



**Report on the
IRRDB 1981 Hevea germplasm
by Cirad (France)
Year 2002**

**Presented at the IRRDB Workshop
on Breeding, Agroforestry and Socioeconomy,
August 28 – September 06, 2002**

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Abstract

This report sums up the different rubber germplasm operations carried out with Cirad participation, mainly in close cooperation with Cnra-Côte d'Ivoire, relating to collections, introduction and dispatching of germplasm, field evaluation, genetic diversity analysis, assessment of recombination. Results issued from research of the last three years are presented. The current position of Cirad and its specific interests related with IRRDB 1981 germplasm are explained. A discussion about possible strategies for the use of IRRDB rubber germplasm is proposed. Specific interest is expressed for genetic variability management, with possible complementary roles of a core collection and of 'working populations' (to be developed), for natural pollination applied to germplasm recombination and for the development of a IRRDB germplasm database.

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1. Introduction

The 1981 international collection of wild genetic resources from *Hevea brasiliensis* species, organised by IRRDB in the 3 Amazonian states of Acre, Rondonia and Mato Grosso with the essential partnership of Brasil, has been the predominant operation aimed at enlarging the genetic base of the cultivated *Hevea brasiliensis* species since the beginning of rubber cropping one century before. Performed on a network basis, this very important operation allowed the creation and maintenance of 2 Conservation Centres in Malaysia and Côte d'Ivoire (assisted by IRRDB funding), and the dispatching of the IRRDB 1981 germplasm to most of IRRDB members.

During the last 20 years, many efforts were devoted to field and lab characterisation of the IRRDB 1981 germplasm and actions for integrating it in rubber breeding. An important and new variability has been provided to breeders, which can be used for improving growth, yield, resistance to leaf diseases, quality of rubber, rubberwood production, etc.

In the same time, new tools issued from biotechnologies, such as genetic molecular markers and genetic engineering have been developed and made available for genome analysis. Molecular markers played a key role in the analysis of the genetic structure of the IRRDB 1981 germplasm. As the available accessions are just specific combinations of the genes of the gene pool of *Hevea brasiliensis*, the future identification of more and more useful expressed genes will probably lead to consider the *Hevea brasiliensis* IRRDB germplasm, as well as the germplasm from allied species, as a valuable reserve of molecular resources.

In the following text, the term 'accession' designates one genotype belonging to basic genetic resources, multiplied by grafting as any other clone. IRRDB 1981 germplasm accessions will be designated as 'IRRDB-Germ' with :

- **IRRDB-Germ/Asia** for accessions of the Asiatic Conservation Centre in Malaysia
- **IRRDB-Germ/Africa** for accessions of the African Conservation Centre in Côte d'Ivoire.

2. Summing up of germplasm operations carried out with Cirad participation

These operations are briefly presented hereafter :

- 1974-1976 : French-Brasilian collection of Amazonian accessions (Acre and Rondonia, *Hevea brasiliensis*). Introduction of 44 clones in Côte d'Ivoire. Introduction in Côte d'Ivoire of some clones issued from allied species and observation in arboretum (the arboretum BMOA15 has now been replanted for other purposes). Introduction in Côte d'Ivoire of 18 MDF clones (Madre de Dios/Firestone) issued from a Firestone collection in Peru.
- 1979-1981 : Participation to the organisation of IRRDB international collection, and participation to the collection of seeds (predominant part) and budwood (around 200 accessions) in the 3 Brazilian States of Acre, Rondonia, Mato Grosso (16 districts and 60 locations) in 1981. Provision of Cirad quarantine station facilities at Guadalupe Island for the transfer of accessions collected in the form of budwood to Asia and Africa.

- 1980-1995 : Creation (hand pollination) and evaluation of Wickham x Amazonian full-sib families in Côte d'Ivoire (in seedling evaluation trials). Evaluation of Wickham x Amazonian clones of different types (IAN, FDR, FX, GU, W x IRRDB-Germ) in different small scale trials.
- 1981-1989 : Study of 20 accessions from Brazilian-French collection in 1974 + MDF accessions in Côte d'Ivoire (BMOA21, 30 trees/clone).
- 1981 : Receipt of IRRDB-Germ/Africa in Côte d'Ivoire, and settling at quarantine site of Divo (greenhouse and field).
- 1983-1984 : Planting of IRRDB-Germ/Africa source bush nursery at Bimbresso central station, near Abidjan.
- 1984 : Receipt in Bimbresso of 147 IRRDB-Germ issued from budwood (AC/I, RO/I, MT/I), transfered from Guadalupe Island quarantine station.
- 1984-1988 : Transfer of IRRDB-Germ/Africa mother-trees from Divo quarantine station to Bimbresso central station and planting of the Gene Pool Garden. But transplanting success was poor and many trees died. This field has now been replanted with commercial clones.
- 1984-1988 : First evaluation of IRRDB-Germ/Africa genetic diversity by the use of morphological characters of leaves and growth units (Lesprit, 1984).
- 1984-1992 : Evaluation of IRRDB-Germ/Africa genetic diversity by the use of isozyme genetic markers (Chevallier, 1988).
- 1985-1998 : Planting of a trial (normal density : 510 trees/ha) with one grafted tree per accession for around 2500 IRRDB-Germ/Africa (Bimbresso, BMOA38). At the closure of the trial, the field was maintained as a conservation arboretum for IRRDB-Germ/Africa.
- 1985-1993 : Planting and evaluation of a trial with 64 IRRDB-Germ Africa (Bimbresso, BMOA33, normal density, 6 trees per accession).
- 1985-1993 : Evaluation of 16 full-sib Wickham x Amazonian families (Bimbresso, BMOA32 ; 2 Wickham female and 14 Amazonian male parents from 1974 Brazilian-French collection + MDF ; 5 clones per family ; 2 x 8 trees per clone).
- 1987 : Introduction in Côte d'Ivoire of 24 CNSAM clones from a bilateral exchange with Cnpsd/Embrapa in February 1985, issued from Brazilian collections in the State of Amazonas between 1976 and 1980 (funded by STD1 European contract TSD-A-182).
- 1986-1990 : Dispatching of IRRDB germplasm budwood from Côte d'Ivoire to Cameroon (1500 accessions) and to Nigeria (2500 accessions).
- 1987-1988 : Introduction in Côte d'Ivoire of a set of 341 accessions from the 2 Columbian sites of Calima and Palmira, issued from collections of Professor R.E. Schultes (funded by STD1 European contract TSD-A-182).

- 1988-1989 : Exchange of around 200 IRRDB-Germ between the two IRRDB Conservation Centres (Malaysia and Côte d'Ivoire) including Schultes accessions from Côte d'Ivoire to Malaysia.
- 1989-1997 : Planting and evaluation of a trial with 122 IRRDB-Germ/Africa representing 8 districts of the collection (Hevego, GOOA1, normal density, 20 trees per accession).
- 1990-1998 : Planting and evaluation of a trial with 23 Cnsam accessions, 77 Schultes-Calima accessions, 40 Schultes-Palmira accessions (Hevego, GOOA2, normal density, 20 trees per accession).
- 1990-1995 : Analysis of the genetic structure of IRRDB-Germ by the use of RFLP genetic molecular markers at nuclear genome level (Besse, Seguin) and at mitochondrial genome level (Luo, Boutry).
- 1991-2001 : Planting and evaluation of 379 IRRDB-Germ/Africa which had been selected for yield in trial BMOA38 (Bimbresso, BMOA50, normal density, 6 trees per accession).
- 1992-2001 : Setting of 3 isolated natural pollination gardens in Divo (Côte d'Ivoire), with 3 samples of IRRDB-Germ/Africa (total of 100 accessions). Methodological analysis of natural pollination in one of them by using microsatellite markers for paternity identification.
- 1992 : Exchange of around 200 IRRDB-Germ between Côte d'Ivoire and Vietnam.
- 1992 : Transfer of 324 IRRDB-Germ from Côte d'Ivoire to French Guyana.
- 1993-2001 : Planting and evaluation of a trial with 124 IRRDB-Germ/Asia, 55 IRRDB-Germ/Africa, 203 Schultes-Calima accessions (Bimbresso, BMOA53, normal density, 6 trees per accession).
- 1993-2001 : Planting and evaluation of a trial with 42 IRRDB-Germ/Africa, 68 IRRDB-Germ/Asia, 11 Schultes accessions, 29 other Amazonian accessions from MDF and 1974 Brazilian-French collection (Bimbresso, BMTA13, normal density, 6 trees per accession). Around 1000 IRRDB-Germ have been evaluated at field level in Côte d'Ivoire with a good level of accuracy (trials at normal density with 6 to 20 trees per accession).
- 1993 : Setting of a full-sib families design for the estimation of Wickham x Amazonian crosses genetic parameters such as additive and dominance variance (Côte d'Ivoire, Hevego, GOOA7, not analysed yet).
- 1995 : Synthesis of informations issued from agromorphologic, isozymic and molecular markers. Provision of a representation of the genetic structure of *Hevea brasiliensis* germplasm (IRRDB-Germ, Schultes and Wickham) with 6 genetic groups (Seguin et al., 1999) : Am1 (G1), Am2 (G2), Am3 (G3), Am4 (G4), Pal (G5), W (G6).
- 1995 : Dispatching of around 150 IRRDB-Germ/Africa (part of working population) to Yunnan Institute for Tropical Crops (YITC in Jing Hong, Yunnan/Xishuangbanna, China).

- 1995-1999 : Planting and evaluation of 25 miscellaneous clones in French Guyana, including 5 IRRDB-Germ/Asia and 1 IRRDB-Germ/Africa (Pinard et al., 1999).
- 1996 : Preliminary approach of diversity evaluation of molar masses of the rubber product for 44 IRRDB-Germ/Africa (Acre and Rondonia) compared with some Wickham clones, with view to evaluate the variability of technological properties within the Hevea germplasm (Bimbresso).
- 1997 : Genetic diversity analysis of IRRDB-Germ/Africa based on agronomic data from trial BMOA38. The 4 genetic groups of IRRDB-Germ are confirmed by this way (Vi Cao, 1997).
- 1997 : Transfer of 49 IRRDB-Germ from French Guyana to Brasil (Michelin-Mato Grosso Estate + Embrapa-Cenargen).
- 1998 : Genetic diversity analysis of IRRDB-Germ/Africa by the use of 17 microsatellite markers, confirming the 4 genetic groups of IRRDB-Germ (Seguin et al., in prep.).
- 1998 : Preliminary methodological approach for the definition of a 'core collection' of rubber tree, combining both agronomic traits and molecular genetic markers data, performed on a restricted sample of 183 accessions, with PCSS method set up by Ird (Institut de Recherches pour le Développement, Montpellier-France, Hamon et al., 1998).
- 1999-2000 : Observations of field resistance of IRRDB-Germ/Africa to *Microcyclus ulei* in French Guyana (298 accessions) and Mato Grosso-Brasil (49 accessions) with two different pathological pressures (Le Guen et al., in prep.).
- 2000 : Evaluation of susceptibility to cassiicoline (Corynespora toxin) of 133 miscellaneous clones including 10 IRRDB-Germ/Asia by toxin-based lab-test in French Guyana (Breton, not published).
- 2002 : Initiation of the building of a database for storing germplasm information and providing easy access to data available for each accession.

3. Last results from Cirad participation to germplasm research

3.1. Yield evaluation in Côte d'Ivoire

In trial BMOA50 planted in 1991 with 379 IRRDB-Germ/Africa previously selected for yield in BMOA38 trial, mean cumulated yield of IRRDB-Germ for the period of 3 years (April 1997 to March 2000) was of 3.3 kg/tree which represents 25.2 % of the production of GT1. Differences between the four genetic groups are small and vary only from 26 % (Am1) to 31 % (Am3). 6 IRRDB-Germ were producing more than GT1, 14 IRRDB-Germ were higher than 80 % of GT1 and 28 IRRDB-Germ were higher than 50 % of GT1. IRRDB-Germ, because of its low production, now has a girth a little higher than that of GT1 (101 %).

Trial BMOA53 includes 124 IRRDB-Germ/Asia, 55 IRRDB-Germ/Africa and 203 Schultes-Calima accessions. Mean cumulated yield of IRRDB-Germ for 3 years (April 1998 to March

2001) was of 20 % of the level of GT1 (Am1 : 11 %, Am2 : 22 %, Am3 : 21 %, Am4 : 25 %). A slight difference is observed between IRRDB-Germ/Asia (20 % of GT1) and IRRDB-Germ/Africa (18 % of GT1) but it must be underlined that the 2 samples have not exactly the same structure. Yield level of Schultes-Calima (which is included in Am1 group according to molecular markers data) is of 13 % of GT1. 2 IRRDB-Germ yield more than GT1, and 13 IRRDB-Germ + Schultes yield more than 50 % of GT1.

Trial BMTA13 includes 162 clones of different types (Amazonian, IRRDB-Germ, Schultes, Wickham x Amazonian). Yield was measured from April 1998 to March 2001 and varies from 3 % to 139 % of GT1. Comparison of groups cannot be made due to small sizes of the different types. 20 accessions yield more than 50 % of the level of GT1.

Due to competition between neighbouring plots, growth and yield evaluation of field trials in Côte d'Ivoire has now reached its end. Around 1000 IRRDB-Germ are now characterized with a good level of accuracy. These trials could still be useful for evaluation in case of a leaf disease attack or for analysing the variability of technological properties. Branching habits can also be observed. As far as these fields in Bimbresso and in Hevego (BMOA38, BMOA50, BMOA53, BMTA13, GOOA1, GOOA2) can be maintained and not replanted for other purposes, they, together with the source-bush nurseries, contribute to reliable conservation of IRRDB-Germ.

3.2. IRRDB-Germ and *Microcyclus ulei*

Among 25 miscellaneous clones set to the field and evaluated for Salb resistance in French Guyana, including 5 IRRDB-Germ/Asia and 1 IRRDB-Germ/Africa (Pinard et al., 1999), all the 6 IRRDB-Germ accessions were found resistant to Salb. RRIM/AM/22/418 (RO/JP/3) never showed any symptom of infection, as well as clone IAN6158. However, these results could be changed in other locations with higher Salb pressure.

Observations of field resistance of IRRDB-Germ/Africa to *Microcyclus ulei* were performed in French Guyana (298 accessions) and Mato Grosso-Brasil (49 accessions), in source bush nurseries with two different pathological pressures (Le Guen et al., in prep.). Two sets of observations were made in French Guyana, in early 1999 and late 2000. One set of observation was made in Mato Grosso in early 1999. A high proportion of Salb-susceptible accessions was found for IRRDB-Germ issued from Mato Grosso origin (from 39 % to 81 % depending on observations), whereas accessions from Acre and Rondonia origins were more often found resistant (from 11 % to 26 % of susceptible accessions depending on observations). The same accessions observed in the two sites exhibit similar classification but with a higher pathologic pressure in Mato Grosso where only 4 accessions (from Acre and Rondonia) are found completely resistant and all accessions from Mato Grosso origin are found highly susceptible.

3.3. IRRDB-Germ and *Corynespora cassiicola*

The susceptibility of 133 clones to cassiicoline (toxin-based lab-test) was assessed in French Guyana in the framework of *Corynespora* resistance analysis (F. Breton, 2000, Cirad unpublished report). The test was performed with 3 levels of toxin and a global score from 0 (highly susceptible) to 3 (resistant) was used. The 3 clones GT1, RRIM/AM/24/242 (issued from the Asiatic Conservation Centre, belonging to RO/C/8 district) and IAN6546 were scored « 3 ». The 13 clones F4512, Harbel29, PFB5, PA31, AC55, AC68, RRIM/AM/36/485

(AC/FA/6 district), FDR76, MDF6, FX2784, FX25, RRIM/AM/22/374 (RO/JP/3 district) and RO55 were scored « 2 ». It must be noticed that F4542 (*Hevea benthamiana*) appeared to be highly susceptible. Another test was performed in the greenhouse of Montpellier with a set of 52 clones including AC58 (supposed to be an offspring of F4542 and RO38 (clone which proved to be identical to FX3899 and so an offspring of F4542)). Submitted to the toxin-test, AC58 appeared resistant but RO38 proved to be very susceptible. This indicates that the genetic determinism of the resistance to the toxin might be due to one major dominant gene.

3.4. IRRDB-Germ recombination by natural pollination

In 1992, 3 isolated natural pollination gardens were planted in Divo (Côte d'Ivoire), each of them covering around 1 hectare, with 50 IRRDB-Germ/Africa parents, each parent being represented by around 6 grafted trees (around 300 trees over each pollination garden). The objective was to produce, at low cost, large lots of seeds issued from Amazonian x Amazonian recombination with parents selected for yield so as to apply a new cycle of selection to progenies. One pollination garden was chosen for methodological analysis of natural pollination and recombination between the accessions by using 8 microsatellite markers for paternity identification (Blanc et al., 2001). Genotyping of the 50 parents was carried out first so as to make possible paternity identification of 797 progeny-seeds collected over the 2 years 1998 and 1999 (with Cervus software). So, it was possible to estimate selfing rate and the quotient of participation of the different parents to pollination and progeny production. Average selfing rate was found to be around 5 %.

Contributions of the different parents to pollination proved to be highly unequal, with 4 parents contributing for 40 % of paternity, 14 parents (28 % of the 50 parents) contributing for 80 % of paternity and 25 parents contributing for 95 % of paternity. This imbalance was found to be mainly due to differences in flowering capacities of the different parents. Accessions issued from Mato Grosso origin (genetic group Am4) were the best pollinators as 20 out of the 25 Mato Grosso accessions participated to 78 % of succeeded pollination. Moreover, the 10 best pollinators, contributing to 67 % of the progeny sample, are from Mato Grosso origin. So, it can be recommended to manage the different Amazonian genetic groups of IRRDB-Germ separately (which had not been made in the three pollination gardens of Divo).

4. Current position of Cirad relating with IRRDB 1981 germplasm

Cirad has participated to many projects and operations aimed at enriching the availability of and knowledge about germplasm since 1974. Cirad participation to the IRRDB international collection of 1981 and to subsequent research has been the predominant one. This was more often conducted in close cooperation with the National Centre of Agricultural Research of Côte d'Ivoire (CNRA) and with many introductions into the source bush nursery of CNRA. Most of field operations were conducted with CNRA scientists and facilities at its Bimbresso central station, near Abidjan, with significant contribution of Hevego (experimental estate based in the South-West of the country). Many field and lab operations have been associating CNRA and Cirad-Montpellier Centre. Characterisation of IRRDB-Germ/Africa was hugely facilitated by continuous funding of European Union during the period from 1985 to 1996 (by the way of 3 successive projects STD1, STD2, STD3). Within the last European project (STD3), IRAD-NRRP (National Rubber Research Programme, Cameroon) and the Catholic

University of Louvain-la-Neuve (Belgium) were participating to the project, together with CNRA and Cirad.

Apart from various scientific publications, results from these participations have been regularly presented to IRRDB (cf references below). Many other scientific reports, written in French language, are available with more details : the richer of them is the final report of the European contract STD3 : TS3-CT92-0133 (Clément-Demange et al., 1997b).

At the end of the STD3 project, cooperation was continued with CNRA until June 2001. CNRA is currently managing IRRDB-Germ/Africa at its Bimbresso station, near Abidjan.

The genetic diversity analysis which has been carried out on IRRDB-Germ/Africa, Schultes and Wickham collections showed a genetic structure with 6 groups overall and 4 groups for IRRDB-Germ on itself. This structure is considered by Cirad as a basis for the strategy of utilisation of IRRDB-Germ.

The list of 287 accessions defining the 'Cnra-Cirad 1997 working population' which was displayed at IRRDB meeting in 1997 in Ho Chi Minh City, October 14-15 (Clément-Demange et al, 1997a), has not been modified so far. It could be modified in the future after further analysis of last collected data. This working population has been defined by taking into account the germplasm available in Côte d'Ivoire and on the basis of field experimentation in this country. Yield was used as the main criterium for selection but diversity related with evidenced genetic groups and with the different geographic origins was also taken into account. This working population is considered worth to focus future research efforts on, as far as other promising approaches such as the building of a core collection have not been implemented so far.

Germplasm in Côte d'Ivoire, apart from Wickham base, is made of 2467 IRRDB-Germ/Africa and 380 IRRDB-Germ/Asia. Wild amazonian germplasm overall also includes 343 Schultes accessions (Calima : 302, Palmira : 41), 24 Cnsam accessions (collected and provided by Embrapa-Brasil), 40 accessions from the 1974 Brazilian-French collection (AC, RO), 19 MDF accessions (Firestone collection in Peru) and 10 accessions from allied Hevea species (not 'brasiliensis'). Total Amazonian germplasm in Côte d'Ivoire is currently of 3283 accessions.

The current inventory of the amazonian wild germplasm under Cirad management in its French Guyana station of Kourou-Combi is of 458 accessions, including 324 IRRDB-Germ introduced from Côte d'Ivoire (300 IRRDB-Germ/Africa and 24 IRRDB-Germ/Asia), 76 accessions from Schultes collection, 28 Cnsam accessions, 21 accessions issued from the 1974 Brazilian-French collection, 6 MDF accessions and 3 accessions from allied Hevea species. 121 accessions of the Cnra-Cirad 1997 working population are included in the sample managed in French Guyana.

The germplasm available in French Guyana is currently being used in the framework of breeding against South American Leaf Blight due to *Microcyclus ulei*. 49 IRRDB-Germ have been transferred to Brasil for testing under the pathological pressure of Michelin estates (Mato Grosso and Bahia) in this country. Budwood of these 49 accessions has also been passed to Embrapa-Cenargen (Brasilia).

A first evaluation of the susceptibility to cassicoline (toxin-based lab-test) of the germplasm available in French Guyana and in Montpellier was performed, in the framework of *Corynespora* resistance analysis. This work could be emphasized in the future.

Cirad is currently providing assistance to a young Thai researcher belonging to Rrit-Doa and performing a Ph.D. research at Kasetsart University, studying the diversity of rubber germplasm by using expressed genes markers as well as neutral molecular genetic markers. Our assistance is related with neutral molecular genetic markers.

Cirad is currently implementing a database with view to make the information available for each accession of IRRDB-Germ (as well as other accessions and cultivated clones) easily accessible by any concerned people.

5. Considerations about the characterization and utilisation of rubber genetic resources

Genetic resources are important for any breeding programme willing to integrate the full biological possibilities of the genus or species submitted to the breeding process. They represent a source of variability which can be exploited for addressing new objectives and so are a guarantee that breeding will maintain its potential in the long term. Moreover, biotechnologies now increasingly help to identify and use molecular tools such as Qtls and genes of interest, and they will be more beneficial if they can be applied to exploration of a wide biological scale.

The Wickham population, or W base (G6), can be considered as the 'first level' of genetic resources, high yielding and highly adapted to current rubber cropping in Asia, Africa and escape areas of Latin America located out of Salb pressure. Breeders feel that the reduced genetic variability of W base is limiting further progress on the improvement of latex yield and growth. So the need to widen it has justified the IRRDB 1981 collection. In most areas of Latin America confronted with Salb, our last results seem to confirm that the sole W base offers no hope of overcoming this disease which is a threat for the whole of the natural rubber industry. Consequently, Brazilian breeders, who had access to wild genetic resources in their own country, and private companies operating in Latin America (Ford, Firestone) were the first to incorporate Amazonian accessions from *Hevea brasiliensis* (Am base equivalent to a second level of genetic resources currently represented by IRRDB-Germ) and from allied species (third level of genetic resources) in their programmes (clones FX, IAN, GU, FDR, MDX, etc.). Observations in Cameroon (Cirad, IRAD) have shown the existence of resistance components to leaf diseases such as *Colletotrichum* or *Corynespora* in Am base (Gobina et al., 1999). Integration of genes from other genus could be considered as a 'fourth level' of genetic resources (this level will not be discussed here).

Out of W base, IRRDB-Germ and more generally Am base can provide new variability for addressing traditionnal objectives such as latex yield productivity and growth as well as rather new objectives such as biomass and trunk growth for rubberwood production, adaptation to stress tolerance, latex technological properties, etc. But the low latex yield level of currently available accessions is probably the main obstacle to overcome for integrating new progenitors in the creation of commercial clones.

Considering breeding related with leaf diseases resistance, utilisation of Am base and probably also of allied species seems indispensable for efficient work. A specific

characterisation of Am base and IRRDB-Germ for identifying resistance sources to the main leaf diseases, independantly from latex yield or other characteristics, seems a priority and must be continued.

Consequently, for improving IRRDB-Germ latex yield, elimination of unfavorable genes (genetic burden) is a priority (at least in the first phase of improvement). Breeding for disease resistance is quite different as it makes necessary the association of favorable genes for building a durable resistance. Moreover, a resistant clone can be useful only if combined with a reasonable level of latex yield.

One more general aspect of genetic resources management relates to ensure conservation and appropriate management of genetic variability issued from initial large-sized collections.

5.1. Genetic resources structure and management

Within the cultivated *Hevea brasiliensis* species, W, IRRDB-Germ and Schultes collections can be structured in 6 genetic groups (Seguin et al, 1999) but in fact 5 groups as far as the small Schultes-Palmira population (G5 = Pal) can be neglected (it must be added that the origin of Palmira population is not clearly known and might include Wickham x Amazonian accessions). Breeding these groups separately is necessary to maintain the existing genetic variability between these groups. It must be reminded that the variability within such categories as locations and districts of collection, assessed by genetic markers, was found more important than the variability 'between' the groups and so allows significant genetic progress. Preservation of these genetic groups along the breeding process makes possible further genetic analysis of crosses between these groups with possible heterotic or complementary effects which could be exploited for the creation of commercial clones.

Am4 (Mato Grosso + RO/PB) and W (Wickham) appear to be very close by taking into account molecular data, but it cannot be imagined to use them as a same category due to the huge gap between the two groups for latex yield. However, the question of the relative interest of Am4, compared with Am1, Am2 and Am3, can be discussed. Am4 clearly appears to be more susceptible to Salb. It was also found more susceptible to *Colletotrichum* in Côte d'Ivoire due to later refoliation (Legnaté et al., 1992). It might be less adapted for rubberwood production. Its contribution to the provision of new variability is lower than that of the 3 other groups which are more different from W and Am4. The small differences in mean latex yield between the groups, before any selection effect (Am1 might be lower), would not have much incidence on future breeding. It would seem more interesting to work on groups Am1, Am2 and Am3 which provide more new variability. On the other side, Am4 might be more able to fit with W. With our current knowledge, the respective potentials of the 4 groups are not predictable. For maintaining maximum variability, it would be advisable not to neglect any Am group.

Unexpectedly, the molecular diversity of Wickham group appeared to be larger than could be thought of due to : a/the supposed restricted size of the Wickham population originally introduced in Asia, b/the intensive selection and use of a limited number of ancestral parents. As a matter of fact, the Wickham population will still be for some time the main basis for commercial clones release. For a better management of its genetic variability, and for limiting genetic relatedness between W progenitors, it could be advisable to split the Wickham population in two sub-populations (i.e. W1 and W2), on the basis of known parentage. With such a scheme, new W clones could be issued from W1, W2 and W1 x W2, but clones W1 x

W2 would never be used as progenitors. It can be assumed that clones issued from W1 x W2 crosses might progressively be the more performant due to the development of a higher level of heterozygosity.

5.2. Breeding Am groups for yield

First evaluation of W x Am or Am x Am does not indicate any heterotic effect for latex yield as W x Am crosses provide progenies with intermediate yield. Conversely, W x Am crosses often provide very vigorous progenies. So, on the basis of additive effects for latex yield, it can be assumed that W x Am crosses could become an efficient source of new performant clones, provided that the mean level of latex yield in the Am groups can be enhanced. Consequently, improving latex yield level in each group, combined with mild selection on other traits and appropriate preservation of genetic variability, could be addressed by rubber breeders. At the level of IRRDB, a shared approach would allow each breeding team to specialise on only one of the Am1, Am2, Am3, Am4 groups, with further exchanges of progenitors between the teams.

The first improvement step of one group is the selection of accessions with a relatively high latex yield and good other characteristics (growth, branching habits). Group Am4 will express more abundant branching. Groups Am1, Am2 and Am3 will frequently express shapes with tall and straight trunks and poor branching, probably more adapted to rubberwood production.

Natural pollination applied to a good number of accessions selected in one group can be an adapted way for abundant recombinations prior to a new cycle of selection for yield. Such recurrent selection seems necessary to discard allelic forms unfavorable for yield or for other characters and so to improve the mean yield of the group, while maintaining enough genetic variability. As selected genotypes are not aimed at being used as commercial clones, selection can be limited at the seedling evaluation stage. After each selection phase, best genotypes could be crossed on Wickham testors by hand pollination for comparing the progenies with other W x W families. Using a mating design, if possible, will help to characterise the genetic properties of such W x Am crosses.

5.3. Breeding for resistance to leaf diseases

The strategy of breeding for resistance must be defined depending on which leaf disease is concerned after in depth studies of the pathologic system. The host-pathogen interaction must be analysed so as to evaluate the diversity of pathogenic strains. Efficient test procedures (lab, field) must be elaborated. Evaluation of the germplasm will make possible the identification of genotypes bearing resistance sources. Some of these genotypes can be used for analysing the genetic determinism of these resistance sources (major gene, multigenic resistance). Added to the traditional analysis of segregation of progenies, genetic mapping and Qtl approach appear now performant for identifying different resistance components issued from the parents of one cross, and localising them on the genome (cf Lespinasse et al., 2000). This work more often seems necessary for preparing the building of durable resistance by associating many independent resistance components within one clone. Molecular exploration can even lead to identification and cloning of resistance genes which could then be integrated in some clones by genetic engineering.

As many resistance sources can be found in Am germplasm and allied species, evaluation of Am accessions for resistance in areas submitted to pathological pressure of important diseases

(*Microcyclus*, *Corynespora*) appears very beneficial for the future of rubber resistance breeding.

5.4. Molecular markers for controlling the breeding process

Many types and a large number of neutral molecular genetic markers have been developed and applied to germplasm studies, notably for analysing genetic diversity. Among them, microsatellites (Single Sequence Repeats) are particularly performant due to their high level of polymorphism (between 10 and 20 alleles per marker) and to their fast and easy implementation thanks to the PCR technology. As DNA molecule is very stable, analyses can be carried out at central labs with sending of fresh leaves samples to the labs by express mail from quite any location in the world.

These markers now make possible the genetic identification and registration of quite any genotype (establishment of identification databases and control of budwood gardens).

Markers can be applied also to the evaluation of alleles presence and frequency. Consequently, they can be used to estimate how the initial genetic variability is maintained or eroded in each group along the process of recombination and selection. They can be applied to sampling procedures aimed at maximising genetic variability for building core collections (Hamon et al., 1998).

In the case of natural pollination, microsatellites proved to be very efficient for paternity testing. If the gardens are not isolated from any outside pollination source, progenies issued from outside pollination can be detected and discarded. Selfing rate can be estimated. The rates of male contribution of the different parents of the garden can be estimated. Moreover, male parentage of progenies can be determined a long time after the production of seeds, which enables paternity identification of best progenies at the end of the selection process.

Consequently, molecular markers in general, and more specifically microsatellites, brought and will probably bring an important contribution to the management of genetic variability and to the improvement and utilisation of rubber germplasm.

5.5. Core collection, working populations

The creation of two IRRDB Conservation Centres (around 10000 accessions for IRRDB-Germ/Asia and around 2500 accessions for IRRDB-Germ/Africa) and the dispatching to most of IRRDB members provided a network approach and global good security conditions for the conservation of IRRDB-Germ. Each country is able to develop its own strategy for managing it in its own pace and for its own specific objectives. However, control of presence/absence of each accession in the different possible locations and coordination of common multilocation evaluation of same samples are difficult to carry out. The composition of local populations in the different countries may evolve and diverge along time far from the initial composition of original IRRDB-Germ with significant variability erosion. Moreover, maintenance of all these conservation budwood garden are relatively costly.

So as to focus part of conservation and further evaluation efforts of breeders on one same reference population of reduced size with a high level of genetic variability and so a good quality of representation of the whole IRRDB-Germ, Cirad suggested that a common 'core collection' of IRRDB-Germ could be built under the aegis of IRRDB (Clément-Demange et

al. 1997, 1999). This collection would generate more common interest for IRRDB-Germ and more communication between the different breeding teams. This would also have the advantage of making the monitoring of IRRDB on IRRDB-Germ conservation clearer and cheaper. A better multilocation characterisation of this 'core collection' would also help recognition of IRRDB role in rubber germplasm preservation by IPGRI and might favor some funding of future research applied to germplasm.

The concept of 'working population' represents a first step of selection among IRRDB-Germ. The need to focus selection activities on different characters depending on the objectives of different programmes (latex yield, resistance to *Microcyclus*, resistance to *Corynespora*, etc.) underlines the necessity of building different types of 'working populations' with well defined specific properties. In Côte d'Ivoire, the objective has clearly been to adapt Amazonian groups for improving their mean latex yield level with view to produce more efficient W x Am crosses. Due to the process of their building, these working populations are particularly subject to possible fast genetic erosion. So, it would be advisable to monitor their genetic variability along selection by the use of microsatellite markers.

5.6. IRRDB-Germ Database

From all evaluation efforts scattered among the different members of IRRDB, many results have been obtained, so providing a good level of knowledge about main genetic properties of IRRDB-Germ. In Côte d'Ivoire, CNRA and Cirad have collected a huge amount of results which were stored in different digital files. Getting available information for one specific accession, selecting accessions fitted to one specific goal are not easy and require time from people fully aware of the experimental context and of the structure of the files. We assume that other countries meet the same problem and we suggest that a common database could be designed and built under the aegis of IRRDB Breeding Group so as to make status of accessions (origin, locations where available, etc.), agromorphologic and molecular data easily available to concerned people. This proposal was already first expressed (by Malaysian Rubber Board) at the IRRDB Breeding Group meeting in October 19, 1999 (IRRDB meeting in Hainan). Cirad fully approves it and wishes that terms of reference could be established for further implementation.

6. Conclusion

The IRRDB international collection of 1981 has been a very important operation for ensuring the long term sustainability of rubber breeding and for addressing objectives such as resistance to some major leaf diseases. It provided more than 12000 IRRDB-Germ accessions which give access to a widely enlarged genetic variability of *Hevea brasiliensis* germplasm, probably close to the whole extent of the variability of this species.

Rubber breeders are confronted with the responsibility of conservation and characterisation of this variability on one hand, and of its exploitation for different objectives of the rubber industry on the other hand. Trying to manage the whole of the collection is a huge task to quite any breeding team, which requires to focus on reduced size populations such as a 'core collection' for maintaining and managing initial genetic variability, and 'working populations' for addressing specific breeding objectives. After 20 years of characterization of IRRDB-Germ, there is a real need for the creation of a common database able to provide any available information at the level of individual accessions.

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