Deciphering rubber biosynthesis using genome editing and artificial miRNA in *Hevea brasiliensis*.

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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).

The rubber tree is the only source of natural rubber exploited on an industrial scale. Natural rubber has been placed on the list of strategic materials for Europe since 2017. 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).

Natural rubber biosynthesis pathway

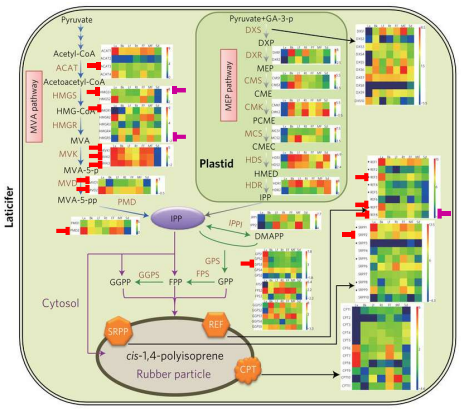

Figure 2:
Natural rubber biosynthesis pathway (Tang et al., 2016; Wu et al., 2021)

The *cis*-1,4 polyisoprene is biosynthesized from sucrose produced by photosynthesis in the leaves and translocated to specialized cells called laticifers. After loading, sucrose is metabolized into isopentenyl pyrophosphate (IPP), a monomer used for elongation of the polymer biosynthesized in the rubber particles of latex cells. All genes involved in the NR biosynthesis pathway have been identified in the genomic sequences of the Chinese rubber clone Reyan 7-33-97 [1] and in clone PB 260 [2], and particularly the genes encoding the Rubber Elongation Factor (REF1–8) and Small Rubber Particle Protein (SRPP1–10) families (Figure 2). Difficulties persist in establishing a functional model for the final step of polyisoprene chain polymerization.

A robust procedure for genetic transformation of rubber tree

An *Agrobacterium tumefaciens*-mediated genetic transformation has been developed based on the successful plant regeneration procedure using *H. brasiliensis* PB 260 somatic embryos [3-5]. The GFP was used as a visual marker (Figure 3) [6-8]. Several functional studies by endogenous or exogenous gene overexpression have been successfully carried out allowing the discovery of new functions in Hevea, notably in laticifer cell differentiation [9-11].

Gene silencing strategies in rubber tree

The identification of a highly expressed *HbMIR408* gene allowed the development of a gene silencing strategy using artificial miRNA leading to a partial inactivation of the *uidA* transcript, present in a transgenic line overexpressing this gene [12] (Figure 4). The same approach is underway with the use of sgRNAs targeted the *uidA* gene in order to obtain total inactivation.

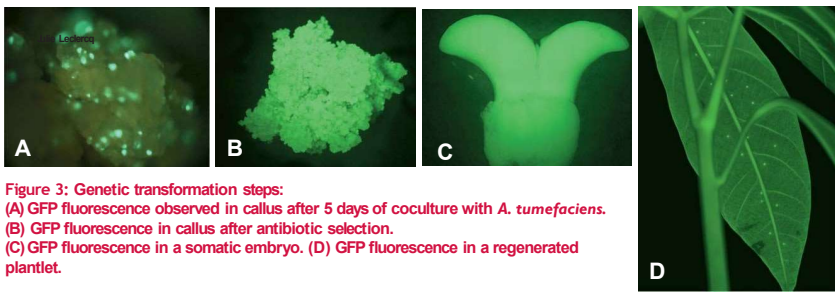

Figure 3: Genetic transformation steps:
(A) GFP fluorescence observed in callus after 5 days of coculture with *A. tumefaciens*.
(B) GFP fluorescence in callus after antibiotic selection.
(C) GFP fluorescence in a somatic embryo. (D) GFP fluorescence in a regenerated plantlet.

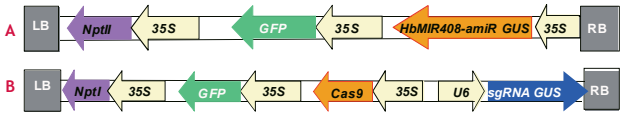

Figure 4:
(A) Construct used for gene expression silencing by artificial miRNA
(B) Construct under developement for genome editing

Conclusion

The partial or total extinction of the expression of the genes coding for the proteins of the biosynthetic complex could make it possible to disentangle the role of each protein that constitutes it by taking into account the functional redundancy.

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