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Isozyme diversity within African *Manihot* germplasm

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Summary

Isozyme diversity is described among a collection of 365 *Manihot esculenta* cultivars plus 109 accessions from wild relatives (*M. glaziovii* and spontaneous hybrids) from Africa. The study is based on 17 polymorphic loci. A natural hybrid swarm is detected between the two species. Although they were recently introduced, *M. esculenta* and *M. glaziovii* show high levels of polymorphism: heterozygosity estimates are 0.225 and 0.252 respectively. For the wild species, diversity is structured at the unilocus level, and the multilocus approach reveals a geographical pattern. The organization of the diversity is not so clear for the cultivated cassava, but a multilocus approach, based on both common and rare alleles, led us to identify different groups of clones with many intermediate genotypes between them. Elements of the secondary diversification process of *Manihot* in Ivory Coast are discussed.

Introduction

The genus *Manihot* Mill. consists of about 100 species representing different geographical areas from Central and South America, most of them are monoecious (Rogers & Appan, 1973). The domestication of cassava, *Manihot esculenta* Crantz, is still not clear (Allem, 1987). Cytological evidence for an allopolyploid origin of the species was given, each one having 2n= 36 chromosomes (see Lefèvre & Charrier, in press, for review). Cassava was first introduced in Africa in the sixteenth century, but the culture did not spread before mid of the eighteenth; some other *Manihot* species were also introduced at the beginning of the twentieth century (Jones, 1959). Two major diseases, among others, are responsible for dramatic yield losses in Africa: the African cassava mosaic disease (Jennings, 1972) and the cassava bacterial blight (Umemura & Kawano, 1983). Resistance to such diseases is one of the main objectives for breeders, and wild species like *M. glaziovii* Muell. Arg. represent interesting sources of resistance genes (Hahn et al., 1980). Most of the breeding programs in Africa are based on the indigeneous germplasm, although information on its genetic diversity is still scarce.

There is an important diversity for morphological characteristics (vegetative organs and flowers) within and among *Manihot* species. These traits should be used with caution in the study of the genetic diversity among cultivated clones: although they do not seem to have any agronomic or organoleptic consequence, some authors have shown that they have been subjected to artificial selection as distinctiveness tools (Boster, 1985). We present here a biochemical approach based on isozyme diversity at the intra- and the inter-specific levels, using 17 polymorphic loci that were described in a previous paper (Lefèvre & Charrier, in press). A methodology was developed to describe the structure of

Table 1. Geographic origin of the *M. esculenta* accessions

Region	Country	No. of accessions
West Africa	Ivory Coast	260
	Ghana	1
	Nigeria	9
	Togo	11
Other African countries	Central Africa	45
	Congo	7
	Kenya	8
	Malagasy Republic	16
South America	Brazil	5
Asia	India	3
Total		365

the diversity in a set of independent clones without any population structure.

Materials and methods

The techniques and the markers were presented by Lefèvre & Charrier (in press). The germplasm collection from ORSTOM[1] consisted of 365 *M. esculenta* clones (Table 1): traditional cultivars collected in the Ivory Coast (Zoundjihekpon, 1986) and traditional or improved varieties from other research centres in Africa. All these clones have different names, however we suspect that a single genotype might be cultivated in various places under different names, resulting in possible duplications in our collection; to avoid such synonymies, this study is restricted to single genotypes with no repetition.

Other *Manihot* accessions were also collected in Ivory Coast: some were old abandoned plantations for latex production, others were frequently used as ornamental trees (Lefèvre, 1989). They were morphologically identified as 24 *M. glaziovii* samples (clones and progenies) and 85 plants derived from a *M. esculenta*× *M. glaziovii* natural hybrid swarm (clones only) (Table 2).

Heterozygosity and its variance were estimated, for the *M. esculenta* and the *M. glaziovii* samples, following the unbiased formulae given by Nei (1978):

$$H = (1/r) \sum_k 2n\,(1 - \sum_i x_{ik}^2)/(2n-1)$$

where:

x_{ik} = estimated frequency of the i-th allele of the k-th locus
n = population size
r = total number of loci

V(H) is calculated according to the values of n and H/(1-H).

Multivariate analyses were used to study the organization of genetic diversity. Each genotype was considered as an individual observation, and the presence of the different alleles for each loci represented the variables (null alleles were discarded). With that choice, a theoretical F1 hybrid would appear in intermediate position between its two parent lines: this seemed relevant for our study of the

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Table 2. Morphological characteristics of the *Manihot* samples in the collection

Character	*M. esculenta*	Interspecific hybrids	*M. glaziovii*
Habit	shrub	shrub or tree	tree
Latex production	moderate	moderate	important
Fruit wings	large	small	none
Leaf velum	none or small	medium	large
Tubers	many	few or none	none
Abscission scars	prominent	medium	non prominent
Lamina shape	linear, lanceolate elliptic, irregular	lanceolate or elliptic	round

diversity among heterozygous genotypes. Factorial correspondence analysis (Benzecri, 1980; also called 'reciprocal averaging' by Hill, 1973) was performed on the contingency table from these variables, and the individuals were projected as extra points. In a multivariate approach, alleles with high or low frequency do not share the same information (Brunel & Rodolphe, 1985). Rare alleles are obviously found in a small number of individuals, and sampling effects may result in linkage disequelibria with other loci: in such cases, the rare alleles may have enhanced contributions to the dispersal of the individuals, whereas they only represent few of them. To take in account simultaneously the information on the general background (common alleles) and the singularities (rare alleles), we proceeded in two steps.

In a first analysis, we only considered the codominant genes with frequencies above 22% (and below 78%): this level corresponded to the probability 0.05 for a rare homozygote, it was also a discontinuity in the frequency distribution (Table 3). In order to identify a possible structure at this step, we took 3 random samples of 99 genotypes each and performed hierarchical clustering (variance criterion) on the principal components: the clusters that were stable among the 3 analysis were then used as primers to aggregate additional individuals through discriminant analysis.

Then a second analysis took into account all the codominant alleles. Thus the genotypes were classified both according to the combinations of common alleles and the presence of rare alleles. Statistical analyses were performed with the NDMS package[2] on a personnal computer.

Results

Characterization of a natural hybrid swarm between M. esculenta *and* M. glaziovii

Only 10 genotypes could be distinguished among the 85 plants of the natural hybrid swarm. For 8 loci (namely: Got-B, Sdh-A, Mdh-A, Idh-A, Pgd-B, Est-A, Pgi-A, and Amp-A) most of the genotypes were heterozygous with one allele from each species. The other loci presented non-specific alleles and no conclusion could be drawn about interspecific heterozygosity. However two accessions were homozygous for *M. esculenta* alleles at some loci: Pgi-A and Amp-A for accession n° H69, and Pgi-A for n° H82 (Fig. 1). No clone with homozygosity for *M. glaziovii* alleles was found. This confirms the existence of various introgression levels among the spontaneous hybrids: in a previous study, the 2 clones H69 and H82 also appeared closer to *M. esculenta* on the basis of qualitative and quantitative morphological traits, domestication characteristics (ability to regenerate from cuttings, plant architecture, tuber formation), and pollen fertility (Lefèvre, 1989).

Mean heterozygosity

The gene frequencies are presented on Table 3. Among the 365 cultivars in the *M. esculenta* collection, we identified 184 different genotypes. Heterozygosity estimate for these 184 genotypes and 20 loci was H= 0.225 with a variance of V(H)= 0.0023. For each of the 11 polymorphic loci, the frequencies of heterozygotes did not differ from panmixia.

Among 41 *M. glaziovii* individuals (clones and half-sibs from the different progenies, interspecific hybrids excluded), 25 different genotypes were detected on the basis of 11 polymorphic loci. Four of these loci showed a significant lack of heterozygotes. Estimated heterozygosity for the 25 geno-

Fig. 1. AMP electrophoregrams; from left to right: *M. esculenta* (E, lanes 1 & 2), *M. glaziovii* (G, lanes 3 to 5), spontaneous interspecific hybrids (H, lanes 6 to 8), *M. esculenta* (E, lanes 9 to 11). Note the typical *M. esculenta* pattern of the 3rd hybrid.

[2]NDMS: Software for data management and statistical analysis. Logor, ORSTOM, Paris.

types and the 19 loci was significantly higher than for *M. esculenta,* with H = 0.252 and V(H) = 0.0027.

Structure of the genetic diversity

The excess of homozygous genotypes among the *M. glaziovii* accessions suggested a non random struc-

Table 3. Gene frequencies in the collection (×100)
+: included in the multivariate analysis
*: only found in the interspecific hybrids
–: undetectable polymorphism for *M. glaziovii* (overlaps)

Allele	*M. esculenta*	*M. glaziovii*	Allele	*M. esculenta*	*M. glaziovii*
Acp-A1	100	100	Est-C3	0	36
Got-A1	100	100	Got-B2	0	8+
Mdh-C1	100	100	Got-B3	0	*
Est-A1	100	0	Got-B4	0	88+
Got-B1	100	0	Got-B5	0	4+
Idh-A1	100	0	Idh-A2	0	72+
Pgi-A1	100	0	Idh-A3	0	28+
Pgi-C2	100	42+	Mdh-A4	0	100
Pgm-B1	100	0	Pgd-A3	0	46+
Amp-A1	92+	0	Pgd-A4	0	4+
Mdh-B0	92	–	Pgd-B3	0	8+
Pgm-A1	90+	0	Pgd-B4	0	78+
Pgd-B2	79+	14+	Pgi-A2	0	100
Idh-B0	66	0	Pgi-C1	0	36+
Pgi-B2	61+	30+	Pgi-C3	0	22+
Est-C1	57+	28+	Pgi-C4	0	*
Acp-B1	55+	16+	Pgi-B3	0	70+
Mdh-A1	53	0	Pgm-B2	0	100
Pgd-A1	48+	0	Sdh-A5	0	80+
Pgd-A0	47	0	Sdh-A6	0	16+
Acp-B2	45+	16+			
Sdh-A2	45+	0			
Est-C2	43	36			
Mdh-A3	39+	0			
Pgi-B1	39+	0			
Idh-B1	34+	100			
Sdh-A3	30+	0			
Pgd-B1	21+	0			
Sdh-A1	16+	0			
Pgm-A2	10+	100			
Amp-A3	8+	2+			
Mdh-A2	8+	0			
Mdh-B1	8	–			
Sdh-A4	8+	4+			
Pgd-A2	5+	50+			
Acp-B3	0	68+			
Amp-A2	0	10+			
Amp-A4	0	54+			
Amp-A5	0	34+			
Est-A2	0	98+			
Est-A3	0	*			
Est-A4	0	2+			

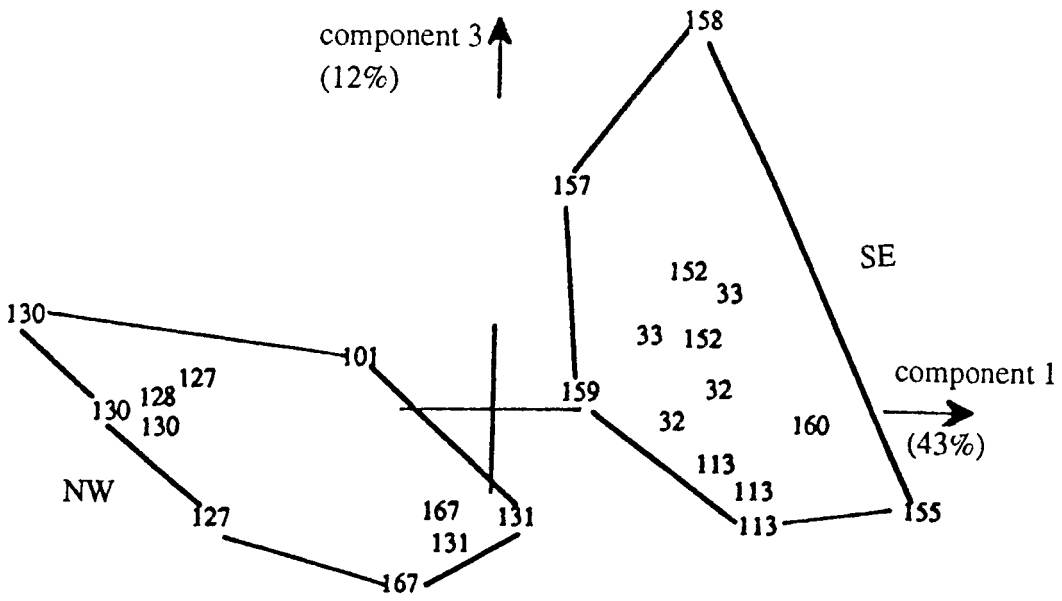

Fig. 2. Projection of the individuals on the plan of principal components 1 and 3 of the factorial analysis on 29 alleles from polymorphic loci in *M. glaziovii.* Each genotype is identified by its sample number (clone or progeny). Individuals from different geographic origins have different enzyme profiles. SE: samples collected in the South-East of Ivory Coast (Abidjan, Sikensi). NW: samples collected in the North (Khorogo) and the West (Gagnoa, Man).

ture of the genetic diversity. This was not a sampling artifact as shown by the factorial correspondence analysis on 29 alleles from the 11 polymorphic loci.

Four principal components accounted for 80% of the total inertia. In the plan defined by components 1 and 3, the south-eastern samples are clearly sep-

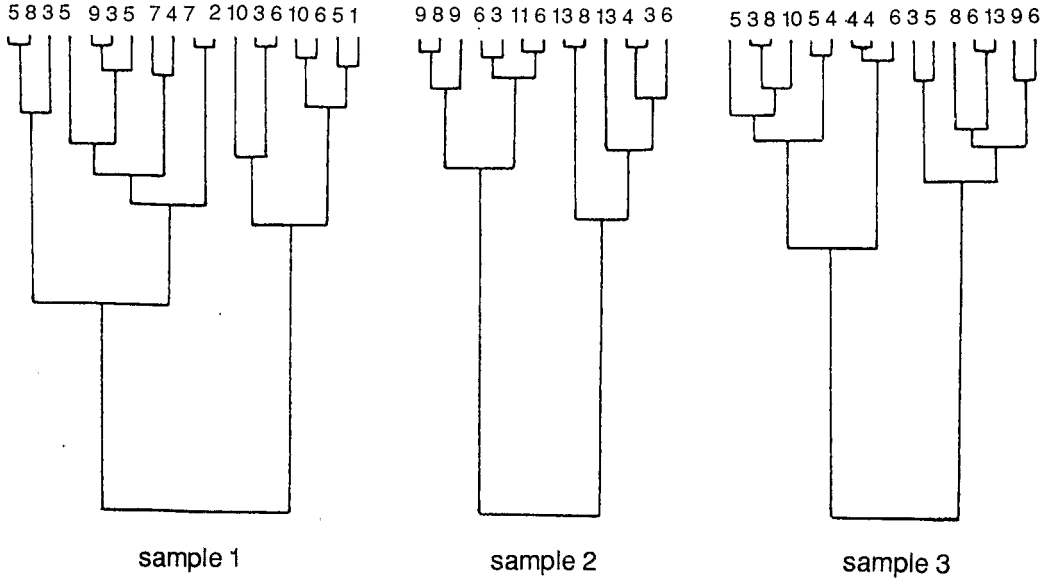

Fig. 3. Hierarchical clustering on 3 random samples of 99 genotypes. Two groups of genotypes are easily distincted in each case. The number of genotypes is indicated for the last levels illustrated.

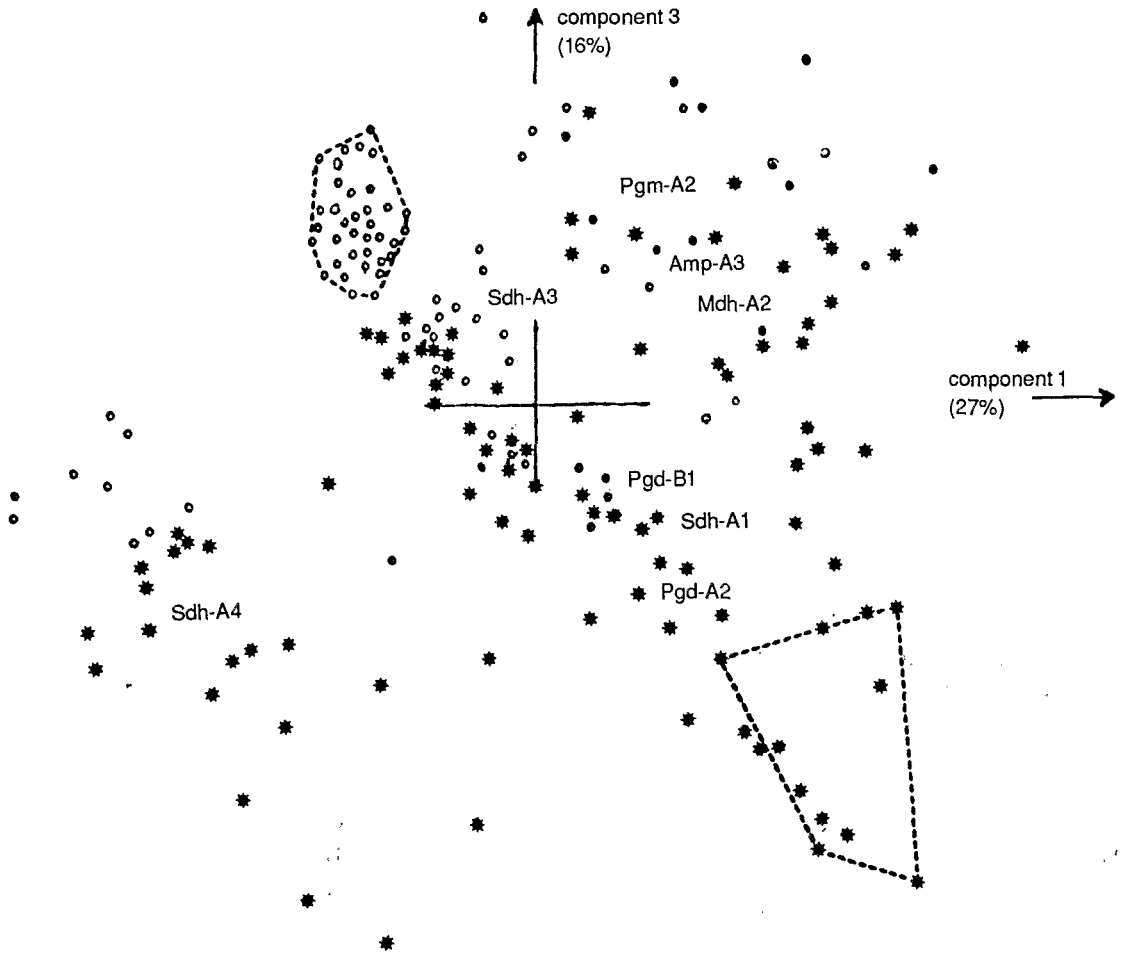

Fig. 4. Projection of the individuals on the plan of principal components 1 and 3 of the factorial analysis on 20 alleles from polymorphic loci in *M. esculenta.* Alleles with major contribution are also represented: they are all 'rare' alleles (frequency <22%) except Sdh-A3. Genotypes from the 'CB' and 'Bonoua' groups are respectively identified by black and white spots; dotted lines delimit the two major clusters (see text).

arated from those collected in the North or in the West of the Ivory Coast (Fig. 2).

For the cassava there was no structure at the unilocus level (panmixia was not rejected for any loci). This collection could not be split into different natural populations, and there was no obvious geographic differentiation among the cultivars: the gene frequencies estimated within the subset of traditional cultivars from the Ivory Coast did not significantly differ from those observed in the whole collection. However, at the multilocus level, gene associations were not random.

As mentioned above, we proceeded in a two step multivariate analysis of the codominant genes. The first factorial analysis included 10 common genes: 4 principal components accounted for 84% of the total variability. Hierarchical clustering was performed on 3 samples of genotypes, and two main clusters clearly appeared in each case (Fig. 3). Then we could define 2 groups of genotypes on the basis of the combinations of common genes: they were called 'CB' and 'Bonoua', from the names of well known cultivars.

The second analysis included 20 codominant alleles. Four principal components represented 76% of the variation, and, as expected, they were almost entirely determined by rare alleles. However the previous classification was still pertinent to these new principal components: discriminant analysis gave 81.5% of well classified genotypes in the 'CB'

and 'Bonoua' groups. Moreover, this two step analysis allowed us to identify two major clusters. One was composed of 11 genotypes belonging to the 'CB' group and sharing the original association of rare alleles 'Sdh-A1+ Pgd-B1+ Pgm-A2' (this association was never combined with other rare alleles), it was opposed to another group of 36 genotypes from the 'Bonoua' group with the particularity of having no rare allele. This is illustrated on Fig. 4 where the 2 major clusters appear on extreme positions of their respective groups 'CB' and 'Bonoua', with a lot of intermediate genotypes between them.

Discussion

Natural introgression between *M. esculenta* and *M. glaziovii* was detected, outside of their centre of diversification. Spontaneous hybrids between these species were previously mentioned in Africa (Nichols, 1947; Cours, 1951). The existence of a natural hybrid swarm among *Manihot* species was also mentioned in Brazil (Nassar, 1984). Such events support the hypothesis of a weak differentiation between *Manihot* species (Rogers & Appan, 1973), and may explain some changes in the systematics of the genus (Allem, 1987). Some of these hybrids were male fertile and might be used in the breeding programs (Lefèvre, 1989).

The two monoecious species appeared highly polymorphic: the heterozygosity estimates are in accordance with the values usually found in outcrossing species (Brown, 1989). This would indicate a secondary diversification process for *Manihot* germplasm in Africa. According to our results, we may assume different forms of evolution for the cassava and *M. glaziovii.* On one hand, cassava was introduced from Brazil 400 years ago: founder effects may be suspected at the time of introduction and also during the recent inland dispersal. Genetic diversity would have probably been lower if the crop was strictly clone propagated. However, Silvestre & Arraudeau (1983) have observed that African cultivators sometimes take the cuttings from the spontaneous seedlings, which in fact have not been submitted to several cycles of strong parasitic pressure. This practise may have favoured the sexual reproduction, whereas vegetative propagation permitted the spread of the best genotypes over different regions: there was no clear geographic pattern of variation for the isozyme markers.

On the other hand, *M. glaziovii* was introduced in the 1900's for rubber production experiments, which were rapidly dropped. Founder effects may also be assumed for this species. Numerous spontaneous seedlings are found in the places of abandonned plantations, which appear like artificial, isolated, populations of very recent origin. The lack of material transfer by man from one of these populations to the other would explain the geographic structure of the diversity of *M. glaziovii* in Ivory Coast.

Whatever the diversification process may be, it results in a great potential for cassava breeders in Africa. Advanced breeding strategies would get the best benefits of intra- and interspecific diversity. We identified different groups of genotypes among the *M. esculenta* cultivars, and the numerous intermediate forms between highly homogeneous clusters which could be the result of gene exchanges between groups. Obviously, isozymes should not be used to predict hybrid vigour (Brunel, 1985), but in the short term they could be used to avoid intercrossing between too similar genotypes.

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