Over-expressing *CuZnSOD* gene for controlling oxidative burst in *Hevea brasiliensis (Müll. Arg).*

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Figure 1 : (A) Application of ethephon to a rubber tree tapping panel.

(B) Tapping panel of a healthy tree.

(C) A sick tree showing tapping panel dryness (TPD).

Hevea brasiliensis (Müll. Arg.) is the main source of natural rubber which is biosynthesized in latex cells. Ethephon application can enhance the biosynthesis activity required for latex regeneration after each tapping (figure 1A), optimizing the yield potential of rubber tree. However, a good management of both tapping frequencies and ethephon applications is required for avoiding excessive metabolic activation which can lead to Tapping Panel Dryness (TPD) (figure 1B, 1C).
This physiological disorder is a consequence of an oxidative stress in the latex cells, leading to membrane damages, flocculation of rubber particles and plugging of the latex vessels (Figure 1C). Three genotypes (PB 260, PB 217 and RRIM 600) with contrasted metabolism activity and response to ethylene stimulation have been selected for further studies.

Expression analysis of *CuZnSOD* gene by *Q-PCR*

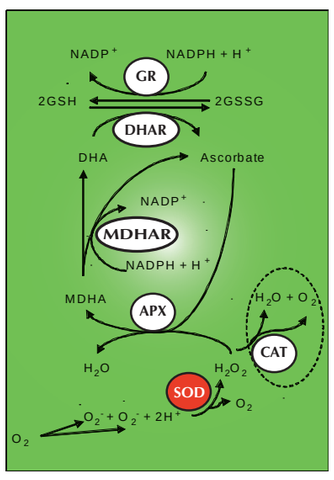


Figure 2: The ascorbate-glutathione cycle with the major antioxidative enzymes (Noctor, 1998; Alscher *et al.*, 2002)

Expression analyses of *CuZnSOD* gene involved in detoxifying the reactive oxygen species (ROS, Figure 2) has been studied in healthy trees, TPD trees and also in young budded plants. During photosynthesis, ROS production is enhanced (Foyer and Noctor, 2003). *CuZnSOD* gene expression is stimulated slightly in PB 260 at 16h00 and strongly in PB 217 and RRIM 600 at 12h00 (figure 3A).
In response to ethylene, the expression of the *CuZnSOD* gene is highly enhanced in PB 260, remains constant in PB 217 but is down-regulated in RRIM 600 even though in this genotype the expression level of *CuZnSOD* is always higher than other ones (figure 3B). In response to wounding, *CuZnSOD* transcript level is reduced in PB 260 and PB 217 (figure 3C). *CuZnSOD* gene has also been shown to be down-regulated in TPD tree compared to its healthy counterpart (unpublished data).

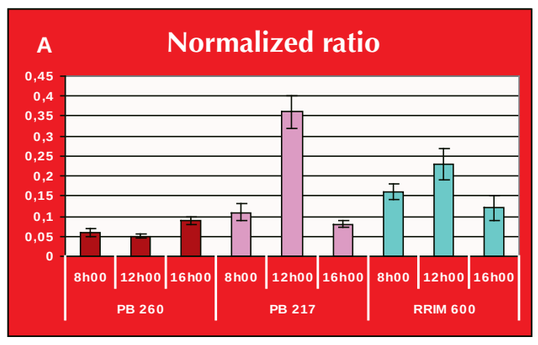
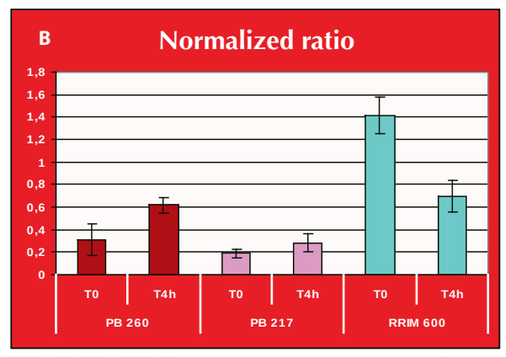
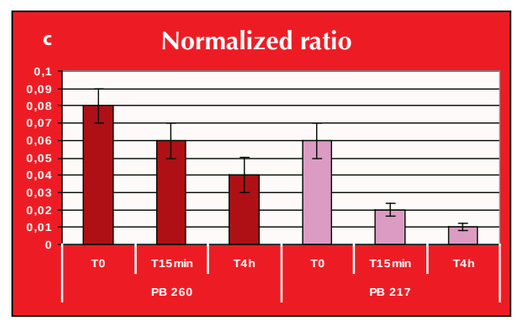


Figure 3: (A) *CuZnSOD* gene expression analysis by Q-PCR during the day in young budded plant.



(B) *CuZnSOD* gene expression analysis by Q-PCR in response to ethylene treatment (T0 = air, T4h = 1 ppm during 4 hours) in young budded plant.



(C) *CuZnSOD* gene expression analysis by Q-PCR in response to wounding in young budded plant (15 min and 4 hours after wounding).

CuZnSOD over-expression by genetic transformation of rubber tree

An *Agrobacterium tumefaciens*-mediated genetic transformation has been developed (Blanc et al., 2006) based on the successful plant regeneration procedure using *H. brasiliensis* PB 260 somatic embryos (Lardet *et al.*, 1999). The GFP was used as a visual marker (Figure 4). The *CuZnSOD* gene placed under the control of *CaMV35S* promoter was over-expressed in transgenic calli (data not shown). Regeneration of transgenic plants harbouring a single copy of the T-DNA is on-going (Figure 5).

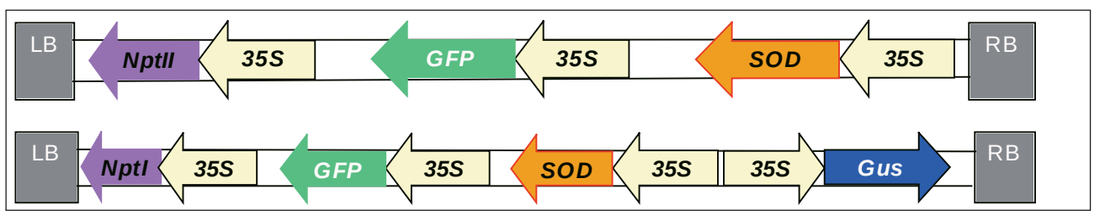


Figure 4: Constructs used for genetic transformation

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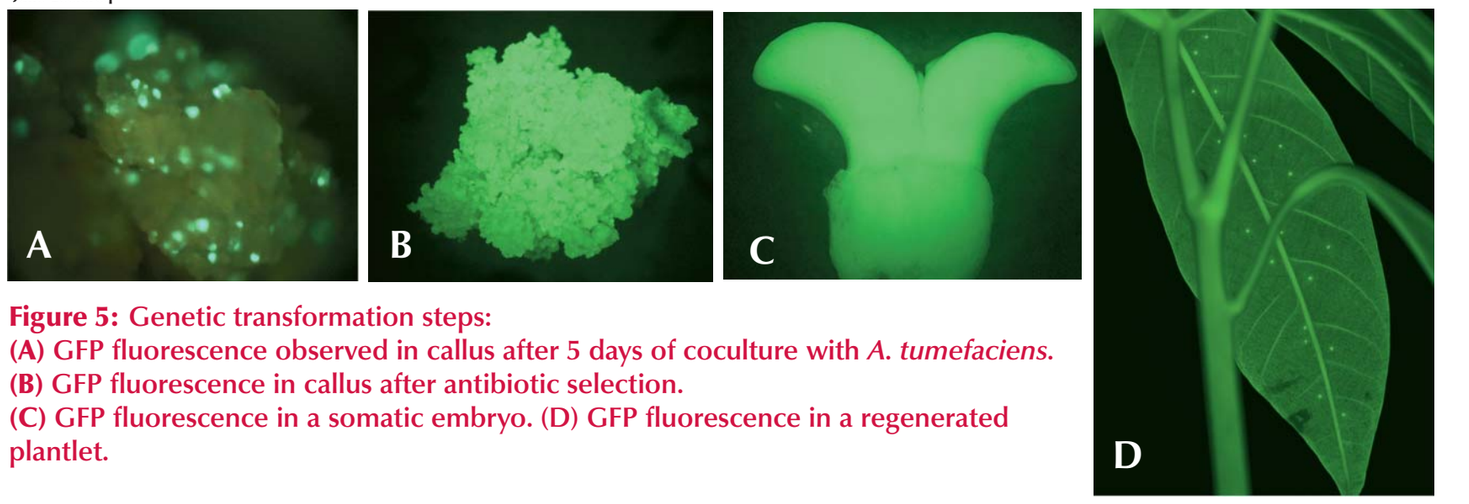

Figure 5: Genetic transformation steps:
(A) GFP fluorescence observed in callus after 5 days of coculture with *A. tumefaciens*.
(B) GFP fluorescence in callus after antibiotic selection.
(C) GFP fluorescence in a somatic embryo. (D) GFP fluorescence in a regenerated plantlet.

Conclusion

The gene encoding *CuZnSOD* displays different expression profiles in the three genotypes in response to day light, ethylene and wounding. Analysis of over-expressing *CuZnSOD* gene transgenic plants will allow a better understanding of the implication of *CuZnSOD* activity in oxidative burst tolerance in rubber tree.

References...

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