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BIOTECHNOLOGY AND DEVELOPMENT OF RUBBER PLANTING MATERIAL

Pascal Montoro[1], Marc-Philippe Carron[1], Ludovic Lardet[1], André Clément-Demange[1], Julie Leclercq[1], Chen Xiongting[2], Olivier Monteuuis[1]

[1] Plant Development & Breeding Joint Research Unit, CIRAD, France
[2] CATAS, Haikou, Hainan Province, China

Abstract

Breeding and dissemination of planting material for rubber plantations are closely linked with 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. Microcuttings were developed from juvenile seedling material and rejuvenated clonal material by reiterated grafting on young seedling or somatic embryogenesis. Somatic embryogenesis procedures were developed on 18 clones worldwide but researches are still needed for large-scale propagation by this method. Both zygotic and somatic embryogenesis allows rejuvenation of plant material, which offers development of new propagation strategies. Thanks to a combination of in vitro culture methods, a whole raft of innovations will be released in the next twenty years for the propagation of higher-yielding planting material. 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.

INTRODUCTION

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

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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.

Consequences of Reproductive Biology in *Hevea*

Cross-fertilization in *Hevea brasiliensis* gives rise to highly heterogeneously seed-issued offspring. Consequently, budding has been the only way to mass produce *Hevea brasiliensis* selected clones, which has so far resulted in a remarkable genetic improvement, observed since the middle of the 20th century, especially with regard to latex yields and disease resistance (Clément-Demange *et al.*, 2007).

The incompatibility of certain intraspecific, but especially interspecific, recombinations makes the introduction of certain traits difficult. Attempts to overcome those barriers have notably led to protoplast fusion and embryo rescue (Cailloux and Lleras, 1979; Sushamakumari *et al.*, 2000). However, that research has yet to find a use in genetic improvement programmes.

Challenges in Planting Material Breeding for Plantations

Even if budded clones led to a dramatic genetic progress, two factors currently limit the improvement of planting material quality. The existence of the graft slows down nutrient exchanges *via* the anarchic vascular system at graft level, and generates varying degrees of stress depending on the genetic incompatibility between the rootstock and its scion. The presence of a non-clonal root system lies behind the still strong heterogeneity existing in clonal plantations. No improvement of the root system is possible to encourage tree growth, latex production, and tolerance of various biotic and abiotic (drought) stresses. Hence, propagation of selected clones on their own root part was undertaken by in vitro culture.

ADVANCES IN BIOTECHNOLOGIES

In vitro culture research has led to three types of micropropagation techniques and genetic modification.

<u>Microcuttings</u>. 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.* Its propagation capacities are limited, but it offers a strong advantage for true-to-type multiplication.

<u>Short-term somatic embryogenesis</u>. 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 (Tabel 1).

Table 1. Clones to which regeneration by somatic embryogenesis has been obtained

Process type	Regenerated clones	Explants used	Country (Institute)	Authors
Primary somatic embryogenesis	PB 260, PR 107, RRIM 600, PB 235, RRIM 703*, IRCA 109*, PB 254*, PB 310*, BPM 24*	Immature seed integument	France (CIRAD)	Carron *et al.* 1995 (*) data not published
	Haiken 1, Haiken 2, GL1,Dafeng 95*,Reyan 7-33 97*,Wenchang 217*, Yun Yan 77-2*	Anther (mononuclear stage)	(CATAS)	Chen *et al.* 2004 (*) data not published
	PR 300	Anther (immature floral buds)	(IRRI, IBRIEC)	Sumarmadji and Darmono 2004
	RRII 105	Anther (immature floral buds) Inflorescence	(RRII)	Thulaseedharan *et al.* 2004
	Non selected clones	Cotydons of zygotic embryos	(CATAS)	Huang Tiandai *et al.* 2005
Maintained somatic embryogenesis	PB 260	Immature seed integument	France (CIRAD)	Carron *et al.* 1998
	RRII 105	Anther (immature floral buds) Inflorescence	(RRII)	Jayasree and Thulaseedharan 2004
	PB 260	Immature seed integument Primary somatic embryos	France (CIRAD)	Lardet *et al.* 2007

Long-term maintained somatic embryogenesis. This is the only technique by which inexpensive mass propagation can be envisaged (Carron *et al.*, 1995b). Although recent work has shown 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.

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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).

Ageing Concept

The ageing or maturation concept is well known from tree physiologists, especially those dealing with species propagated by cloning. According to (Fortanier and Jonkers, 1976), ageing can be divided into chronological, ontogenetical and physiological ageings.

Chronological ageing refers to the time needed to the structure under consideration to reach its current stage of development. For a tree as a whole, this is the time elapsed since the original seed from which the genotype derives has germinated to develop an increasingly complex architectural pattern, some parts of this expanding tree remaining physiologically more juvenile than others, more far away from the root system (Bonga, 1982; Schaffalitzky de Muckadell, 1959).

Ontogenetical ageing can be defined as the establishment of the successive phases of the ontogenetic process during the course of time. Ontogenetical ageing effect can be easily observed in heteroblastic plants, or species exhibiting foliar dimorphism according to their successive stages of development (Schaffalitzky de Muckadell, 1959). Such morphological changes reflect the maturation process affecting the organogenetic shoot apical meristem from which all the elements of the caulinar structure originate (Robinson and Wareing, 1969). The importance of cumulative effects of repeated mitotic divisions on ontogenetical ageing has been hypothesized (Fortanier and Jonkers, 1976; Schaffalitzky de Muckadell, 1959).

Physiological ageing is responsible for physiological changes associated with the maturation process such as flowering, fruiting, modification of foliar morphology, decline of ability for adventitious rooting, variations of growth habits, rates and cycles (Bon *et al.*, 1994; Hackett, 1985). The list is not limitative (Bonga, 1982), although the onset of flowering is classically recognized as characterizing the end of the juvenile phase (Hackett, 1985), discordances to this precept according to species refute any too dogmatic opinion and confirm the view that the juvenile state remains difficult and ambiguous to define. This is particularly true for complex structures like trees characterized by a within-individual physiological basipetal gradient of juvenility increasing with the proximity of the root system, the more distal part, ontogenetically older, being also the more physiologically mature (Bon *et al.*, 1994; Bonga, 1982).

Ageing has been an intriguing topic for many years (Krenke, 1940; Schaffalitzky de Muckadell, 1959), giving rise to various concepts and exchanges of opinions. The prevailing current view is that the shoot apical meristems are the core components of this phenomenon, mainly under physiological control as the result of correlative systems responsible for space-time variations (Krenke, 1940; Monteuuis, 1988), and under certain conditions for reversion to a more juvenile status (Borchert, 1976 ; Monteuuis, 1988).

Practical manipulations or treatments have been used for years for counteracting the negative effects of the maturation process on the true to type ability of selected genotypes which, when initially mature and depending on the species can even be rejuvenated (Franclet, 1983; Monteuuis, 1992). For instance, millions of cuttings have been clonally produced from mature selected "Plus" trees of teak or eucalypts. The relevant horticultural techniques commonly used for proper stockplant management include varying intensity of pruning, hedging or even pinching, grafting onto juvenile rootstocks and serial propagation by cuttings. It is interesting to notice that all these treatments aim at maintaining a suitable physiological balance between shoots and roots by limiting artificially a too large expansion of the branching system and enhancing therefore the influence of the root system. Such practices support Borchert's opinion (1976) about the influence of physiological correlative systems on maturation considering the root system as a supply or "source" of metabolites en endogenous substances, and the organogenic apical meristems as "sinks". In vitro culture procedures can be from the physiological standpoint interpreted in the same way, the root system being replaced by the synthetic medium acting as a "source".

In *Hevea,* the juvenility concept was very soon established but propagation by cuttings failed as consequence of a non-mastered phase change. It was not until the end of the 1980s that juvenility was studied with a view to developing the microcutting technique. Indeed, besides, somatic embryogenesis, like zygotic embryogenesis, must be duly considered as the most accomplished or sublime rejuvenation process, from an ontogenetical point of view at least. This plant material offers development of new propagation strategies.

Potential Cloning Strategies

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

- *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).

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- *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).

- *Budded clones on clonal root-stocks*. This last strategy, whose feasibility is still being studied through seedling microcuttings (Carron *et al.,* see Poster IRCE 2007), would fit in with new selection programmes for the root system, which it has never been possible to study.

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 (aerial and root genotypes), or a combination of root and aerial genotypes that are more compatible with each other and therefore conducive to rubber tree growth and production.

What Planting Material Will be Potentially Available in the Short Term for Plantations?

Self-rooted clones

Propagation of one-part trees by microcuttings is possible from material rejuvenated by short-term somatic embryogenesis. That method was tested by CIRAD at the beginning of the 1990s and a small-scale trial on RRIM 600 clone (data not published) was planted in Ivory Coast. Nowadays, that propagation method is also used by CIRAD to multiply transgenic material.

In the meantime, the method has been tested by CATAS. Buds of young *in vitro* plantlets obtained by somatic embryogenesis from anthers were introduced into the microcutting procedure. The seven clones propagated by that method are PR 107, Haiken 2, Dafeng 95, Wenchang 217, Yun Yan 77-2, RRIM 600, and Reyan 7-33-97. Trials on different rubber farms currently occupy around twenty hectares set up since 1997. The cost of an *in vitro* plantlet has been estimated at around 5 Yuans. The plants display conicity at the base of the trunks.

Rejuvenated clones

The propagation of juvenile clones offers several comparative advantages due to the ease with which it can be implemented and the limited risks taken with the quality of the planting material produced. This method requires the installation of juvenile budwood gardens, which are set up with emblings obtained by short-term somatic embryogenesis. A dozen clones are reactive to short-term somatic embryogenesis, making it possible to propose a range of rejuvenated cultivated clones rapidly. The quality criteria are:

- optimum budding success (100%),
- technical ease of multiplication (budding identical to the conventional method),
- planting material in plantations conserving both the juvenility criteria in terms of growth and trunk conicity, and a production potential at least equivalent to the mature clone,
- interaction between a juvenile root system (seedling) and a juvenile aerial system is still unknown, but should theoretically be conducive to resistance to environmental and man-made stress.

The question of juvenility conservation in budwood gardens remains whole, due to the high multiplication rate envisaged for replacing all the current industrial budwood gardens. However, current work on juvenility management in budwood gardens is based on knowledge acquired in other species, notably forest species, for which there are several decades of experience on this subject.

CONCLUSIONS AND PROSPECTS

Consortium on Biotechnology

The time taken to assess rubber tree clones is a major constraint for rapid development of new types of planting material for plantations. Substantial investments need to be made to model rubber tree growth and rubber production, and to acquire efficient biotechnologies.

Although a series of innovations is under way, it will take about twenty years of clone evaluation before recommendations can be made for the market, so long as we do not have efficient modelling techniques, and progressive assessment tests, up to true planting material production conditions, are needed. Involving growers and agro-industrialists upstream of the innovation process is needed to encourage the diffusion of new technologies in today's rubber cultivation. Indeed, there is a true risk of a breakdown in the natural rubber market. Current climate change and the threat of certain diseases like SALB might cause unprecedented damage in rubber growing. As for research on other species, substantial resources need to be pooled to invite tenders from the best biotechnology research teams to solve the fundamental issues that will enable research to efficiently take up the challenges of tomorrow.

Outputs of Biotechnologies

Biotechnologies are in a position to propose solutions for supplying plantations with improved planting material within a reasonable time lapse. If research and research-development resources were to be strengthened, biotechnologies could be incorporated into the disseminated material as follows:

- **Planting material for planting 5-10 years away.**
  - o Juvenile budwood garden for recommended clones Large-scale trials in multi-site areas.

- **Planting material for planting 10-15 years away.**
  - o Microcuttings from emblings
    - Scaling-up the production of microcutting plants in laboratories.
    - Evaluation in large-scale multi-site trials
    - growth and latex yield
    - root disease incidence (cultivated clones are **unknown phenotypes** as root parts)
- **Planting material for planting 15-20 years away.**
  - o Emblings (one-part tree)
  - o Rootstock clones
- **Planting material for planting 20-25 years away.**
  - o GMOs (resistance to diseases, drought tolerance, production of medium-value proteins, secondary metabolites, etc.).

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