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# Genetic diversity among wild and cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis

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**Abstract** Restriction fragment length polymorphism was assessed in wild and cultivated populations of *Hevea brasiliensis* using random probes from an *Hevea* nuclear library. One-hundred-and-sixty-four individuals were surveyed, and the results discussed in the light of previous work performed on isozyme variation. Both studies show that germplasm collections have led to an effective enrichment of the genetic resources available for *Hevea* breeding, and that cultivated clones have conserved a relatively high level of polymorphism, despite their narrow genetic base and their high level of inbreeding. An equivalent level of polymorphism is revealed by random nuclear probes and isozymes. However, the genetic structuring of the diversity appears more striking using RFLP markers. Wild accessions can be divided into three genetic groups according to their geographical origin. The present results are an essential guide to the incorporation of wild material in breeding schemes.

**Key words** *Hevea brasiliensis* · RFLP · Nuclear probes · Isozyme · Genetic diversity

## Introduction

In terms of the history of crop species, it is obvious that in many cases the first introductions represent only a restricted part of the genetic variability available in the area of origin. *Hevea* is no exception to this rule. Indeed, all clones cultivated today in Africa and the Far East (named Wickham clones), originate from a few seeds coming from a specific location in the Amazon basin, the area of origin of *Hevea brasiliensis*. This represents a very small part of the considerable variability available in the Amazonian forest. It thus rapidly became clear that the genetic base of the cultivated clones was not sufficient to carry on obtaining substantial genetic improvements in rubber production or in disease resistance (e.g., to South American Leaf Blight). For this reason, a survey was carried out in 1981 by different IRRDB (International Rubber Research and Development Board) member countries in three different Brazilian states: Acre, Rondonia, and Mato-Grosso. The eventual incorporation of the material obtained into on-going breeding programs, implies, concurrent with an agronomical evaluation (Legnate and Clement-Demange 1991), an estimation of the genetic variability present in these new clones compared with that of cultivated material.

The use of RFLPs (Restriction Fragment Length Polymorphisms) for generating powerful markers for genetic relationship studies, as well as for mapping purposes, has been described by Tanksley (1983), Beckmann and Soller (1986), and Soller and Beckmann (1983). Molecular markers have already proved useful as tools for *Hevea* breeders. As alternatives to isozymes (Chevallier 1988; Lebrun and Chevallier 1990), DNA fingerprints (Besse et al. 1993) provide powerful markers for the individual identification of cultivated Wickham clones.

We present here the results of a genetic diversity study performed with low-copy nuclear probes on wild and cultivated *H. brasiliensis* populations, in the light of previous work on isozyme variation (Chevallier 1988; Chevallier et al. 1988; Seguin et al. 1994).

## Materials and methods

### Plant material

A total of 92 amazonian accessions (44 Acre, 20 Rondonia, and 28 Mato-Grosso) has been examined as a sample of the clones previously

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studied by isozyme analysis (Chevallier 1988; Chevallier et al. 1988; Seguin et al. 1994). The geographical localization of the three states and the different districts surveyed are presented in Fig. 1.

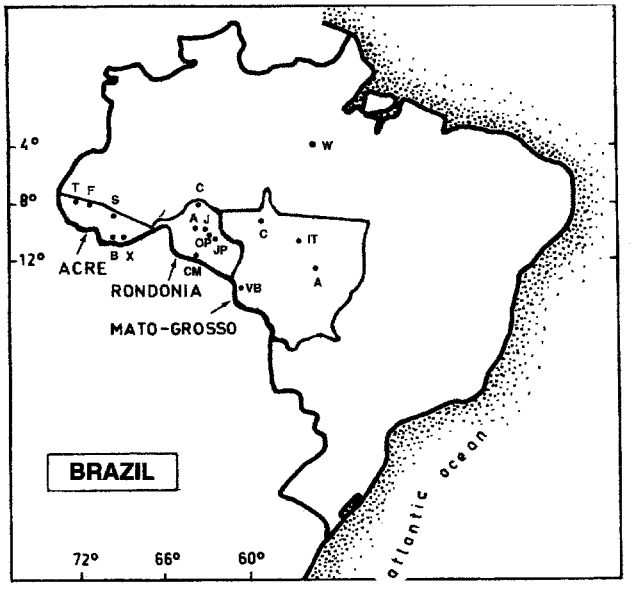
Additionally, 72 cultivated Wickham clones, previously identified by DNA fingerprinting (Besse et al. 1993, except for clone RRIC 103), have been added to the analysis in order to compare their variability with that of the wild material. The cultivated clones all originate from a few seeds (9–22) collected by Wickham near the Tapajos river (Fig. 1).

### Library construction and characterization

Enriched nuclear DNA was extracted from the leaves of Wickham clone GT1 using a protocol adapted from Grisvard (1985). Nuclear DNA was digested with *Pst*I according to the supplier's instructions (Bethesda Reseach Laboratories), and fragments ranging from 0.5 to 2.2 kb were electroeluted by Biotrap® (Schleider and Schuell Inc.). Ligation in pUC 18 vector and transformation in DH 5α *E. Coli* strains were both performed according to Sambrook et al. (1989). Recombinant clones were selected on IPTG-X gal plates (Sambrook et al. 1989) and plasmids were isolated by a miniprep procedure (Birnboim and Dolly 1979). Probes were consecutively numbered as pHbCIR#.

Recombinant plasmids were digested with *Pst*I, run in an agarose gel and then Southern-blotted onto Nylon membranes (Hybond $N^+$, Amersham) as described previously (Besse et al. 1993). Genomic *Hevea* DNA (for extraction procedures, see Besse et al. 1993) was labelled with $^{32}P$ by random priming (Feinberg and Vogelstein 1983), and hybridized to the filters in order to detect inserts containing repeated sequences. A highly-repetitive sequence (pTA71, the wheat rDNA probe of Gerlach and Bedbrook 1979) and a single-copy gene (the maize ADH1 gene, Gerlach et al. 1982) were used as references. Inserts showing no hybridization signal were assumed to represent low-copy sequences and were selected for RFLP analysis.

**Fig. 1** Geographical localization of the different states surveyed. The detailed name of each district (the number of individuals studied per district is indicated in parenthesis) is as follows: Acre district: T, Tarauaca (6); F, Feijo (7); S, Sena Madureira (13); B, Brasileia (10) and X, Xapuri (8). Rondonia district: C, Calama (5); A, Ariquemes (1); J, Jaru (5); OP, Ouro Perto (1); JP, Jiparana (3) and CM = Costa Marques (5). Mato-Grosso district: C, Juruena (15); IT, Itauba (7); A, Aracatuba (3) and VB = Vila Bela (3) The letter W indicates the area of origin of cultivated Wickham clones




### RFLP procedures

Total DNA extraction, digestions and blotting were carried out as described previously (Besse et al. 1993). Migraations were performed in 0.8% agarose gels, with a TAE buffer, and run for 10 h at $1.5\ V \cdot cm^{-1}$. Internal lambda markers (24.8 and 1.5 kb) were added as standards for visualisation of migration distortion, and were revealed when desired by adding $^{32}P$-labelled $\lambda$ with the probe.

Single-copy probes were PCR-amplified directly from bacterial culture or after the minipreparation procedure. This PCR amplification appeared essential to avoid a hybridization signal produced by the pUC vector on *Hevea* genomic DNA blots. After migration in a low-melting-point 1% agarose gel, and migration with a TAE buffer, probes were picked up from the gel, and directly $^{32}P$-labelled by random priming (Feinberg and Vogelstein 1983) after resolubilization at 65 °C for 5 min.

Prehybridization, hybridization, and washings were performed as recommended by CIMMYT (Hoisington 1992). Removal of the probes was performed in 1% SDS at 68 °C for 30 min.

### Statistical analysis

Gene diversity, as an estimation of the variability revealed within each population, was calculated as (Nei 1987):

$$h = 1 - \sum_{i}^{m} \bar{X}_i^2,$$

where $\bar{X}_i$ is the frequency of the $i$th profile for each probe/enzyme combination (or isozyme locus). $H$ is then calculated as the average value for all the $h$ obtained for each probe/enzyme combination (or isozyme locus).

Each RFLP fragment was scored 1 for presence and 0 for absence. A multivariate analysis, termed factor analysis of correspondences (FAC) (Benzecri 1973) was performed on this binary matrix after disjunction of the variables (to give the same weight to the different individuals). In this analysis, variables as well as individuals contribute to the definition of the different axes. This analysis provides a synthetic view of the variability by regrouping the most informative data on the first axes. Very rare variables (i.e., with a frequency below 3%) were not considered as active variables in order not to unbalance the analysis. Dendrograms were constructed based on FAC coordinates (on the five first factors), with dinter-cluster variance maximization as agregation criteria. All these computations were performed using appropriate programs of ADDAD software (Lebeaux 1983).

Similarities between individuals were calculated for all possible pairwise comparisons using the D Dice index (Dice 1945) according to Nei and Li (1979).

## Results

### Characterisation of the nuclear PstI library

Screening of the library for low-copy DNA inserts revealed that 96% of the clones were low-copy, showing that a methylation-sensitive enzyme strategy is as efficient in *Hevea* as in many higher plants, such as maize (Burr et al. 1988; Helentjaris et al. 1988), tomato (Miller and Tanksley 1990a, b), *Brassica* (Figdore et al. 1988), peanut (Kochert et al. 1991), *Phaseolus vulgaris* (Chase et al. 1991) and tall fescue (Xu et al. 1991). Therefore, about 660 low-copy probes are available in our library.

A subset of 14 individuals representative of the overall variability was constituted in order to allow a preselection of informative clones fulfulling the follow-

ing criteria: (1) giving strong hybridization signals, (2) providing easily scorable fragments, (3) showing a polymorphic pattern among the individuals surveyed. Seven 6-cutter restriction enzymes have been tested. The level of polymorphism revealed appeared to differ according to the enzyme used. Therefore, two enzymes were selected for the diversity study in order to compare their relative abilities in providing a genetic description of the diversity. *Eco*RI and *Sst*I were chosen since they reveal the highest level of polymorphism (data not shown).

Finally, 88 random single-copy probes have been screened, and 25 appear to be suitable for a diversity study, giving 31 probe/enzyme combinations (20 with *Eco*RI and 11 with *Sst*I, and with six probes involving both enzymes).

## Restriction profiles

Most of the probes give simple restriction profiles (with one or two bands per individual) suggesting a single locus with different alleles (Fig. 2a). However, other probes gave more complex patterns (Fig. 2b) due either to restriction site polymorphism or to the existence of duplicated loci in *Hevea*. Even so these probes can be considered as low copy, as determined by library screening. Since making assumptions on genetic determinism appears too risky without segregation analysis (Gillet 1991), fragments were scored only as present/absent. All the results obtained were compared with previous work on isozyme variation (Chevallier 1988; Chevallier et al. 1988; Seguin et al. 1994) by extracting the corresponding sample of individuals and treating them like RFLP data.

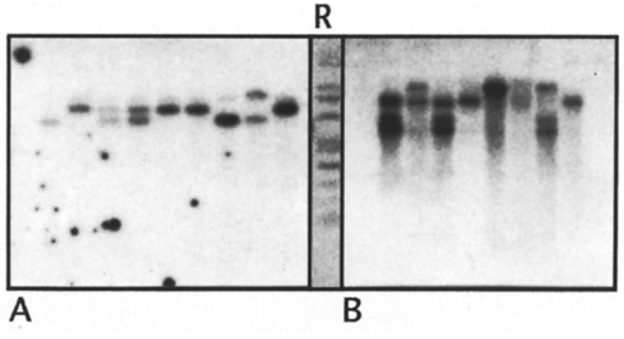



**Fig. 2** Examples of the hybridization patterns obtained. **A** for probe-enzyme combination pHbCIR 494/*Eco*RI. **B** for probe-enzyme combination pHbCIR 482/*Eco*RI. R is a Raoul (Appligene) molecular weight marker

## The classification of variable restriction fragments

Using the 31 probe/enzyme combinations, a total of 124 variable restriction fragments can be detected in the whole population. Only ten of them are specific to single individuals (eight being restricted to one particular individual, RO/C/8/9), and 13.7% of the remaining fragments have frequency below 5%. Each probe enzyme combination detects from two to eight RFLP fragments, and from three to 32 different profiles (for probe/enzyme combinations pHbCIR 470/*Eco* RI and pHbCIR 41/*Sst*I respectively). The classification of the 124 restriction fragments (Table 1) shows that 50 of them (i.e., 40.3%) are specific to the wild accessions, while 34.7% of isozyme alleles were found to be specific to wild populations. This gives a mean of 2.35 and 3.97 RFLP fragments per probe/enzyme combination, versus 2.90 and 4.45 alleles per isozyme locus for cultivated and wild clones respectively.

Some restriction fragments specific to a particular State can be detected (Table 2); most of them have a frequency higher than 3% whereas all the specific alleles revealed by isozymes were rare. One fragment specific to the Wickham collection was identified, but it concerns only one individual (clone Harbel 2).

## Genetic variation

Genetic diversity (Nei 1987), as a measure of genetic variation within each population, was estimated by looking at the different RFLP profiles, and was compared with the gene diversity obtained from isozyme data also based on the different patterns revealed. Gene diversity values are given in Table 3. The Wickham gene diversity value obtained by RFLP appears lower than that obtained using isozymes, mainly because nine probe/enzyme combinations gave monomorphic pat-

**Table 1** Number and distribution of the different RFLP fragments and isozyme alleles

| Number of | Cultivated clones (Wickham) | Wild amazonian accessions | | |
|---|---|---|---|---|
| | | Acre | Rondonia | Mato-Grosso |
| RFLP fragments 31 probe/enzyme combinations | 73 | 95 | 102 | 96 |
| Total:124 | 73 | | 123 | |
| Isozyme alleles 11 loci (enzymatic systems | 32 | 41 | 40 | 41 |
| Total: 49 | 32 | | 49 | |

**Table 2** Distribution of specific restriction fragments and isozyme alleles

| Number of | Wickham (Wickham) | Amazonian accessions | | |
|---|---|---|---|---|
| | | Acre | Rondonia | Mato-Grosso |
| RFLP-specific fragments | 1<br>0[a] | 1<br>1[a] | 17<br>3[a] | 4<br>2[a] |
| Isozyme-specific alleles | 0<br>0[a] | 2<br>0[a] | 1<br>0[a] | 1<br>0[a] |

[a] Without fragments, or alleles with a frequency below 3%

**Table 3** Average gene diversity values (Nei 1987) as a measure of within-population gene diversity and maxima and minima for RFLP and isozyme data. The number of probe/enzyme combinations giving monomorphic patterns in each population (i.e. $h = 0$) is indicated in parentheses. (Rondonia values are given without clone RO/C/8/9)

| Data | Wickham | Amazonian accession | | |
|---|---|---|---|---|
| | | Acre | Rondonia | Mato-Grosso |
| RFLP | | | | |
| *H* (average) | 0.383 + / − 0.310 | 0.450 + / − 0.271 | 0.509 + / − 0.247 | 0.545 + / − 0.213 |
| *h* min | 0(9) | 0(2) | 0(3) | 0(1) |
| *h* max | 0.923 | 0.915 | 0.855 | 0.889 |
| Isozyme | | | | |
| *H* (average) | 0.458 + / − 0.251 | 0.604 + / − 0.175 | 0.497 + / − 0.212 | 0.508 + / − 0.216 |
| *h* min | 0.054 | 0.250 | 0.087 | 0.206 |
| *h* max | 0.734 | 0.872 | 0.888 | 0.853 |

terns in this population. All the cultivated Wickham clones studied can be identified using 13 probes associated with the restriction enzyme *Eco*RI, with an average XD value of 0.725.

Wild populations appear to be more polymorphic than cultivated clones, with Rondonia and Mato-Grosso being the most variable with RFLP whereas with isozyme markers Acre proved most variable.

## Diversity organization

A factor analysis of correspondences performed on wild clones based on RFLP data is shown in Fig. 3. The 1–2 plane represents 30.3% of the total variability, with a major contribution from 55 out of 124 restriction fragments. A striking geographical structuring is revealed. The first axis separates Mato-Grosso from Acre and Rondonia, and the second axis separates Acre from Rondonia. This structure is clearly defined by frequency variations between States, and not by specific fragments. Indeed, an FAC performed without these specific fragments shows the same organization (data not shown).

Dendrogams based on FAC coordinates give a more general view of the major part of the variability, since the first five factors taken into account represent 48.2% of the total variability, with a major contribution form 83 out of 124 RFLP fragments. The same geographical structuring is evident. Mato-Grosso appears well separated from the rest of the population, and two distinct

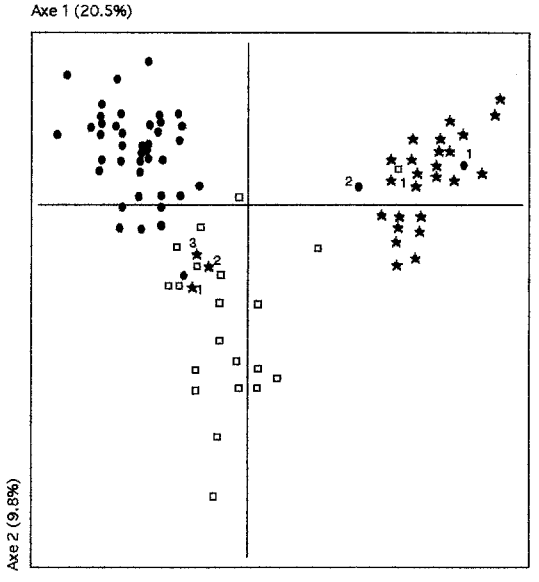



**Fig. 3** Representaation of the 1–2 plane of the FAC (representing 30.3% of the overall variability) performed on wild accessions (Acre ●, Rondonia □, Mato-Grosso ★) with RFLP data

Acre and Rondonia groups are represented. However, a small heterogeneous group consisting of some Acre and Rondonia clones is revealed (Fig. 4).

When cultivated Wickham clones are added to the analysis (Fig. 5), their variability appears included in that of the Mato Grosso clones.

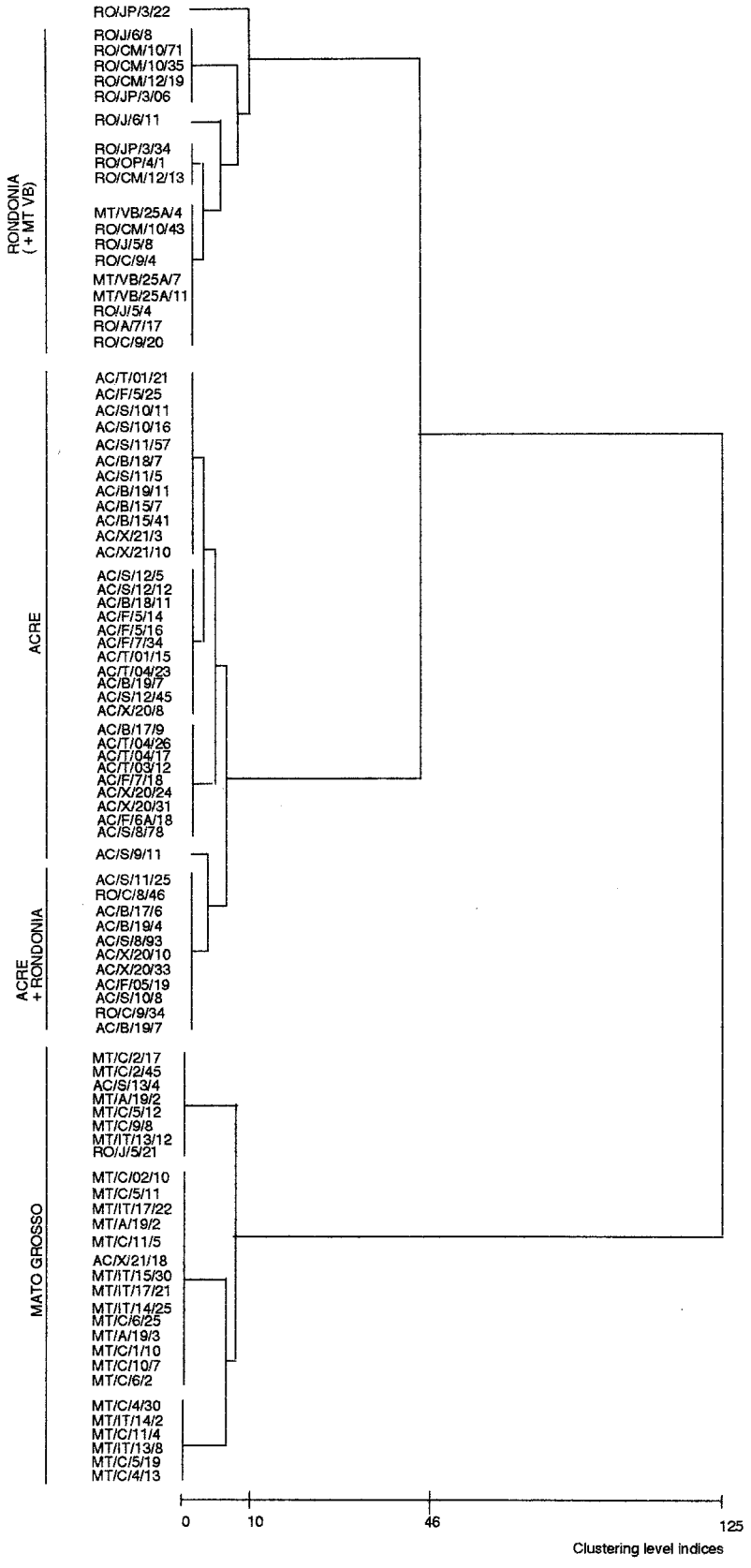



**Fig. 4** Dendrogram based on FAC coordinates (on the five first factors) performed on wild accessions (without clone RO/C/8/9) with RFLP data. *AC* is Acre, *RO* is Rondonia, and *MT* is Mato-Grosso. District symbols are as indicated on Fig. 1

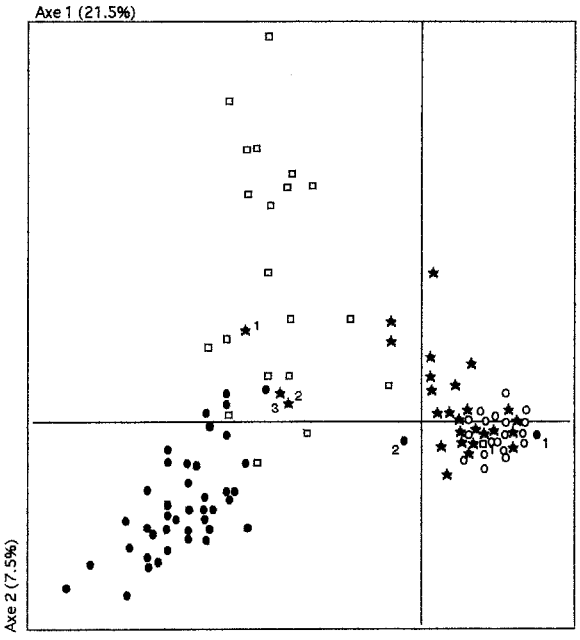



**Fig. 5** Representation of the 1–2 plane of the FAC (representing 29% of the overall variability) performed on wild accessions (same symbols as Fig. 3) and cultivated Wickham clones (○) with RFLP data

Factor analyses of correspondences were also performed on isozyme data taken from previous work (Chevallier 1988; Chevallier et al. 1988) for the individuals used in the RFLP analysis (Fig. 6). The same general organization as that found with RFLP markers is revealed, although Rondonia clones are less differentiated from Acre clones. As in the case of the RFLP analysis, this organization is defined by allelic frequency variations between States (Chevallier et al. 1988)

In order to assess the respective ability of each restriction enzyme in the detection of genetic diversity organization, an FAC was also performed separately with each enzyme for the six common probes. This study involves only a few probes compared with the global analysis, and thus gives a less-striking organization. It shows, however, that the overall structure in the three geographical groups is conserved whichever enzyme is used (Fig. 7a, b).

Multivariate analysis, while quite appropriate for detecting a global structuring, is not always suitable for discussing particular points. The comparison of analyses performed with isozyme and RFLP data, however, lights up some significant details that had not been noticed in the previous work on isozymes. Since these particularities are revealed with both type of markers, they certainly have a genetic significance and cannot be considered as an artefact. Thus, Acre clone AC/S/13/4 and Rondonia clone ROJ/5/21 (indicated as 1 in Figs. 3, 5 and 6) appear with both analyses to be included in the Mato-Grosso group. Acre clone AC/X/21/18 (indicated as 2 in Figs. 3 and 5) appears much closer to Mato-Grosso than to Acre with RFLP. This clone also showed a particular genotype for the DIA (diaphorase) isozyme locus. Moreover, three Mato-Grosso clones (indicated as 1, 2, 3 in Figs. 3, 5 and 6) appear always to be grouped with the Rondonia clones. These three individuals are all from the Vila Bela (VB) district (see Fig. 1). In the light of this particularity, an FAC has been carried out using isozyme data from a larger set of individuals (including eight accessions from Vila Bela) and without ambiguity shows, that all these Vila Bela clones are much closer to the Rondonia populations (Seguin and Chevallier, personal communication).

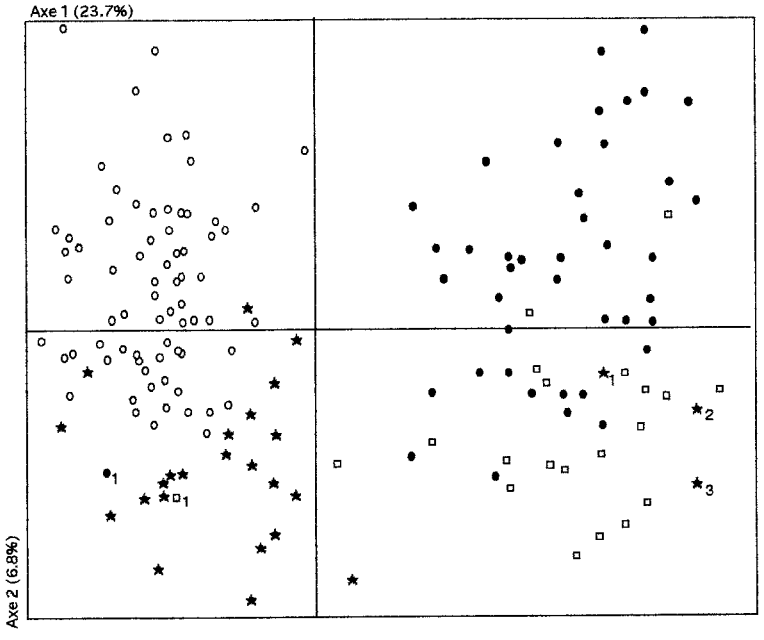



**Fig. 6** Representation of the 1–2 plane of the FAC (representing 30.5% of the averall variability) performed on wild accessions and cultivated Wickham clones (same symbols as Figs. 3 and 5) based on isozyme data

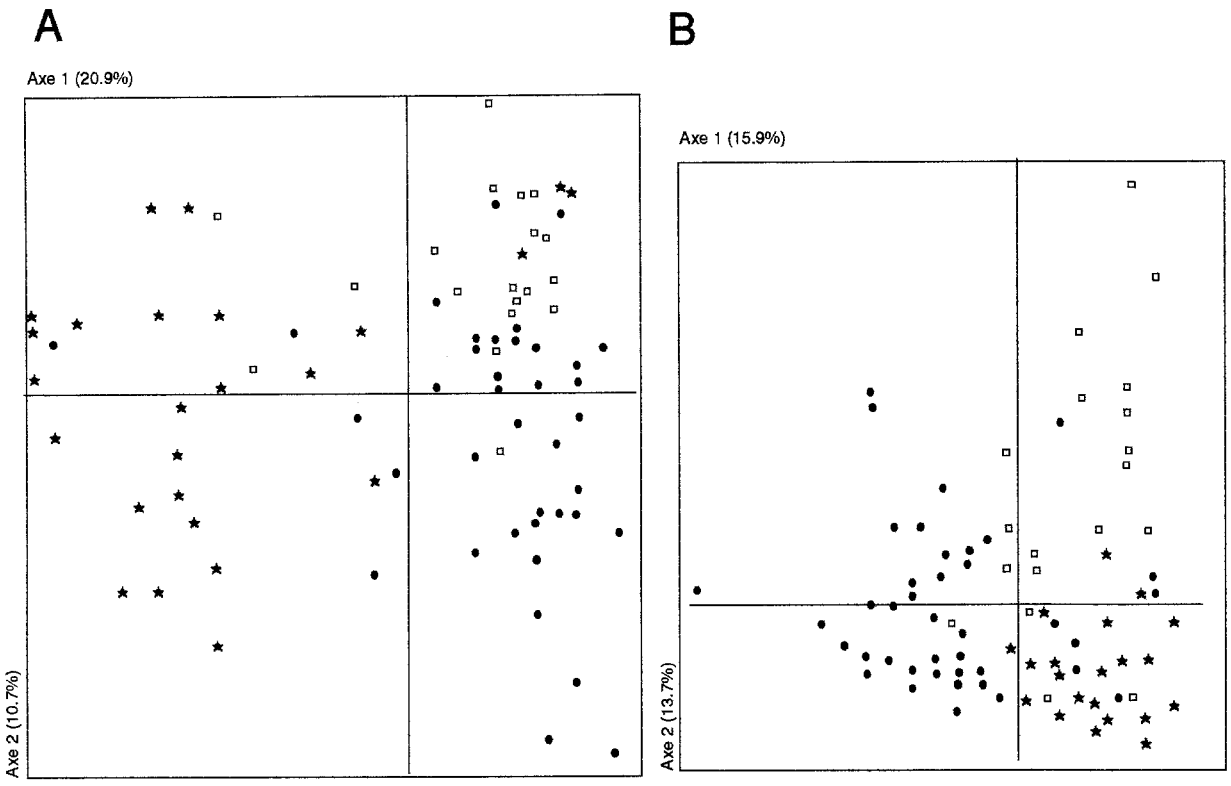



**Fig. 7 A, B** Comparison of the ability of two restriction enzymes in genetic diversity organization detection, based on RFLP data for six common probes. Representation of the 1–2 plane of the FAC performed on wild accessions (same symbols as Fig. 3) based on: **A** *Sst*I RFLP data (31.6% of the total variability) and **B** *Eco*RI RFLP data (29.6% of the total variability)

## Discussion

### Isozyme/RFLP comparison

Only few studies are available on a detailed comparison of RFLP and isozyme data. However, contrary to species like lentil (Havey and Muelbauer 1989), maize (Messmer et al. 1991), and *Brassica campestris* (McGrath and Quiros 1992), a comparison of the polymorphism revealed by isozymes and RFLPs in *Hevea* shows very similar levels, like *Capsicum* (Prince et al. 1992), *Glycine max* (Keim et al. 1989) and *Phaseolus vulgaris* (Chase et al. 1991). This can be explained by the fact that all the DNA clones used in this study are random nuclear clones. By contrast, cDNA clones have been found to detect more polymorphism than random clones in lettuce (Landry et al. 1987), lentil (Havey and Muelbauer 1989), tomato (Miller and Tanksley 1990a, b), and peanut (Paik-Ro et al. 1992). The use of cDNA clones may increase the detectable polymorphism in *Hevea* as well. Indeed, in *B. campestris* (McGrath and Quiros 1992), the use of cDNA clones revealed three-times more polymorphism than did isozymes.

### Restriction enzyme ability in detecting genetic diversity organization

Comparison of the efficiency of the different enzymes showed that the same general organization is conserved whichever enzyme is used. Similar results were obtained for rice (Wang and Tanksley 1989), but in that case the different enzymes tested revealed the same polymorphism (insertion/delection type). In *Hevea*, the first screening on seven restriction enzymes, as well as the present study, showed that variations are mostly due to base-change mutations, since the variations revealed by a particular probe/enzyme combination are not the same as those revealed by the same probe associated with another enzyme. Despite this, the two enzymes studied by us give the same pattern of genetic variability, agreeing with results obtained on *P. vulgaris* (Chase et al. 1991). Therefore, the combination of two restriction enzymes for a diversity study does not provide a better structuring of that diversity than the use of a single enzyme. However, it saves considerable time in probe screening, especially for species involving base-change-type mutations.

### Genetic variation in wild and cultivated *H. brasiliensis*

The presence of nine probe/enzyme combinations showing a monomorphic pattern in the cultivated population

gives an indication of their restricted origin. However, despite their narrow genetic base and their high level of inbreeding, cultivated Wickham clones show a high level of polymorphism. This result agrees with that obtained with different types of genetic markers, including isozymes (Chevallier 1988) and minisatellites (Besse et al. 1993), although their relative evolutionary rates are very different. It is thus now clear that the mass selection performed on the first Wickham introduction has been able to retain a high level of variability within the cultivated clones. Moreover, all the Wickham clones studied can be identified using 13 probes associated with the restriction enzyme *Eco*RI, providing another powerful tool for *Hevea* breeders, as an alternative to minisatellite (Besse et al. 1993) and isozyme markers (Chevallier 1988). The corresponding average D-value (XD = 0.725) shows, however, that the polymorphism revealed by these low-copy probes is less than that revealed by fingerprint analysis (XD = 0.414, Besse et al. 1993).

Contrary to isozyme studies, one specific Wickham fragment, restricted to the clone Harbel 2, has been detected. This result illustrates the power of RFLP technology in detecting genetic peculiarities. Since the Wickham population comes from a small number of seeds collected near Santarem, on the Tapajos river bank, it represents only a restricted sample of the diversity available in this area. More numerous specific restriction fragments would almost certainly be detected if we could resample the area of origin of the Wickham seeds. Santarem area populations could be genetically different from those of Acre, Rondonia and Mato-Grosso, and thus could also represent an interesting *Hevea* genetic resource.

The comparison between wild and cultivated clones shows without ambiguity that cultivated clones are less variable than wild accessions, and that they are very close to the Mato-Grosso clones, as already demonstrated by isozyme analysis (Chevallier 1988; Chevallier et al. 1988; Seguin et al. 1994).

Both isozyme and RFLP data show the considerable extent of variability available from the new collections since 39% of the RFLP fragments or isozyme alleles detected are specific to the wild populations. Acre was shown as the most polymorphic by the isozyme study. With RFLP markers, the most polymorphic states are Rondonia and Mato-Grosso, mainly because of the occurrence of more specific fragments in these States than in Acre. However, with both type of markers combined, it seems that an equivalent variability is available within each State.

The genetic variability of the wild *H. brasiliensis* populations surveyed is clearly organized on the basis of the geographical location of the areas sampled. Compared with previous work on isozyme variation, the present study has generated some new results. First, it shows a clear-cut division between the three populations originating from Brazil: Acre, Rondonia, and Mato-Grosso. On the basis of the isozymatic analysis (Chevallier 1988; Chevallier et al. 1988) Rondonia clones have long been considered by breeders as being like Acre clones. In the light of our results, although there is evidence of a common genetic background for some individuals (as revealed by the small heterogeneous group on dendrograms), Rondonia and Acre clones appear to be well individualized. Moreover, specific fragments have been detected. Secondly, the results of the present study suggest that Mato-Grosso accessions from Vila Bela district are much closer to Rondonia. This is agrees with the geographical localization of this district (see Fig. 1).

Our results also show that some particular clones from Acre and Rondonia are genetically much closer to those from Mato-Grosso. These particularities have to be taken into account in the choice of parents for breeding programs. Moreover, clone RO/C/8/9 shows eight specific restriction fragments and also showed a specific MDH (malate dehydrogenase) allele (Chevallier personal communication). This indicates that this clone might have an interspecific origin.

In conclusion, the general organization of diversity in *Hevea* germplasm as revealed by RFLP markers appears more striking than that obtained with isozymes. RFLPs, by virtue of the larger number of loci studied, can cover more of the genome, and thus lead to a better representation of the structuring of germplasm diversity (Tanksley 1983). This was also the case for studies on rice (Wang and Tanksley 1989), maize (Messmer et al. 1991), *Capsicum* (Prince et al. 1992) and *P. vulgaris* (Chase et al. 1991). Indeed, the present study shows clearly that the *H. brasiliensis* germplasm collection can be divided into three geographical groups, involving respectively the Acre, Rondonia (plus Mato-Grosso clones from Vila Bela) and Mato-Grosso clones. This will be of great help in guiding the choice of convenient populations to be used in recurrent breeding schemes being currently developed. In a like manner, the high resemblance of cultivated and Mato-Grosso clones has to be taken into account in Wickham × Amazonian crosses.

RFLP study has enlarged the number of available molecular markers, and has detected fragments specific to particular States. Hence, by the increasing number of fragments detected, RFLP can help to provide powerful tools for genetic studies, such as paternity testing in seed gardens (Simmonds 1986). Molecular markers are essential for both identification purposes and diversity studies on *Hevea* since no distinctive morphological traits exist. The perennial crop characteristics of *Hevea* also make RFLP essential to provide early selection markers that could considerably reduce the long duration of breeding schemes. In this sense, the present study can help in defining a genetic mapping strategy for the *Hevea* genome. Such a linkage map is now in progress.

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