GENESALB (2007-2010): GENEtic analysis of resistance to South American Leaf Blight (SALB – Microcyclus ulei) in rubber tree (Hevea spp.)

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=> Abstract P78
Rubber tree
(*Hevea brasiliensis*)

Rubber world consumption (24 Mt in 2010)
- 42% of natural rubber (*i.e.* from *Hevea*)
- 58% of synthetic rubber (petroleum)

Natural rubber: cis-polyisopren

\[
\begin{align*}
&\text{CH}_2 \quad \text{CH}_2 \\
&\text{CH}_3 \quad \text{CH}_3 \\
&\text{CH}_2 \quad \text{CH}_2 \\
&\text{CH}_2 
\end{align*}
\]
Rubber tree (*Hevea brasiliensis*)

- Rubber stored in latex = cytoplasm of laticiferous cells,
- Harvested by bark tapping, 2-3 times per week, during ~30 years

- 9 millions ha
- Production: 10.2 Mt (2010)
  -> 80% by small holders
  -> 20% by private companies
Rubber tree (*Hevea brasiliensis*): areas of origin and of current production of natural rubber

**Sources:** IRSG 1998

- **Asia:** 91%
- **Africa:** 5%
- **Latin America:** 4%

**Main area of breeding (1877-1977) in SALB free environment => all elite cultivars are fully susceptible to SALB**

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**South American Leaf Blight (SALB) - *Microcyclus ulei* (Ascomycota)**

**Legend:**
- > 1 million t
- > 500,000 t
- > 100,000 t
- < 100,000 t

Sources: IRSG 1998
*Microcyclus ulei* (Ascomycota): repeated defoliations $\Rightarrow$ death of the trees.

South American Leaf Blight: a threat for world natural rubber production
1992 -> Cirad – Michelin partnership (CMB): => Create cultivars (clones) combining high yielding (latex) and resistance to SALB genetic improvement of SALB resistance  
   o genetic mapping – genetic determinism 
   o phytopathology - epidemiology

2008-2010 GENESALB - ANR/Genoplante  
   o genetic mapping:  
      diversity of genetic determinisms  
   o genomic resources  
   o for MAS development
GENESALB: in connection with the Cirad – Michelin breeding program

SALB resistance:
- field evaluation
- controlled inoculations

Hand pollination
- SALB resistance:
  - field evaluation

Clermont-Ferrand: co-coordination

Montpellier, Cirad, UMR-AGAP:
- Genetics & Genomics
  - genotyping
  - QTL mapping
  - genomic resources: SSR coll. ESTs, BAC library

SALB resistance:
- field evaluation

Genomics: ESTs

UESC univ.
GENESALB project (2008-2012)
3 sub-projects:

1. Genetic mapping and QTLs detection => diversity of genetic determinism and markers for early selection (MAS)

2. Markers development => for mapping; expressed genes integration in genetic maps, search for co-location with resistance loci/QTLs

3. Disease related ESTs / SSH libraries cloning, sequencing and expression analyses => candidate genes
GENESALB sub-project 3: candidate genes identification

Cloning of ESTs differentially expressed during SALB infestation:

• **11 SSH libraries** from leaves:
  resistant vs susceptible or healthy vs infected
  at several times after controlled inoculation

• **6700 ESTs** cloned and sequenced

• **490 candidate genes**: putative functions and expression profiling by macro-arrays

Garcia et al. (2011)
GENESALB sub-projects 2: markers development in ESTs and mapping

Search for SSR in ESTs sequences available:

• 760 SSR sequences identified
• 261 SSR screened for polymorphism by bin mapping
• **125 polymorphic EST-SSRs** identified
• 80 EST-SSRS mapped on GENESALB mapping populations
GENESALB sub-projects 1: genetic mapping and QTL detection

Previous knowledge on genetic determinism of SALB resistance was based on only 1 mapping population:

- PB260xRO38: a multigenic resistance bypassed in French Guiana and Brazil

=> genetic mapping of 3 additional diversified sources (Hevea accessions) of resistance: MDF180, FX2784, FDR#
Previous results:
Genetic determinism of SALB resistance of RO38: a bypassed resistance despite its multi-genic determinism

QTL with LOD > 3:
R² = 10% ▼▼▼
R² = 20% ▼▼▼

M13-1bn: a major resistance locus governing qualitative resistance

(Lespinasse et al. 2000, Le Guen et al 2007)
### Qualitative trait loci (major genes) and Quantitative Trait Loci (QTLs) identification and mapping - resistance evaluation in field trials -

<table>
<thead>
<tr>
<th>Mapping pop.</th>
<th>Field location</th>
<th>pop. size</th>
<th>markers</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible x Resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB260 x MDF180</td>
<td>French Guiana</td>
<td>351 F1</td>
<td>gSSR, AFLPs</td>
<td>durable in Brazil &amp; French Guiana</td>
</tr>
<tr>
<td>PB260 x MDF180</td>
<td>Bahia</td>
<td>171 F1</td>
<td>gSSR, EST-SSR</td>
<td></td>
</tr>
<tr>
<td>PB260 x FX2784</td>
<td>French Guiana</td>
<td>125 F1</td>
<td>gSSR</td>
<td>bypassed in Bahia</td>
</tr>
<tr>
<td>PB260 x FX2784</td>
<td>Mato Grosso</td>
<td>295 F1</td>
<td>gSSR</td>
<td></td>
</tr>
<tr>
<td>PB235 x FDR#</td>
<td>Bahia</td>
<td>200 F1</td>
<td>gSSR, EST-SSR</td>
<td>durable in Brazil &amp; French Guiana</td>
</tr>
<tr>
<td>PB260 x RO38</td>
<td>French Guiana</td>
<td>500 F1</td>
<td>RFLP, AFLP gSSR</td>
<td>bypassed in Brazil &amp; French Guiana</td>
</tr>
</tbody>
</table>
PB260xFX2784 progeny in Mato Grosso (Plantation Edouard Michelin, PEM)
**Preliminary result (Cirad):** in French Guiana, FX2784 exhibit a **qualitative resistance** governed by a single resistance locus (2nd major locus, we named *M2-FX*) located on linkage group (LG) g2.

PB260xFX2784
Linkage map
LG g2

Resistance locus integrated in the map as a genetic marker
GENESALB: identification and mapping of the same \textit{M2-FX} locus governing qualitative resistance to SALB of \textit{FX2784} in Brazil (Mato Grosso) - this resistance is bypassed in Bahia state -

\[ \Rightarrow \text{reliability and accuracy of field resistance evaluations} \]

Microsatellite / SSR markers
Resistance locus / marker

\textit{Le Guen \textit{et al.}} In prep
GENESALB sub-project 1: construction of saturated maps for MDF180 and FDR# resistant clones (gSSR & EST-SSR markers)

MDF180 genetic map covering the 18 linkage groups / chromosomes
GENESALB: Genetic determinism of SALB resistance of MDF180:
a durable resistance governed by only 2 major loci
- identification of a 3rd major / qualitative resistance locus *M15-md* -

QTL with LOD > 3:
- $R^2 = 10\%$
- $R^2 = 20\%$

Le Guen *et al.* 2011
GENESALB: the durable resistance of FDR# is governed by a single major QTL in Brazil (Bahia)
GENESALB: simple genetic determinisms of resistance to SALB in rubber tree with a great diversity of resistance factors (qualitative R loci or QRLs)
Conclusions / original results:

- Richness of genetic resistance factors to SALB in *Hevea* genetic resources

- **Simple genetic determinisms** of even durable resistances (do not fit with classical models !)

- Four unlinked major resistance loci

- A favorable situation for **resistance introgression and pyramiding** in few generations of inter-crosses

- Additional **genomic resources** for genetic, breeding and functional genomic studies
Prospects

- Development of marker aided selection (MAS)
- Genetic analysis of additional sources of resistance
- QTL analysis of latex yield and growth vigor
- SNP markers development for expressed and candidate genes mapping
- Q-PCR validation of candidate genes
Thank you for your attention