

## Regulation of the expression of ethylene biosynthesis genes in *Hevea brasiliensis* shoots

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### ABSTRACT

Ethephon, an ethylene generator, is applied to the bark of rubber trees to increase natural rubber production by stimulating both latex flow and regeneration. A good command of both ethephon concentration and its frequency of application is required to avoid an oxidative burst into latex cells resulting in tapping panel dryness (TPD) and a loss of production. Although a little is known about the molecular response to ethylene stimulation further studies on ethylene biosynthesis and its regulation were needed to gain a better understanding of the mechanisms involved in latex production.

The ethylene biosynthesis pathway is well characterised in many plant species. *S*-adenosyl-methionin (SAM) is produced from the methionin (Met) by the enzyme SAM synthase. Then, SAM is converted in 1-aminocyclopropane-1-carboxylic acid (ACC) by the ACC synthase (ACS) (Kende, 1993; Yang & Hoffman, 1984). ACC is converted to ethylene by the enzyme ACC oxidase, the production of which is also highly regulated. These three enzymes are encoded by a multigene family and their expression regulated differentially by various developmental, environmental and hormonal signals (Barry *et al.*, 2000; Ge *et al.*, 2000; Hacham *et al.*, 2007; Llop-Tous *et al.*, 2000)

Several genes involved in the ethylene biosynthesis were previously isolated in rubber tree from cDNA library screening or PCR amplification of conserved regions using degenerated primers then RACE extension technology (Kuswanhadi, 2006). A differential gene expression was observed during plant development (Kuswanhadi *et al.*, 2005) and a kinetic in response to ethephon, ethylene and wounding in 3-month-old budded plants (Kuswanhadi *et al.*, 2006). In this study, we have monitored the expression of eight genes encoding four enzymes (SAMS, ACS, ACO and  $\beta$ CAS) by real-time RT-PCR. These expression levels in bark tissues of budded plant 3-month-old flushes were compared from three *Hevea* clones with contrasting metabolism.

The variation in gene expression depends on the clones and the genes. The accumulation of *SAMS* transcripts is dramatically increased in line with the decrease of the clone metabolism. In addition, this gene is induced during the daytime. *ACS* genes are poorly expressed in any clones. By contrast, *ACO1* transcripts are accumulated in clone PB 260 and its gene is down-regulated during the daytime. *ACO2* gene is more expressed at time 8 am in PB 217 than in other clones, and then induced during daytime. *ACO3* gene is expressed at a very high level in PB 260.  $\beta$ CAS transcripts are accumulated in all clones and induced by the daytime in RRM 600 and PB 260.

*SAMS*, *ACS1*, *ACS-F3*, *ACS-F10* and *ACO2* transcripts were dramatically accumulated after wounding treatment in the three studied clones. Interestingly, *ACS1* gene is the most up-regulated one. An overnight pre-treatment with an inhibitor of the ethylene action, the 1-MCP, partially blocked the induction of this gene by wounding in clones PB 217 and RRIM 600.

The expression of *SAMS*, *ACO1*, *ACO2*, *ACO3* and *βCAS* genes was very slightly modified upon ethylene treatment whatever the clone. By contrast, *ACS* gene is regulated by ethylene. Transcripts were accumulated for *ACS1* and *ACS-F10* in clone RRIM 600, *ACS-F3* in clones PB 217 and RRIM 600,

These data suggest that the studied clones respond more actively to wounding than to the ethylene treatment. The low expression level of the *SAMS* gene recorded in clone PB 260 might be a limiting factor for the ethylene production. Expression of *ACS* genes is also very low and transient in all studied clones. *ACS* is the first committed and generally rate-limiting step in ethylene biosynthesis (Kende, 1993; Zarembinski & Theologis, 1994). This inhibition and the early induction of *ACS1* might suggest that wounding effect uses the ethylene signalling pathway through the control of the degradation of the *ACS* by the proteasome dependent pathway. The differential expression of genes between studied clones is a source of variability for genetic studies using Single Nucleotide Polymorphism for instance. The production of cyanide is concomitant to ethylene. The release of toxic HCN from damaged plant tissues is generally considered as a constitutive plant defence. The expression of *βCAS* gene is not a limiting factor for cyanide detoxification.

**Acknowledgement:** This work was supported by the *Institut Français pour le Caoutchouc*.

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