Mass production of self-rooted *Hevea brasiliensis* clones by tissue culture and nursery methods

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THE SOCFIN GROUP

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- Belgian group created in 1909
- Rubber and Oil Palm plantations in Africa and Asia
- Total of **180,000 ha** out of which **65,000 ha** are planted with rubber trees

  (up to **3,000,000** plants produced per annum)
- Rubber R&D activities mainly in **SoGB**, Ivory Coast (17,500 ha of rubber trees)

Hevea brasiliensis

BOTANY

Big size tree (50m tall, 1m in girth), Rauh architectural model, with vigorous vertical taproots and horizontal lateral roots

Diploid (2n=36), perennial, monoecious and cross-pollinated tree species

Family: *Euphorbiaceae*

Origin: Amazonian rainforest forest

H. Brasiliensis in situ

Domesticated since the XIXth century for **natural rubber production**

PLANTING STOCK ORIGIN

SEEDS

Seedlings: till the end of the XIXth century, rubber tree plantations were established from seeds exclusively

Pros:

- **Natural, easy and cheap** way of producing rubber tree planting stock
- **Good vigor, soil anchorage, overall resistance to diseases and site adaptability** thanks to the high genetic diversity of the seedling-derived plantations

Seedlings plantation

Cons: Tremendous **tree-to-tree variations** in rubber yield

Rationale for developing clonal plantations:

- ✓ **higher productivity,**
- ✓ **crop uniformity,**
- ✓ **and easier management**

PLANTING STOCK ORIGIN
INDUSTRIAL CLONES GRAFTED

Grafts: since 1920s, industrial plantations have been established with grafted clones

Budwood garden

Technique: patch bud grafting

Scion origin: Industrial selected clones

Rootstock origin: Seedlings of various sources (“illegitimate monoclonal”)

Budgrafting steps

PLANTING STOCK ORIGIN
INDUSTRIAL CLONES GRAFTED

Pros:

- **Efficient** technique for mass propagating *H. brasiliensis* mature clones
- **Convenient** : does not require sophisticated and well equipped facilities
- **Good root system vigor and soil anchorage** thanks to the tap root of the seedlings used as rootstocks
- **Higher soil adaptability and less root disease risks** due to the genetic diversity of the seed-derived rootstocks

PLANTING STOCK ORIGIN

INDUSTRIAL CLONES GRAFTED

Cons:

- ***Genetically composite*** individuals
- Risks of **graft union weakness** and of **one sided/unbalanced development**
- Risks of **incompatibility and intraclonal variability**
- Need to produce rootstocks and scions of suitable size and physiological status for proper matching

SELF ROOTED INDUSTRIAL CLONES
<u>AN ALTERNATIVE TO GRAFTING</u>

Rationale:

- **Earlier and higher latex yields are expected** as rubber trees on their own roots look more vigorous and well balanced than grafted ones
- **Overcome the « 2-part plants » graft-induced drawbacks**

Background:

- **1900-1950:** unsuccessful attempts of multiplying rubber trees vegetatively on their own roots by **marcots or rooted cuttings**
- **Since the 1970's** emphasis has been given to **tissue culture** techniques, both by axillary budding and somatic embryogenesis.

19 year old microcutting at SoGB

MICROPROPAGATION
BY AXILLARY BUDDING

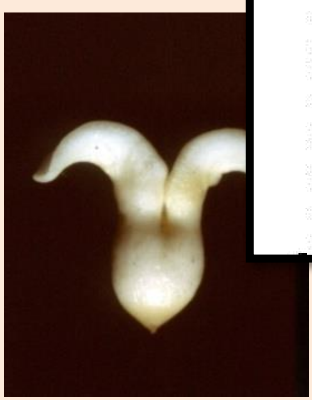
Summary of micropropagation research work on *Hevea brasiliensis*

Explants used	Results	References[a]
Shoot apices	PD, RD	Paranjothy and Ghandimathi 1975, 1976
Somatic embryos	PD	Carron and Enjalric 1982
Axillary buds from young trees	PD	[illegible]82
Rubber rootstocks	PD	Carron and Enjalric 1983
Shoot tips from immature seedling	PD	Gunatilleke and Samaranayake 1988
Shoot tips, internodes	PD, RD	Te-chato and Muangkaewngam 1992
Apices from mature shoots	PD, RD	Perrin *et al*. 1994
Axillary buds	PD, RD	Seneviratne *et al*. 1995
Nodal explants from juvenile plants	PD, RD	Seneviratne and Flegmann 1996
Shoot tips	PD	Seneviratne 1991
Axillary buds from mature clones	PD, RD	Perrin *et al*. 1997
Axillary buds	PD	Seneviratne and Wijesekara 1996
Axillary buds	PD	Lardet *et al*. 1999
Nodal explants	PD	Lardet *et al*. 1999
Axillary buds	PD, RD	Mendanha *et al*. 1998
Shoot tips	PD	Kala *et al*. 2004

Paranjothy and Ghandimathi 1975, 1976

Drawn from Venkatachalam *et al*, 2007.
PD: Plant development; RD: Root development

MICROPROPAGATION
BY SOMATIC EMBRYOGENESIS



Summary of somatic embryogenesis research work on *Hevea brasiliensis*

Explants used	Results	References[a]
Anther	CD, ED, PD	Paranjothy 1974
Anther	CD, ED	Paranjothy and Ghandimathi 1975
Anther	CD, ED, PD	Paranjothi and Rohani 1978
Anther	CD, ED	Wang *et al*. 1980, 1984
Anther	CD, ED	Carron and Enjalric 1982
Anther	CD, ED, PD	Wan *et al*. 1982
Integument tissue	CD, ED, PD	Carron and Enjalric 1985
Integument tissue	CD, ED	El Hadrami *et al*. 1991, 1992
Integument tissue	CD, ED, PD	Etienne *et al*. 1993a, 1993b
Integument tissue	CD, ED	Montoro *et al*. 1993
Integument tissue	CD, ED	Veisseire *et al*. 1994a, 1994b
Integument tissue	CD, ED, PD	Asokan *et al*. 1992
Stamens	CD, ED, PD	Wang and Chen 1995
Immature anthers	CD, ED, PD	Kumari Jayasree *et al*. 1999
Immature inflorescence	CD, ED, PD	Sushamakumari *et al*. 2000a, 2000b
Leaf	CD, ED, PD	Kala *et al*. 2005
Roots	CD, ED, PD	Sushamakumari *et al*. 2006

Drawn from Venkatachalam *et al*, 2007. CD: Callus development; ED: Embryo development; PD: Plant development;

NB: *H. Brasiliensis* is one of the very rare tree species for which SE can be obtained from mature genotypes

IN VITRO MICROPROPAGATION

TO DATE PRACTICAL ACHIEVEMENTS

Out of some ***50 superior industrial grafted clones*** *massively planted worldwide*

And after more than **40 years of heavy investments** involving several researcher teams worldwide…

Less than 10 *have been successfully micropropagated to be field tested (at an experimental scale) !*

IN VITRO MICROPROPAGATION

TO DATE PRACTICAL ACHIEVEMENTS

With special mention for the **medium-yield and more prone to windbreakage clones RRIM600 and PB260**

Whereas no rooted plantlet of clone **PB217**, **the most prized for industrial plantations**, has been transferred to *ex-vitro* yet

IN VITRO MICROPROPAGATION
MAIN LIMITATIONS

- **Genotype-dependent recalcitrance** to micropropagation and **insufficient efficiency of the tissue culture protocols** applied so far

- **Tissue culture facilities are located too far away** from natural growing conditions: hinders culture initiation and rooting/acclimatization process

- **Cost**

ROOTED CUTTINGS

TO DATE PRACTICAL ACHIEVEMENTS

In vitro culture limitations prompted SOCFIN in **2009** to work on propagating *H. brasiliensis* industrial clones by **rooted cuttings**.

3 ys later, **several thousands of rooted cuttings** were produced in SoGB, from *in vitro*-issued stock plants, to be field-established for further assessment (Masson et al., 2013).

ROOTED CUTTINGS

TO DATE PRACTICAL ACHIEVEMENTS

It was the first time that industrial clones of *H. brasiliensis* could be so massively and readily produced by rooted cuttings in nursery conditions

SoGB keeps improving its know how, with significant practical achievements :

- All the **36 mature genotypes** tested so far have been rooted
- The best clones like **PB217** are currently being propagated on their own roots at SoGB
- An increasing number of **Field trials** are being planted with such materials

ROOTED CUTTINGS

<u>PROSPECTS AND QUESTIONS</u>

Self-rooted clones can be used :

- as **stock plants in clonal garden** for producing rejuvenated material to be grafted

- for **mass producing rooted cuttings** for industrial clonal plantations

Resorting to improved tissue culture protocols judiciously applied could help in this respect.

ROOTED CUTTINGS
PROSPECTS AND QUESTIONS

Field behavior of such rooted-cutting derived clones compared to the grafted ones is another determining issue

All these aspects are currently being investigated at SoGB.

Grafted trees

Self rooted trees

«One can do nothing with nothing,

but one can do plenty with little.»

Adrien Hallet, SOCFIN's cofounder