Molecular genetics and functional genomics studies for identifying QTLs and genes underlying tolerance to SALB and latex production in *Hevea brasiliensis*

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CIRAD teams have implemented molecular genetics and functional genomics studies for identifying QTLs and genes underlying tolerance to the South American Leaf Blight (SALB) and latex production in *Hevea brasiliensis* in partnership with the Rubber Research Institute of Thailand, the Manufacture de Pneumatique Michelin and the University of Santa Cruz in Brazil.

Latex production is a complex genetic trait involving a large number of regulatory and metabolic pathways. Identification of QTLs and genes was carried out using a phenotype/genetic analysis of a RRIM 600 x PB 217 progeny (collaboration with the RRIT) and a functional genomics approach based on the characterization of target genes from biological models (Rattanawong et al. 2009; Seguin et al. 2003).

Transcript sequences were produced for various *Hevea brasiliensis* clones grown under various abiotic (tapping, wounding, ethylene, water deficit) and biotic (*Microcyclus ulei* infection) stresses in order to uncover genes involved in SALB tolerance and ethylene signaling and response. First, 8593 expressed sequence tags (ESTs) were obtained from 8 SSH libraries for several Hevea tissues (latex, bark, leaves, roots, juvenile plant, flower, fruit) of commune and semi-domesticated genotypes. More recently, transcript sequence databases were generated from RNAseq using the 454 pyrosequencing technique. 34,572 and 199,174 contigs were obtained from leaf of clone RRIM 600 and from various tissues of clone PB 260, respectively (Montoro et al. 2010).

Given the involvement of the ethephon and ethylene in the production of natural rubber, genes involved in the ethylene-biosynthetic pathway (Kuswanhadi et al. 2010) and signaling (Duan et al. in preparation; Duan et al. 2010) were characterized in *Hevea brasiliensis*. Ethylene-insensitive transgenic lines were established in order to study in detail the involvement of ethylene in the various signalling and metabolic pathways related to the latex production. Related to the Tapping Panel Dryness (TPD), the oxidative stress is one of the earliest responses to biotic and abiotic stresses. The reactive oxygen species- (ROS) scavenging system was strengthening in transgenic *Hevea* plants. Over-expressing a cytosolic isoform of the *HbCuZnSOD* gene in *Hevea* changed its response to water deficit and also impacted both plant regeneration by somatic embryogenesis and plant growth (Leclercq et al. Submitted). More recently, conserved miRNAs and their putative targets related to the control of redox status during plant development and abiotic stress were identified in *Hevea brasiliensis* (Gébelin et al. in preparation).

SALB resistance is also a complex trait which is differentially expressed in various genotypes. For this reason, our studies aiming to characterize the genetic basis of SALB resistance were carried out on several resistant cultivars in various environments and with different strains of *Microcyclus ulei* (Le Guen et al. 2008). By analyzing the segregation of resistance among progenies between resistant and susceptible cultivars and comparing it with molecular-based genetic maps, we were able to identify in the genome the loci responsible for this trait (Le Guen et al. 2003). For each of the four studied cultivars, a distinct major gene or a strong resistance QTL were characterized, each one located on a different chromosome (Le Guen et al. 2007). The effect of these major loci was generally slightly modulated by the presence of minor resistance QTLs. Our results were original, as they did not show any direct relationship between monogenic and complete resistance on the one hand, and polygenic and partial resistance on the other hand, contrarily to usually admitted resistance models. This situation is particularly favourable for the utilization of marker-assisted-selection in rubber tree breeding schemes.

Information gained from these studies may be useful for whole genome sequencing programme. Whatever sequencing methods and strategy are used in a whole genome sequencing project, a crucial step after the assembly of the sequences is to anchor these sequences (contigs, meta-contigs or scaffolds) along the linkage groups of the species. For this, it is necessary to get a high-quality genetic map, based on numerous genetic markers. For a large genome as the one of rubber tree, it would be ideally necessary to get 4,000 markers located onto the genetic map, which would represent in average one marker per centiMorgan or one marker every one million of base pairs. Cirad's researchers have already mapped 450 microsatellite markers on a synthetic map based on the study of 4 mapping populations implying 6 different parental genotypes. This synthetic map could be the skeleton for the establishment of the high-resolution genetic map necessary for the anchorage of the sequences. In that way, the Cirad contribution could be both (i) the preliminary search for other markers, as for example SNP markers, and their location on the rubber tree genetic maps, and (ii) the characterization of the polymorphism of microsatellite sequences produced by the sequencing project and their location on the map. Our added value is our possibility to exploit simultaneously various populations, with a large number of progeny individuals, thus increasing the probability to detect polymorphism of the markers and to map them. In that way, the Cirad contribution could be both (i) the preliminary search for other markers, as for example SNP markers, and their location on the rubber tree genetic maps, and (ii) the characterization of the polymorphism of microsatellite sequences produced by the sequencing project and their location on the map. Possibility to exploit simultaneously various populations with a large number of progeny individuals increases the probability to detect polymorphism of the markers and to map them. Furthermore, knowledge on certain biological processes would ease the annotation of related genes. ESTs resources may be used to validate gene prediction and then perform the structural annotation of the rubber genome. Based on transcripts of several families of genes sequenced and functionally characterized, high quality cDNA sequences may serve to support automatic annotation of genes on the genome.

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