Production of mutants affected in hormone signalling to dissect defence mechanisms in *Hevea brasiliensis*: the case of ethylene

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Cellular & Molecular Biology of Stress Responses in Tropical Woody Species

IRRDB Rubber Conference
29-30 October 2012 – Kovalam, Kerala, India

*Agrobacterium tumefaciens*-mediated genetic transformation in *Hevea brasiliensis* for improving agronomical traits

- **Antioxidant systems:** CuZnSOD (CIRAD), GCL1 (CIRAD), MnSOD (RRII)
- **Rubber biosynthesis:** HGMR1 (RRII)
- **Water stress tolerance:** Sorbitol P-dehydrogenase, osmotin (RRII)
- **Cold tolerance:** CBFs (CATAS)

- **Medium-added value proteins:** Human serum albumine, antibodies (RRIM)
Towards the dissemination of genetically modified rubber trees over-expressing Mn-superoxide dismutase in India


Genetically modified rubber trees with the cytosolic copper zinc superoxide dismutase to improve tolerance to oxidative stress

Over-expressing a cytosolic isoform of the CuZnSOD gene in Hevea brasiliensis PB260 cultivar changed its response to drought deficit. Plant Mol Biology
Latex production involves complex regulations

Emerging connections in the ethylene signaling network
Sang-Dong Yoo1,2, Younghee Cho1, and Jen Sheen1 - Trends Plant Sci. 2009 May; 14(5): 270–279
Model for ethylene signal transduction that incorporates biochemical features of the pathway components

Mutants of the Ethylene Signal Transduction Pathway identified in Arabidopsis thaliana

WT  etr1-1  ein2  ctr1-2

The effect of ethylene upon the induction of the triple-response in dark grown seedlings is shown for wildtype, the ethylene-insensitive mutants etr1-1 and ein2, and the constitutive ethylene-response mutant ctr1-2.

The dominant mutant receptor etr1-1 from Arabidopsis confers ethylene insensitivity in heterologous plants

A platform for routine functional analysis of candidate genes

Long-term somatic embryogenesis

Agrobacterium tumefaciens-mediated genetic transformation using the green fluorescent protein as an efficient selection marker

Establishment of ethylene-insensitive Hevea transgenic lines expressing the etr1-1 mutant receptor

<table>
<thead>
<tr>
<th>Mature embryos</th>
<th>Plantlets 0 month</th>
<th>Plants 12 months</th>
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<tbody>
<tr>
<td>TS13T1A10</td>
<td>98</td>
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<td>TS13T1A11</td>
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<td>TS13T2A40</td>
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<td>TS13T2A56</td>
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<tr>
<td>Control CI07060</td>
<td>52</td>
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</tbody>
</table>
*etr1-1* transgenic lines showed normal growth and morphology up to one year after acclimatization

Tolerance of *etr1-1* transgenic lines to ethephon treatment

Comparison of ethylene sensitive (35S:GUS) and insensitive (35S::etr1-1) lines one week after ethephon 2.5% application on 8-month-old transgenic plants
Conclusion & prospects

- Efficient and routine genetic transformation method for both application and academic studies,
- 7 independent transgenic lines & 7 plant regenerant lines & 5 tested transgenic lines showed ethylene-insensitivity
- All etr1-1 plants showed normal growth up to one year after acclimatization & tolerance to ethephon treatment

Further characterization:
- Identification of ERF (Ethylene Response Factors) that control the response to ethylene in terms of defence mechanisms
- Identification of ethylene-responsive genes
- Phenotyping for specific biological process related to ethylene response and abiotic stresses (drought, etc.)

<table>
<thead>
<tr>
<th>EIN2</th>
<th>EIN3/EILs</th>
<th>ERFs</th>
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<tbody>
<tr>
<td>CTR1</td>
<td>ETR1/ETR2</td>
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</tbody>
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Defence Proteins (antioxidant systems, secondary metabolism), Transporters (water, sugar, phosphate), Hormone biosynthesis & receptors, etc.

Latex production vs TPD, Drought tolerance, etc.

Acknowledgements

CIRAD, BURST group
Florence Dessailly
Julie Leclercq
Florence Martin
Pascal Montoro
Maryannick Rio
Eve Lorenzini

University of Florida
Harry Klee

Institut Français du Caoutchouc
French Rubber Institute