Addressing the fears of the natural rubber supply chain regarding the dissemination of genetically modified rubber trees

Pascal Montoro

Abstract

The development of genetic engineering in *Hevea* is designed to speed up the breeding process for traits that are essential to the sustainable production of natural rubber. By using cisgenesis, rather than the transgenesis generally used for commercial varieties resistant to herbicides and insect pests, researchers are seeking to regulate genes already present in *Hevea* in order to improve the best recommended clones. Faced with the concerns of the natural rubber supply chain regarding the dissemination of transgenes via pollen and the use of genes responsible for antibiotic tolerance, research has been launched to find solutions. The first work set out to target genetic modifications outside the reproductive organs, or to induce rubber tree sterility to prevent dissemination via pollen. The second involved a genetic modification method using a selection process other than by the antibiotics needed to identify genetically modified cells. Lastly, with cisgenesis it is possible, among other things, to activate natural defences in the rubber tree or plug some potential deficiencies of certain clones with supernumerary genes taken from *Hevea.* In order to improve a complex trait such as natural rubber production, research is focused on modifying regulation factors coordinating a set of genes. Thus, a whole metabolic pathway, such as rubber biosynthesis, could be modified by controlling a single gene or just a few genes. In most countries, the remit of public research organizations includes studies on the phenomena linked to GMO use. Armed with research on gene flows, gene regulation network modelling and gene transfer techniques, studies could be conducted to evaluate the risks and potential advantages of genetically modified rubber trees that are giving concern to the natural rubber supply chain. These studies should help the public authorities decide what applications should be accorded to biotechnologies.

Article

Genetic engineering has been developed in *Hevea* in order to speed up the breeding process for traits essential to sustainable natural rubber production. Conventional breeding crossing elite parents leads to a genetic mix that generates variability in the performance of individuals within the same progeny. Molecular selection techniques can be used to identify an individual, or individuals, bearing the required agronomic traits, but the genomic disruptions introduced necessitate an assessment of numerous descendants during a selection process, in order to be sure that the new genetic combination is suitable under the desired eco-climatic conditions. These approaches, which are applied with annual species, remain difficult, lengthy and costly in species with a long biological cycle. For *Hevea,* creating a segregating population is limited by the poor success of hand pollination. In addition, it usually takes 20 to 25 years before a new clone/cultivar can be recommended. In this context, gene transfer methods would make it possible to modify the target trait without disrupting the other traits of a selected clone. Such modifications do not bring into play actual transgenesis, with the introduction of a gene from another organism, but cisgenesis, for which only the expression of an existing gene is modulated. However, epigenetic variations, resulting from stress and *in vitro* culture, have an impact on the quality of the planting material produced, making an assessment of the transgenic material necessary. To date, three research teams have published articles on the

production of genetically modified rubber trees; these are, in chronological order: Malaysia, India and France (Arokiaraj et al. 1996; Blanc et al. 2006; Jayashree et al. 2003). The study target in *Hevea* is mainly improved tolerance of abiotic stress (cold, drought, tapping, etc.) to control Tapping Panel Dryness. The production of recombinant proteins of medium added value, such as human albumin serum, is also a target for the creation of new *Hevea* products (Arokiaraj 2000). Natural rubber production is a complex agronomic trait involving numerous genes. Research work is no longer geared towards a single gene or just a few genes linked to a given metabolic pathway, but towards regulation factors that ensure upstream coordination of the set of genes in a biological process (Arokiaraj and Jones 2001; Chen et al. 2003; Duan et al. 2010; Peng et al. 2009; Zhu et al. 2006).

For more than a decade, the emergence of genetically modified organisms (GMO) has raised new ethical, commercial and scientific issues within civil society and among politicians. The article by Jim Smith in Tire Review Magazine on 20 April 2011 reported on and detailed these concerns in the natural rubber supply chain (Smith 2011). Indeed, such issues are on the agenda for rubber growing and natural rubber production because genetic engineering techniques are operational for *Hevea*. The progress achieved in the 2000s heralds future genetic engineering applications in *Hevea*. Alongside these studies, scientists are also preparing to respond to the concerns of the natural rubber supply chain, such as hybridization between transgenic and non-transgenic rubber trees, or the contamination of rubber tree by-products. As rubber is not a foodstuff, genetic modification of *Hevea* did not seem to raise any particular fears at the outset. However, the consumption of honey produced from rubber tree flowers, and the consumption of seeds by cattle raises a problem for farmers in some countries, such as India. In addition to these rubber tree by-products, the dispersal of transgenic pollen is a source of transgene flows towards non-transgenic clones.

The first reaction of researchers was to make sure that genetically modified rubber trees could be produced without using antibiotic resistance genes. Transferring such antibiotic resistance genes from a plant to another organism, such as humans, would make the latter resistant to antibiotics, which would no longer be effective in fighting certain diseases. Although no proof has been demonstrated of such a transfer, it has been shown in *Hevea* that this risk can easily be avoided by a technique that by-passes the need to use antibiotic resistance genes (Leclercq et al. 2010).

The second reaction was to target the transgene product in a single organ or tissue, to prevent the presence of the transgene product in pollen. This was particularly important for the production of Bt type insecticide proteins, which could be toxic for bees. Such targeting is achieved by a transgene expression regulator known as a promoter. For instance, the promoter of the gene encoding hevein, an agglutinin responsible for the coagulation of rubber particles in laticifer cells, has been used with success in *Hevea* (Montoro et al. 2008). Although rubber trees have a low fruiting rate, the solution envisaged to prevent any dissemination of transgenes to non-transgenic rubber crops is the production of sterile trees. As rubber trees are propagated by budding on seedling rootstocks, targeting a cytotoxic gene in the flowers ought to prevent flowering and pollen production. This technique is being studied in other woody species such as poplar (Wei et al. 2006). To overcome leaf diseases, crown-budding is also used to graft canopy of resistant clones. Such technique can be easily carried out to graft crown of non-transgenic material to generate budded clone with only transgenic trunk.

A third public concern often arises from the fact that transgenes come from other organisms. This is the case for so-called Bt crops resistant to insects or tolerant of insecticides. For example, the Bt transgene encoding a toxin for insect larvae was originally isolated from the bacterium *Bacillus thuringiensis*. Research on the physiology and molecular biology of rubber trees is opening up much vaster prospects. The idea is not to use transgenes from other organisms, but to activate or deactivate certain functions specific to *Hevea.* This method, which does not use any foreign genes, is called cisgenesis, as opposed to transgenesis. These new-generation GMOs can be used, among other things, to activate the natural defences of rubber trees or plug potential deficiencies in some clones. For example, controlling oxidative stress generated by various types of abiotic stress, including rubber tree exploitation stress (tapping and ethephon stimulation) is currently being investigated by Indian and French teams (Leclercq et al. Submitted; Sobha et al. 2003). For instance, activating the rubber tree antioxidant system leads to better growth and tolerance of a water deficit in juvenile plants (Leclercq et al. Submitted). In order to gain a clearer understanding of how such modifications affect trees, field trials have been announced by the Indian team (India 2010 ; Jacob 2011; Kumar 2010).

To conclude, researchers possess tools for improving *Hevea* clones by genetic engineering. Awareness of public concerns has led to work intended to limit the risks associated with the dissemination of genetically modified rubber trees. Inhibiting flowering, by-passing the use of antibiotic resistance genes and developing cisgenesis are some of the solutions proposed. However, with the emergence of genetically modified rubber clones, it will be necessary to overcome public fears in order to launch field trials with genetically modified rubber trees, whilst respecting national regulations, and to obtain the funding needed for biotechnology research. The time needed to assess these genetically modified clones will be similar to that for traditional clones. Thus, long-term investments will be required without any guarantee of returns on that investment, due to the fact that *Hevea* clones are propagated by budding, which does not guarantee any control over the planting material. If these constraints are removed, the first recommendations for genetically modified rubber tree clones could be made, in the best of cases, in the 2020s and thus, along with the progress made in conventional rubber tree breeding, respond to the increased demand for natural rubber.

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Program
2011 IRRDB International Rubber Conference
14 - 17 December 2011 in Chiang Mai Thailand

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14 December 2011

14.00 – 17.00 **: Registration**

15 December 2011

Room 1

08.00 – 08.45 **: Registration**

09.00 – 09.50 **: Opening Plenary**

- Welcome Address by Vice Governor of Chiang Mai Province, Mr.Woragan Yokying
- Statement by the Secretary General of the IRRDB
- Opening Speech by Deputy Director – General, Department of Agriculture, Ms.Mantana Milne
- Presentation of the IRRDB B.C. Sekhar Award for Research Excellence
- Citation to be read by Mr. Hubert Omont

09.50 – 10.20 **: Coffee break**

Chairman: Mr. Hubert Omont, CIRAD

10.20 – 10.45 **: Invited paper 1** Facing Challenges in Rubber Diseases (Dr. C.K. Jayasinghe)

10.45 – 11.10 **: Invited paper 2** Progress on Rubber Breeding of Thailand (Dr.Napawen Lekawipat)

11.10 – 11.35 **: Invited paper 3** How Rubber will Bounce in a Volatil World (Dr. Stephen Evans, Secretary General, IRSG)

11.35 – 12.00 **: Invited paper 4** Factor Determing NR Prices in the Short and Medium Term (Jom Jacob, Senior Economic, ANRPC)

12.00 – 13.00 **: Lunch**

Exploitation And Physiology

Chairman: Dr. James Jacob, Director RRI India,

13.00 – 13.15 **: Paper 1** Low Frequency Tapping Systems as for the Solution of Labour Crisis (Panus Paechana)

13.15 – 13.30 **: Paper 2** Influence of Tapping System on Rubber clone ; RRIT 251 (Sajeerat Raemlee)

13.30 – 13.45 **: Paper 3** Assembly of gss-cut Exploitation System on Slow Starter Clones and Stimulantion With Ethylene GAS (Atminingsih)

13.45 -14.00 : **Paper 4** The "Double Cut Alternative" (DCA) Tapping System : An Innovative Tapping System Designed for Thai Rubber Smallholdings Using High Tapping Frequency.
(Eric Gohet)

14.00 – 14.15 : **Paper 5** Carbohydrate Variation of Hevea Induced by Tapping Systems
(Pisamai Chantuma)

14.15 – 14.30 : **Paper 6** Effects of Drought and Tapping for Latex Production on Water Relations of Hevea Brasiliensis Trees
(Mr.Sumit Kunjet)

14.30 – 14.45 : **Paper 7** A Modified Stimulation Method in *Hevea* Brasiliensis for Reducing Oxidative Stress
(R. Krishnakumar)

14.45 – 15.00 : **Paper 8** Sudden Dieback of Young Budded Rubber (*Hevea brasiliensis* Muell. Arg.) Plants at Nursery Stage Under Hot and Dry Climatic Conditions
(A. M. W. K. Senevirathna)

15.00 – 15.30 : **Coffee break**

Chairman: Dr. Chairil Anwar, Director Indonesian RRI

15.30 - 15.45 : **Paper 9** Wood Production and Lumber Recovery of 4 Recommended Rubber Clones in Thailand
(Krissada Sangsing)

15.45 – 16.00 : **Paper 10** Analysis of Wood Quality in Hevea Drasiliensis : Estimation and Quantification of Lignin bio-Polymer and Cell Wall Phenolics
(Dr. C.P.Reghu)

Plantation Agronomy

Chairman: Mr. Suchin Maenmeun, Director RRI Thailand, Chairman of the IRRDB

16.00 – 16.15 : **Paper 11** Model of Developing and Strengthening of Rubber Nursery Institutional to Improve the Quality of Plnting Material and Productivity of Indonesian Rubber Smallholdings
(Lina Fatayati Syarifa)

16.15 – 16.30 : **Paper 12** Modelling of Hevea Yield Production Based on Clone, Soil, And Climate Potential
(Imam Susetyo)

16.30 -16.45 : **Paper 13** Growth Characteristics of Five Rubber Clones at High Elevation Area in South Sumatra
(Thomas Wijaya)

16.45 -17.00 : **Paper 14** Growths and Carbon Stocks of Para Rubber Plantations on Phonpisai Soil Series in Northeastern Thailand
(Chakarn Saengruksawong)

18.00 : **Dinner**

15 December 2011

Room 2

Pests And Diseases Management

Chairman: Prof. Dr. Liu Guodau, Director of RRI CATAS, China

13.00 – 13.15 : **Paper 1** Corynespora Leaf Fall on Rubber in Vietnam, Current Status and Recent Studies
(Phan Thanh Dung)

13.15 – 13.30 : **Paper 2** Pathogenicity Assay of Corynespora Cassiicola Isolates From Rubber Tree and Other Hosts in Vietnam
(Nguyen Don Hieu)

13.30 – 13.45 : **Paper 3** Epidemic of *Corynespora* Leaf Fall on Rubber Orchard in Cote D'Ivoire : Assessment *in Vitro* of Clonal Resistance and Fungicides Effectiveness.
(Wahounou P. J)

13.45 -14.00 : **Paper 4** Differential Expression Analysis by CDNA-AFLP of *Hevea brasiliensis* After inoculation with the Pathogen *Corynespora Cassiicola*
(Huang Guixiu)

14.00 – 14.15 : **Paper 5** Efficacy of Fertilizers to Control White Root Disease of Rubber Caused by *Rigidoporus Microporus* at the Early Planting Stages
(Mrs.Arom Rodesuchit)

14.15 – 14.30 : **Paper 6** Study on the Potency of Serratia Bacteria Used to Control White Root Disease in Rubber
(Tri Rapani Febbiyanti)

14.30 – 14.45 : **Paper 7** The Cell Differentiation Observation at the Early Infection Stage of Oidium heveae and a Method of RNA Extraction of This Pathogenic Fungus
(Mr. Wan Sanlian)

14.45 – 15.00 : **Paper 8** Establish the Indoor Identification Model of Rubber Tree (*Heveae brasiliensis*) to Powdery Mildew (*Oidium heveae*)
(Tu Min)

15.00 – 15.30 : **Coffee break**

Chairman: Dr. Yin Song, Director Cambodian RRI

15.30 -15.45 : **Paper 9** Disease Caused by Botryodiplodia Theobromea Pat on Rubber Tree in Vietnam : Current Status and Recent Studies
(Tran Anh Pha)

15.45 – 16.00 : **Paper 10** Cultural and Morphological Characterizations of *Fusicoccum* sp., the causal agent of rubber (*Hevea brasiliensis*) leaf blight in Malaysia
(Nyaka Ngobisa Aurelie)

Plantation Agronomy

Chairman: Prof. Osayanmo Eguavoen, Director RRI Nigeria

16.00 – 16.15 : **Paper 11** Forestation With Rubber for Carbon Markets ; Yield Tables Under Srilankan Conditions
(E. S. Munasinghe)

16.15 – 16.30 : **Paper 12** Estimation of Rubber Stand Age in Typhoon and Cold Weather Afflicted Area With Landsat Tm Data : A Case Study in Hainan Island, China
(Bangqian Chen)

18.00 : **Dinner**

16 December 2011

Room 1

Breeding and Biotechnology

Chairman: Dr. Lai Van Lam, Director RRI Vietnam

08.30 – 08.45 : **Paper 1** Performance of RRIT 251 Clone in Traditional and Non-Traditional Area
(Ms.Patra Kinnaret)

08.45 – 09.00 : **Paper 2** Adaptation of Rubber Tree (*Hevea Brasiliensis*) Clones to Marginal Areas of Cote D'Ivoire
(ELABO A.A.E.)

09.00 – 09.15 : **Paper 3** *Hevea Brasiliensis* : Results From The Cambodian Large Scale Clone Trials in 2011
(Dr. Phen Phearun)

09.15 - 09.30 : **Paper 4** Potential of IRR 200 Series Promising Rubber Clones on Further Trials in Indonesia
(Aidi Daslin)

09.30 – 09.45 : **Paper 5** Performances of Elite Rriv's Clones Derived From 1994 Hand Pollination Program
(Le Mau Tuy)

09.45 -10.00 : **Paper 6** Genetic Variabilty of Interspesific Crossing Result Between RRIM 600 X PN 1546 Rubber Parental Clones
(Sekar Woelan)

10.00 – 10.30 : **Coffee break**

Chairman: Mr. N'diaye Oumar N'gor, FIRCA, Cote d'Ivoire

10.30 – 10.45 : **Paper 7** Climate Change: Study on Stability of Rubber Clones in Field Trials
(Dr. Nasaruddin Md. Aris)

10.45 – 11.00 : **Paper 8** Polycross Breeding Towards Evolving Genetically Diverse Hevea Clones for Sustainbility
(Dr. Kavitha K. Mydin)

11.00 -11.15 : **Paper 9** Attempts to Evolve Compact Crown Clones of Hevea Brasiliensis
(T. Gireesh)

11.15 – 11.30 : **Paper 10** Early Performance of a Few Indigenous and Exotic Clones of *Hevea Brasiliensis* in a Large Scale Trial
(V.C. Mercykutty)

11.30 – 11.45 : **Paper 11** Evaluation of Some Clones (Hevea Brasiliensis) in a Small Scale Trial in the Southern Part of Guatemala
(Gremial de Huleros)

11.45 – 12.00 : **Paper 12** Application of QTL Mapping for Early Selection on Growth and Latex Yield Traits in Rubber Breeding.
(Ms. Ratchanee Rattanawong)

12.00 – 12.15 : **Paper 13** Identification of Drought Tolerant Genes by Quantitative Expression Analysis in *Hevea Brasilensis*
(Mohamed Sathik)

12.15 – 13.00 : **Lunch**

Chairman: Dr. Mohd Nasaruddin Md. Aris, Director of Production Development, Malaysian Rubber Board

13.00 – 13.15 : **Paper 14** Genetic Analysis and Population Structure of Rubber Tree for Association Mapping.
(Dr.Thitaporn Phumichai)

13.15 – 13.30 : **Paper 15** Identification of Rubber Clones (Hevea Brasiliensis) Using Inter Simple Sequence Repeat (ISSR) Markers
(Hoang Thi Lieu)

13.30 – 13.45 : **Paper 16** Development and Characterization of EST-SSR Markers From *De Novo* Transcriptome Sequencing Data in Rubber Tree (*Hevea Brasiliensis*)
(Dejun Li)

13.45 – 14.00 : **Paper 17** Progress of RRIT's *Hevea* Somatic Embryogenesis
(Wittaya Prommee)

14.00 – 14.15 : **Paper 18** Exploitation of in Vitro Induced Zygotic Polyembryony for Genetic Transformation in *Hevea Brasiliensis*
(Rekha. K)

14.15 – 14.30 : **Paper 19** Unfertilized Ovule - A Potential Explant for Somatic Embryogenesis in *Hevea Brasilensis*
(Jayashree,R.)

14.30 – 14.45 : **Paper 20** Histochemical and Immunohistochemical Identification of Laticifer Cells in Callus Cultures Derived From Anthers of *Hevea Brasiliensis*
(Deguan Tan)

14.45 – 15.00 : **Paper 21** Improvement and Application of the Technique of Mini-Seedling Budding of *Hevea Brasiliensis*
(Lin Weifu)

15.00 – 15.30 : **Coffee break**

Chairman: Mr. Nicomedes Eleazar, Director BAR, Philippines

15.30 – 15.45 : **Paper 22** Identification of *HbNIN2* as the Key Invertase Responsible for Sucrose Catabolism in Rubber-Producing Laticifers, A Rate-Limiting Step Determining Rubber Productivity
(Chaorong Tang)

15.45 -16.00 : **Paper 23** Transgenic plants over-expressing *HbCuZnSOD* cytosolic isoform are more tolerant to a water deficit
(P. Montoro)

16.00 – 16.15 : **Paper 24** Addressing the fears of the natural rubber supply chain regarding the dissemination of genetically modified rubber trees
(Pascal Montoro)

Plantation Agronomy

16.15 – 16.30 : **Paper 25** Effect of Slow Release Fertilizer on Three-Whorl Polybag Rubber Planting.
(Ramli Abd Majid)

16.30 – 16.45 : **Paper 26** Establishment of Standard Values for Nutritional Diagnosis in Soil and Leaves of Immature Rubber Tree
(Mrs.Saichai Suchartgul)

16 December 2011

Room 2

End Use And Processing Technology

Chairman: Dr. R.B. Prenadasa, Director Ganeral, Rubber Development Department, Sri Lanka

08.30 – 08.45 : **Paper 1** Solar Drying Chamber with Furnace for Rubber Sheet
(Mrs.Preprame Tassanakul)

08.45 – 09.00 : **Paper 2** Biomolecules Contents and Rubber Properties of Some *Hevea Brasiliensis* Clones
(Dr.Chatchamon Daengkanit Nathaworn)

09.00 – 09.15 : **Paper 3** The Latest Development in the Application of the Seismic Rubber Bearing Technology
(Kamarudin Ab Malek & Le Jiang Jun)

09.15 - 09.30 : **Paper 4** The Design of Compound Formulae Based on Natural Rubber Blend for Engine Mounting Preparation
(Dadi R. Maspanger)

09.30 – 09.45 : **Paper 5** Porous Pipe Production for Agriculture
(Ms.Sumana Jammeuan)

09.45 -10.00 : **Paper 6** A Method for Producing Carbon Black Silica Master Batch
(Rosamma Alex)

10.00 – 10.30 : **Coffee break**

Chairman: Dr. Jerome Saint Beauve, CIRAD

10.30 – 10.45 : **Paper 7** Utilization of waste fo Rubber Plantation and Crumb Rubber Factory as a Source of Bioenergy.
(Didin Suwardin)

10.45 -11.00 : **Paper 8** SWIM-BED Reactor for the Treatment of Latex Concentrate Wastewater.
(Nguyen Ngoc Bich)

11.00 – 11.15 : **Paper 9** Synthesis of Silvernanoparticles Using *Hevea* Leaf and Latex
(Jayasree Gopalakrishnan)

Social - Economic

Chairman: Dr. Stephen Evans, Secretary General IRSG

11.15 – 11.30 : **Paper 10** The Development of Forward Market Model
(Ms.Athiwee Daengkanit)

11.30 – 11.45 : **Paper 11** Future Prospect for Udon Thani as an SEZ for Rubber Industry
(Suthee Intraskul)

11.45 – 13.00 : **Lunch**

Chairman: Dr. Karyudi, Head Sungei Putih Research Centre, Indonesian RRI

13.00 – 13.15 : **Paper 12** Performance Evaluation of Rubber, Hevea Brasiliensis, Towards Commercialization in Quezon Province.
(Dr. Cecilia N. Gascon)

13.15 – 13.30 : **Paper 13** Nong Khai Rubber Learning Centre Oriented by Famers' Participation
(Mr.Kaset Nabsanit)

13.30 – 13.45 : **Paper 14** Integrated Farming in Rubber Plantation Along Chai Pattana Foundation
(Mr. Thongchai Kamkote)

13.45 – 14.00 : **Paper 15** Transfer of Technology Project in Davao Region, Philippines.
(Alfredo Cayabyab)

14.00 – 14.15 : **Paper 16** Community-Based Participatory Action Research (CPAR) on Rubber-Based Farming System in the Philippines.
(Roger O. Bagaforo)

14.15 – 14.30 : **Paper 17** Grassroots Level Rubber-Based Agroforestry Initiatives in the Philippines: Capitalizing On Lessons and Experiences
(ROSELYN F. DAELMO (UPLB))

14.30 – 14.45 : **Paper 18** Anticipated Constraints for Sustainable Smallholder Rubber Farming: A Case Study in the Moneragala District of Srilanka.
(Mrs.Wasana Wijesuriya)

14.45 - 15.00 : **Paper 19** JDM88 Agro-Ventures Rubber Based Farming System (Jerry Gil S. Murao)

15.00 – 15.15 : **Paper 20** Rubber- Based Agroforestry in Five States : Effort Towards Conserving Farmers' Priotized Medicinal Plants in Nigeria. (Mrs. Eseosa S. Osazuwa)

17 December 2011

09.00 -17.00 : **Field Trip to The International Horticulture Exposition Royal Flora Ratchapruek 2011**