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

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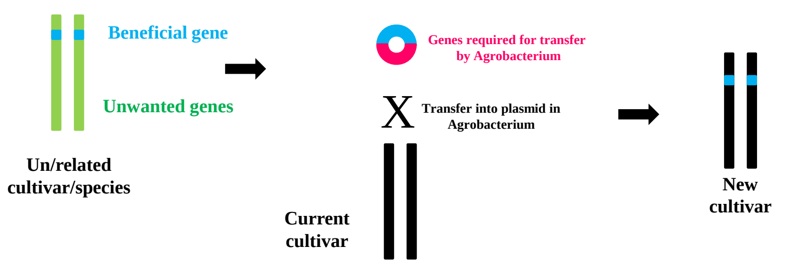
Cellular & Molecular Biology of Stress Responses in Tropical Woody Species

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

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Jayashree R, Rekha K, Venkatachalam P, Uratsu SL, Dandekar AM, Kumari Jayasree P, Kala RG, Priya P, Sushma Kumari S, Sobha S *et al*: **Genetic transformation and regeneration of rubber tree (Hevea brasiliensis Muell. Arg) transgenic plants with a constitutive version of an anti-oxidative stress superoxide dismutase gene**. *Plant Cell Reports* 2003, **22**(3):201-209.
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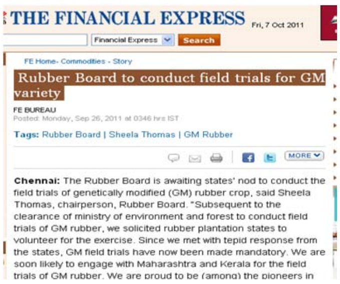
Miao Z, Gaynor JJ: **Molecular cloning, characterization and expression of Mn-superoxide dismutase from the rubber tree (Hevea brasiliensis)**. *Plant Molecular Biology* 1993, **23**(2):267-277.


THE FINANCIAL EXPRESS Fri, 7 Oct 2011

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Rubber Board to conduct field trials for GM variety

FE BUREAU
Posted: Monday, Sep 26, 2011 at 0346 hrs IST

Tags: Rubber Board | Sheela Thomas | GM Rubber

Chennai: The Rubber Board is awaiting states' nod to conduct the field trials of genetically modified (GM) rubber crop, said Sheela Thomas, chairperson, Rubber Board. "Subsequent to the clearance of ministry of environment and forest to conduct field trials of GM rubber, we solicited rubber plantation states to volunteer for the exercise. Since we met with tepid response from the states, GM field trials have now been made mandatory. We are soon likely to engage with Maharashtra and Kerala for the field trials of GM rubber. We are proud to be (among) the pioneers in


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Genetically Modified Rubber Trees and Blingy Wheels

By Jim Smith email
Editor, Tire Review magazine
April 20, 2011

Email Print 0 Comments Bookmark this website

Interesting story for those into horticulture AND concerned about the fast-rising price of natural rubber.

Seems that scientists in India have been playing around with genetically modified rubber. Rather, they have been attempting to genetically modify latex-producing trees in an effort to hasten the amount of time it takes between planting and tapping.

Currently, it takes seven years from the point when a rubber tree is germinated to that plant's ability to produce usable latex milk. Scientists are trying to shave a few years off of that mark, which will greatly help improve supply and (hopefully) reduce market prices.

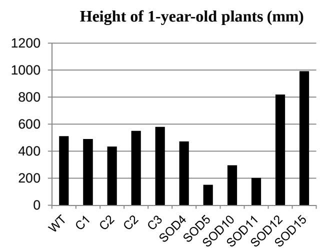
In addition, GM trees are said to be heartier, more resistant to weather and prolonged drought. These trees, it is hoped, could also be raised in other parts of the world; at present latex-bearing trees can only be raised in hot, equatorial regions of the world.

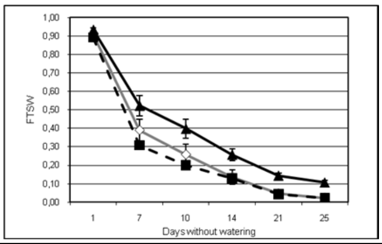
Even without moving to new growing areas, GM rubber plants could boost NR production by 10%, experts claim.

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

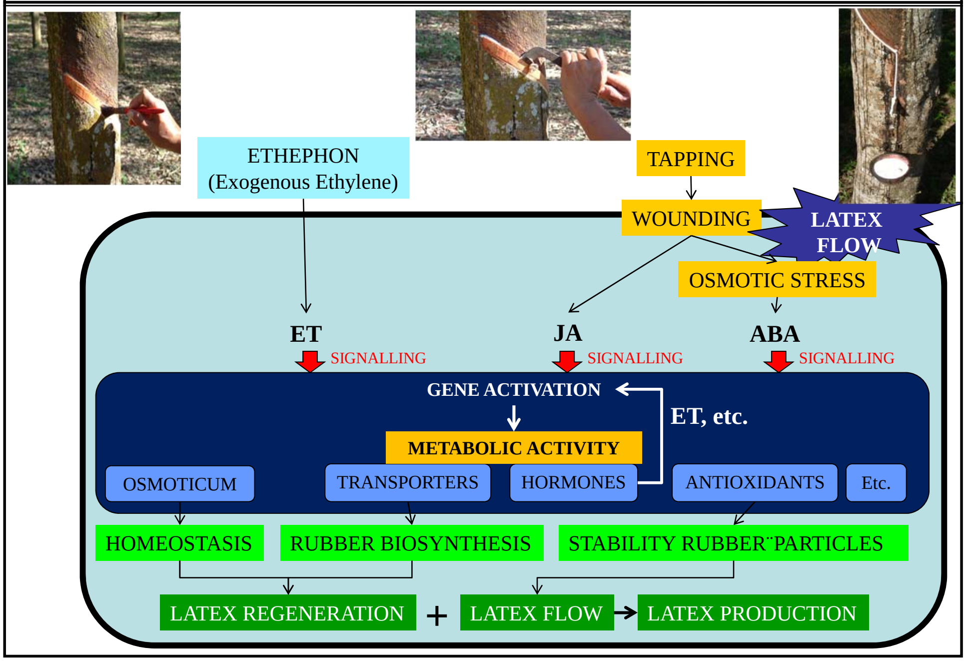
Leclercq, Martin, Sanier, Clément -Vidal, Fabre, Lardet, Ayar, Peyramard, Montoro (2012). Over-expressing a cytosolic isoform of the CuZnSOD gene in Hevea brasiliensis PB260 cultivar changed its response to drought deficit. **Plant Mol Biology**


SOD overexpressing line with higher growth rate than control



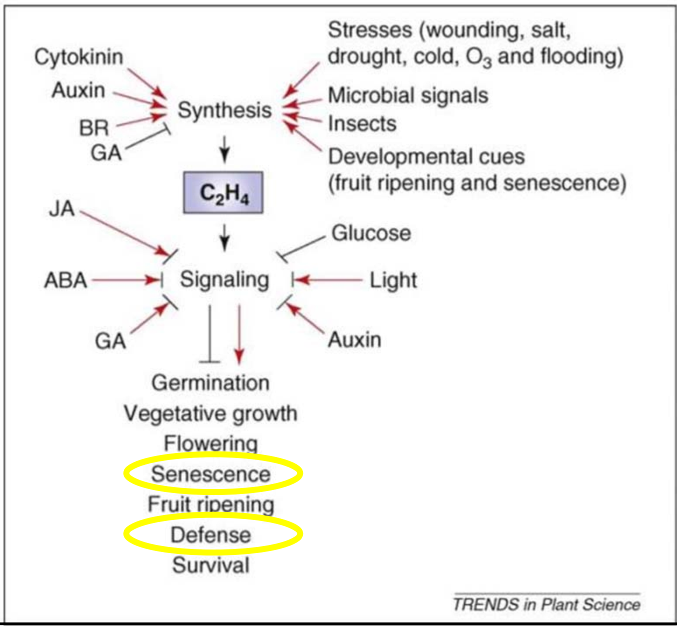


Latex production involves complex regulations



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

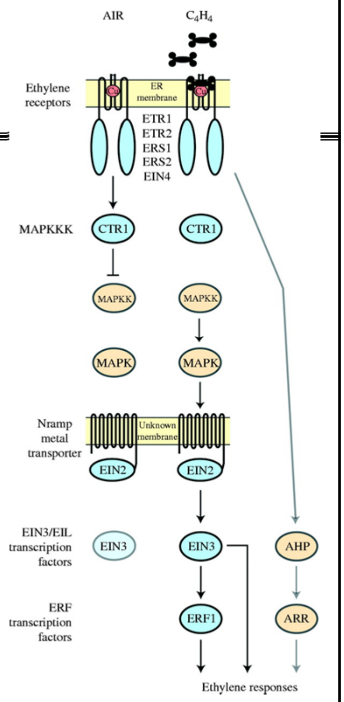


Model for ethylene signal transduction that incorporates biochemical features of the pathway components

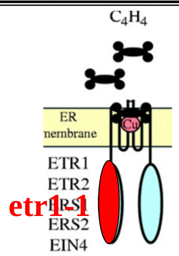
Mutants of the Ethylene Signal Transduction Pathway identifed in *Arabidopsis thaliana*

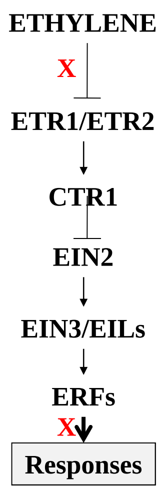
CHEN Y et al. Ann Bot 2005;95:901-915

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





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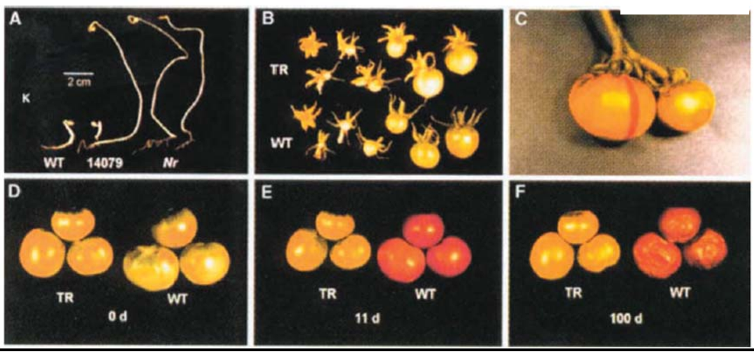
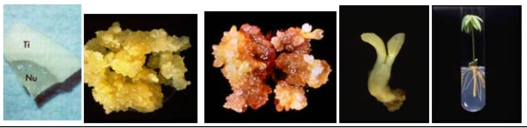


A platform for routine functional analysis of candidate genes



Long-term somatic embryogenesis

Montoro et al. (2000). Plant Cell Reports **19**, 851-855
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Blanc et al. (2006). Plant Cell Reports **24**, 724-733
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Lardet et al. (2011). Plant Cell Reports , **30**, 10, 1847-56
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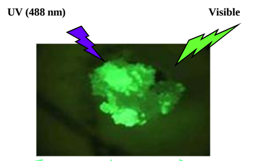
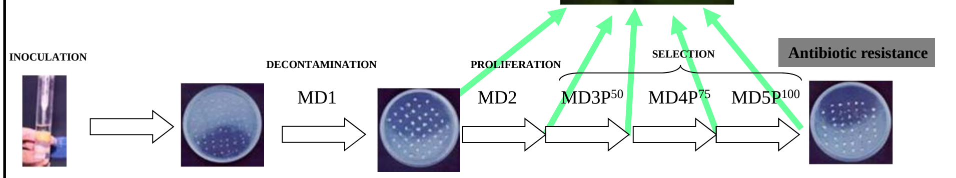
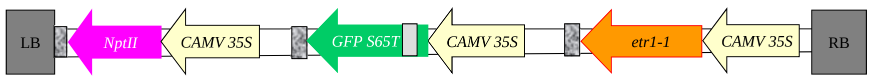


GFP activity



***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

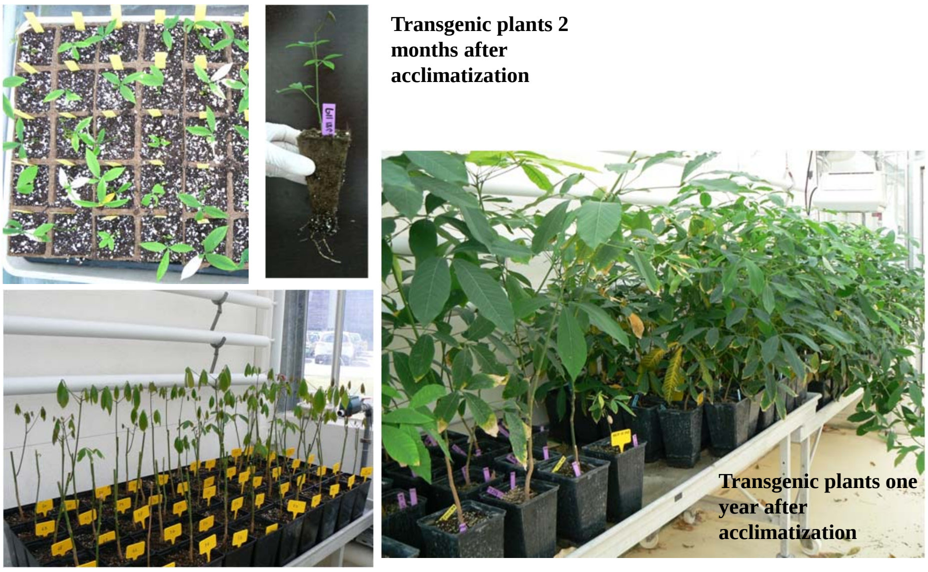


- **7 independent transgenic callus lines**
- **7 regenerant lines**

	Mature embryos	Plantlets 0 month	Plants 12 months
TS13T1A10	98	39	30
TS13T1A11	243	70	56
TS13T1A27	187	50	44
TS13T2A40	117	59	33
TS13T2A42	250	44	41
TS13T2A44	213	65	32
TS13T2A56	16	4	3
Control CI07060	52	27	20

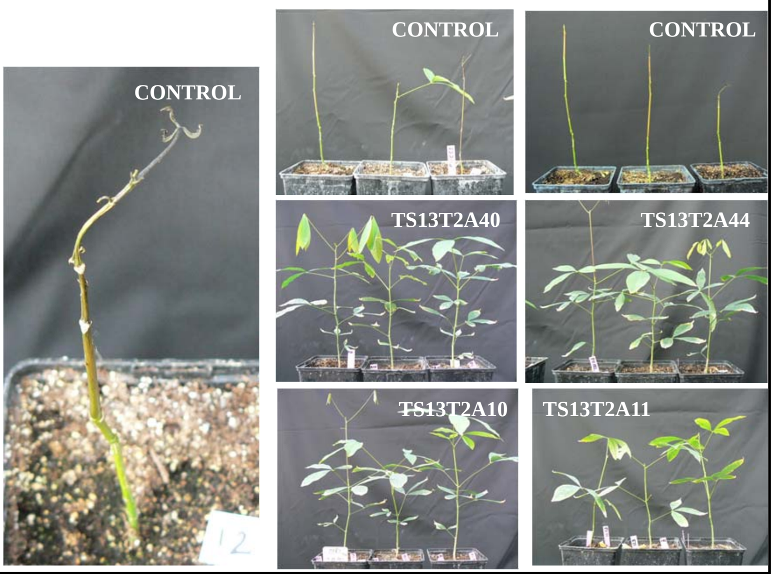
etr1-1 transgenic lines showed normal growth and morphology up to one year after acclimatization

Transgenic plants 2 months after acclimatization

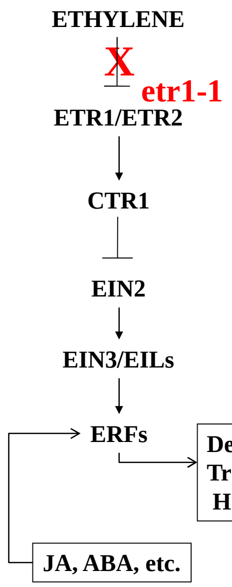


Tolerance of *etr1-1* transgenic lines to ethephon treatment

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.)

Defence Proteins (antioxidant systems, secondary metabolism), Transporters (water, sugar, phosphate), Hormone biosynthesis & receptors, etc.

Latex production vs TPD, Drought tolerance, etc.

Acknowledgements

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