetic factors and location *of physiological components* determining the productivity of *natural rubber crops assessed* using the QTL approach *in Thailand*

Natural rubber is a renewable agromaterial derived from rubber trees—this product competes with but also complements synthetic rubber. It has specific technological qualities that are highly appreciated, especially for manufacturing tires. In Thailand, which is the top world producer, natural rubber trees are cropped almost exclusively on family smallholdings.

The Genmap project *(cf. Partnerships)* focused on the genetic basis of rubber tree *(Hevea brasiliensis)* functioning with the aim of developing more productive varieties (clones). Tapping a natural rubber tree diverts sugars produced by photosynthesis, to the detriment of tree growth while enhancing latex production in latex cells located in the bark. The research project developed in Thailand included a major assessment of the physiological mechanisms of latex production. Latex tapping systems were streamlined on the basis of previous tools and results, especially a biochemical analysis of the state of latex tissues ('latex diagnosis') whereby the metabolic functioning of different *Hevea* clones (grafted varieties disseminated by vegetative propagation) was characterised.


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Research carried out by CIRAD from 1995 to 2000 led to the publication of the first *Hevea* genetic map. This map is useful for analysing the genetic basis of resistance to South American leaf blight. The Genmap project then focused on constructing another genetic map with a new generation of microsatellite markers—the aim was to use this map in a genetic study of latex production factors, especially latex diagnostic parameters.

Conclusion and prospects

Long-term breeding of new clones has enabled growers to use the physiological potential of the species to boost performance. This is why a combined genetics and physiology approach was adopted in this research on natural rubber trees in Thailand.

Plant breeding has become increasingly complex since Mendel's simple laws were first discovered—mathematical models of constantly changing genetic traits (so-called quantitative traits that are assumed to be controlled by many genes) are now used to enhance the breeding potential. The phenotypic expression of these traits is highly modified by environmental variations, thus affecting their heritability and therefore the breeding efficiency. However, with the recent development of genetic mapping *(cf. Molecular genetic markers and genetic mapping),* for the most heritable agronomic traits, loci linked with genes that are highly involved in the expression of these characters can now be pinpointed on the genome. Genomic analysis is increasingly sophisticated and important genes can thus be readily identified, cloned and their functioning studied. •••

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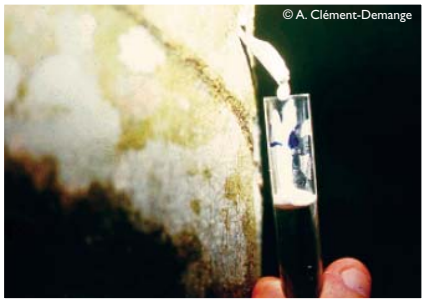
▲ *A plot with adult rubber trees in Côte d'Ivoire*



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◀ *Tapping a rubber tree*
▶ *Harvesting latex*

Hevea rubber trees

Rubber trees (*Hevea brasiliensis*, Euphorbiaceae family) produce natural rubber—a renewable agromaterial that is used by the industrial sector and which accounts for 40% of all elastomers (natural and synthetic rubber) used worldwide. Latex is not a sap but rather a milky emulsion containing rubber particles and consisting of the cytoplasm of latex cells present in trunk bark. In the last 20 years, *Hevea* wood has also been utilised to an increasing extent, mainly for furniture making, and has become a secondary product of cultivated natural rubber trees, currently accounting for 15% of the profits from rubber tree plots. Rubber trees have been intensely utilized since the discovery (in 1839) of vulcanization, i.e. a process that maintains the elasticity of natural rubber, which is an essential feature. 70% of this production is absorbed by the tire manufacturing industry.

Rubber trees were initially cropped after their domestication in 1876, with the first transfer of seeds from Brazil to Southeast Asia. The paradox is that rubber cropping has never actually been developed in South America, i.e. the area of origin of *Hevea brasiliensis*, due to the presence of South American leaf blight caused by the fungus *Microcyclus ulei*. Rubber cropping was first developed by agroindustrial groups in Asia during the colonial era and then gradually became a key activity on family smallholdings of less than 5 ha, subsequently accounting for 75% of the total cropping area. In 2004, Thailand supplied 35% of the 8.4 million t of natural rubber produced worldwide.

Grafted clones obtained by vegetative propagation currently represent the best adapted material available for rubber cropping. A rubber tree plot is tapped 6-8 years after the grafted trees are planted and they can then produce for 20-30 years. Tapping (harvesting latex) is periodically done every 2-5 days. Tapping techniques have gradually changed to optimise the cycle involving latex flow, coagulation and regeneration in latex tissues in the bark. Annual per-hectare dry rubber production can range from 300 to 2500 kg, depending on the production system and plantation age.

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▷▷ Visiting scientists

The Genmap project is a short-term component of a longer-term cooperative integrated research programme involving CIRAD, the Rubber Research Institute of Thailand (RRIT) and Kasetsart University in Thailand, with the support of Agropolis International and the French Ministry of Foreign and European Affairs. This programme, which covers the 1998-2010 period, was aimed at increasing the productivity and quality of products and adapting rubber growing to the ecological and socioeconomic conditions on plantations.

Kanlaya Prapan (a young Thai researcher of RRIT) visited France for 12 months in 2001-2002 to genotype progeny and construct most of the genetic map of the studied hybrid.

Napawan Lekawipat (RRIT) came to France to finalise the genetic mapping work over a 5 month period in 2005 (this visit was financially supported by a fund that complemented that of the Agropolis advanced research platform), so it was thus possible to meet the objective initially set out in the Genemap proposal.

Genomics now involve combinations of different yet complementary methods, and information obtained on one species can be used for other species (research is thus being mutually enriched and efforts are gradually converging). The method implemented in the Genmap project combined genetic mapping with a field analysis of the *Hevea* family studied (screening for QTLs associated with agronomic traits measured in field trials in Thailand).

The Genmap project included the following successive phases:

• **Performing a 'model' cross** (year 2000) by manually pollinating the two RRIM600 and PB217 parents, i.e. two cultivated clones that represent two different metabolic types. The aim was to obtain a broad range of variability in 334 progeny of this cross in order to fulfil the scientific objectives, and also to find clones combining complementary qualities in this progeny.

• **Genotyping 334 progeny** for 267 available and relevant microsatellite markers. For each progeny, the two alleles occurring at the same locus were identified (PCR and electrophoresis).

• **Genetic mapping** of the cross *(cf. Genmap genetic map)* via software processing of genotyped progeny data (*JoinMap3* software), focused on the joint or separate presence of alleles of different markers. Loci corresponding to the different markers were thus found to be located on each of the 18 *Hevea* chromosomes, with the distances between two markers corresponding to the crossover percentages noted between them.

• **Field analysis** (from 2002 to 2010) of the main agronomic traits of 196 progeny, especially growth and latex production traits, biochemical traits associated with metabolic types of production and rubber quality traits.

• **Establishment, by software processing,** of progeny classes associated with each allele per marker, calculation of the values of the agronomic traits of these classes, and identification of loci that determine most of the variation in certain traits (QTLs).

The Agropolis advanced research platform supported phases 2 and 3 of this project. The *Hevea* genetic mapping took 17 months of research time. The use of microsatellite markers represented substantial progress relative to the past use of RFLP markers in terms of research time (PCR efficiency), number of genotyped individuals, distribution of mapped loci on the genome and the polymorphism of assessed alleles.

Partnerships

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Molecular genetic markers and genetic mapping

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▶ *Kanlaya Prapan preparing a polyacrylamide gel to separate PCR-derived microsatellite markers*

So-called 'molecular' genetic markers directly concern the DNA molecule in which they correspond to noncoding zones (loci). They are said to be genetically 'neutral' because they do not influence the evolution of the species, and identification of their alleles in an individual does not depend on the tissue studied or on the medium conditions. The so-called linkage disequilibrium genetic mapping technique has developed considerably since a high number of these markers have become available. This technique involves investigating the extent of character recombination in progeny due to crossover (intra-chromosomal rearrangements that occur during meiosis). Because of these rearrangements, alleles from two loci that were initially present on the same chromosome have a certain degree of probability of being separated in two different gametes (i.e. when a crossover occurs in the space between them).

There are DNA zones (called linkats) where very little crossover occurs and where the same combinations of alleles for neighbouring loci are preserved. Genetic mapping involves analysing the frequency of association of respective alleles from two loci, for all available marker pairs. The markers can thus be located in relative positions to each other, while identifying the linkats and detecting 'super-linkats' corresponding to different chromosomes from the studied species (*Hevea brasiliensis* is a diploid species with 18 chromosome pairs). Each distance between two markers is expressed as a crossover percentage (1 centimorgan = probability of 1% crossover during meiosis), and genetic maps are drawn up on this basis.

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Transfer, development and project follow-up

The expected technical milestones were achieved through this project—progeny genotyping and genetic mapping of the cross—and specific methods were developed for natural rubber trees, such as the adaptation of an accelerated genotyping technique using a DNA sequencer.

The training obtained by the two hosted Thai researchers led to the transfer of expertise to the original Thai institute and promoted a dynamic collaboration with the Thai partners in this project. The Thai institute now implements the PCR method and microsatellite markers developed by CIRAD, so the conformity of the planted material can now be efficiently controlled in Thailand. Essential sequences were posted for free public access on the EMBL/GenBank website. In addition, field operations in Thailand are being continued with the support of CIRAD scientists.


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The RRIT-DOA institute has also just awarded a thesis bursary to a new young researcher (Ms. Rachanee Ratanawong) so that she can work within the framework of the Genmap project to obtain field measurements and screen QTLs, under the academic supervision of Kasetsart University, with training provided by CIRAD. In addition to the scientific aims, the Genmap project thus provides an efficient research training support for groups of 5-10 Thai researchers and technicians.

Other short- and medium-term milestones to be achieved through this project include determining the possibilities of utilizing the expected complementarity between clones with different metabolisms via hybridization, identifying QTLs that can serve as early breeding criteria for the studied hybridization and selecting new efficient relevant clones from the progeny of this cross.

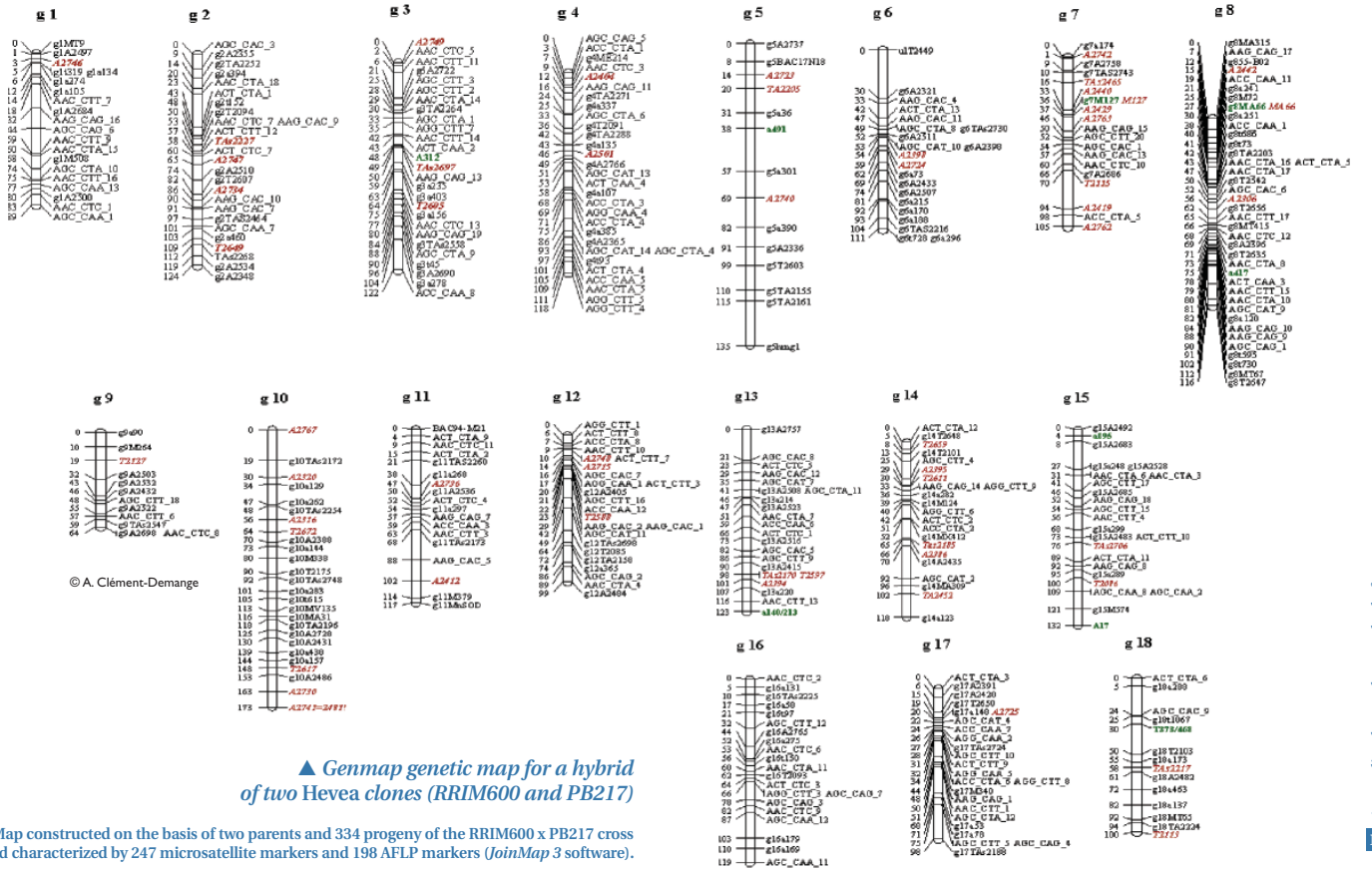
▷▷ Publications

Lekawipat N., 2005. Development of genetic map of RRIM600 x PB217 based on microsatellite markers. Cirad-Biotrop, Montpellier, January-May 2005. Doras-Rubber :Towards the improvement of the productivity of the rubber tree. Genmap component :"Variability analysis and genetic determinism of some physiological characteristics of the rubber productivity in Thailand".

Prapan K., Clément-Demange A., Teerawatanasuk K., Rodier-Goud M. and Seguin M., 2004(a). Genetic mapping and field study of a full-sib family (RRIM600 x PB217) in *Hevea brasiliensis* (Genmap project). First results." Towards the improvement of the productivity of the rubber tree ". Kasetsart University - RRIT-Doa - INRA-Piaf - CIRAD Seminar, Bangkok, 27-28 May, 2004.

Prapan K., Seguin M., Rodier-Goud M. and Clément-Demange A., 2004(b). *Hevea brasiliensis* : Déterminisme génétique et localisation de composantes physiologiques de la productivité de l'hévéaculture par approche QTL en Thaïlande. Réalisation d'une carte génétique ancrée sur marqueurs microsatellites. Programme Agropolis "Plate-Forme de Recherches Avancées", composante Genmap du projet Doras-Rubber, CP_SIC 1676, January 2004.

The long-term milestones are still the main focus of this research, involving the development of a *Hevea* genome documentation database to locate genes of interest that are regularly identified by other genetic and molecular physiological research initiatives on this species. ■



▲ *Genmap genetic map for a hybrid of two* Hevea *clones (RRIM600 and PB217)*

Map constructed on the basis of two parents and 334 progeny of the RRIM600 x PB217 cross and characterized by 247 microsatellite markers and 198 AFLP markers (*JoinMap 3* software).