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Biotechnologies in rubber tree (*Hevea brasiliensis*)

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Rubber tree breeding and the dissemination of planting material for plantations are closely linked to propagation methods. Since the progress made by switching from multiplication by seed to propagation by budding, the development of new techniques, such as micropropagation, has been awaited. An analysis of genetic diversity sets out to identify the agronomic traits to be incorporated into the best clones. More widely, genetic modification is a tool that will enable the introduction of new agronomic traits that are not available in the genetic diversity being assessed, and also to optimize the metabolism of the best cultivated clones in a targeted manner. In the next twenty years, a whole raft of innovations is set to contribute to better quality planting material through more efficient rubber tree breeding and propagation processes. Among those innovations, the establishment of a new generation of so-called juvenile budwood gardens is a possibility within the next five years. That transfer will be decisive for assessing the degree to which new technologies are taken on board in modern rubber growing. The involvement of growers and agro-industrialists upstream of the innovation process is decisive for the success of such an undertaking, as for the progress made last century.

In vitro culture research has led to three types of micropropagation techniques and genetic modification:
Microcuttings. This technique was developed from juvenile seedling material and rejuvenated clonal material by reiterated grafting on young seedling or somatic embryogenesis (Carron *et al*., 2003). The capacity of that technique therefore depends on the juvenility of the material treated in vitro. Although this procedure is labour consuming, it offers a strong advantage for true-to-type multiplication.
Short-term somatic embryogenesis. This technique is now available for about 18 clones worldwide. Although the quality of the emblings is good, the multiplication rate is limited with this method.

Long-term maintained somatic embryogenesis. This was the only technique by which mass propagation can be envisaged (Carron *et al*., 1995b). Although recent work shows this avenue to be highly promising, it is necessary to assess the emblings produced, since the risks of somaclonal variations can be detrimental to the quality of the plant material produced.
Genetic modification. This technique combines gene transfer and plant regeneration techniques by micropropagation. In Hevea, gene transfer is possible by particle bombardment, but *Agrobacterium tumefaciens* is more commonly used, combined with regeneration by somatic embryogenesis (Blanc *et al*., 2006; Montoro *et al*., 2000; Montoro *et al*., 2003).

Several cloning strategies can be considered, based on those different micropropagation techniques (Carron *et al*., 2005).

i. One-part-tree or self-rooted clones. Clones propagate directly by long-term maintained somatic embryogenesis, or indirectly by microcuttings from emblings produced by short-term embryogenesis (Carron *et al*., 1995a).
ii. Juvenile budded clones. Cultivated clone rejuvenated by somatic embryogenesis and multiplied by budding onto seedling type rootstocks. The buds come from juvenile budwood gardens established with in vitro plantlets obtained by short-term somatic embryogenesis (Carron *et al*., 1995a).
iii. Clonal root-stocks. This last strategy, whose feasibility is still being studied through seedling microcuttings, would fit in with new selection programmes for the root system, which it has never been possible to study (Carron *et al*., 2000).

In addition to the juvenility provided by these new methods, these three levels of cloning open the way for the selection of new clones in future breeding programmes, either one-part-trees (same aerial and root genotype), or a combination of root and aerial genotypes that are more compatible with each other and therefore conducive to rubber tree growth and production.

A reliable cryopreservation technique was developed for friable embryogenic callus lines of Hevea brasiliensis. This technique is based on regenerating friable embryogenic

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calli, maintained by regular subcultures at 2-week intervals. However, establishing friable embryogenic callus lines remains a limiting step given 6 to 12 months are needed to obtain a proliferating callus on the one hand, and the frequency of such callus production is low (about 1 per initial thousand explants) on the other hand. In addition, long-term maintenance of friable embryogenic calli lead to loss of callus regeneration competence in rubber tree (Blanc *et al.*, 2004) and increase the risk of somaclonal variations as it was observed in some other species (Cassells & Morrish, 1987; Sussex & Frei, 1968; Yang e*t al.*, 1999). All these problems associated with long-term cell proliferation could be overcome by long-term storage in liquid nitrogen.

An *Agrobacterium tumefaciens*-mediated genetic transformation has been developed, and has lead to genetically transformed plant expressing the *gus*A reporter gene driven either by a CAMV 35S or the Hev*2.1* promoters (Blanc *et al.*, 2006; Montoro et al., 2000; Rattana *et al.*, 2001).

Hopefully, the GFP selection would allow the avoidance of antibiotic selection step and the use of binary vector containing no antibiotic resistance gene. All those tools will be applied for generating genetically transformed plant either over-expressing or silenced for candidate genes. Besides, GFP gene can be fused to genes of interest. Both transcriptional and translational fusions are useful approaches to follow the expression of genes driven by their own promoter through the GFP activity and the subcellular localization to have a better understanding of gene function in rubber tree cells.

This functional analysis demonstrates that the *HEV*2.1 promoter has the ability to direct a strong gene expression in latex cells of root, stem and leaf (Montoro *et al.*, 2006; Montoro *et al.*, 2007). This promoter is also inducible by light, and consequently drives expression in all cells of leaves. By taking this characterization further, it might be possible to specify the role of hevein in latex and leaves, which is already known to be involved in the coagulation of rubber particles and defence against fungi (Gidrol *et al.*, 1994); (Van Parijs *et al.*, 1991). This promoter will be also useful for applications *i*n genetic engineering programmes of rubber tree, in particular to control gene expression in latex cells.

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