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Heredity of seventeen isozyme loci in cassava (*Manihot esculenta* Crantz)

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Summary

Starch gel electrophoresis was used to assess isozyme polymorphism in two *Manihot* species. Crude extracts were obtained from leaves and pollen. Ten enzymes were examined for their polymorphism in a germplasm collection of 365 cultivated plus 109 wild accessions, mainly from Africa. The inheritance of these enzymes was examined using 13 intra and interspecific progenies. Seventeen polymorphic loci were found for the ten enzyme systems, with 59 alleles. All the markers showed disomic heredity and three linkage groups were identified.

Introduction

Cassava, *Manihot esculenta* Crantz is an important species for tropical agriculture. Besides traditional cultivation techniques, intensive management techniques are also used for this crop. Advanced breeding strategies are applied, including recurrent selection and biotechnology (Hahn et al., 1975; Roca et al., 1987). An important gene pool is available for the breeders, but we still lack some basic information about the genetic diversity within the genus (Charrier & Lefèvre, 1987). The use of oligogenic and 'neutral' markers is of great help for such studies.

The first attempts to use enzyme electrophoresis on cassava showed a high level of polymorphism within the species (Zoundjihekpon & Touré, 1985; Hussain et al., 1987; Ramirez et al., 1987). We used starch gel electrophoresis as a cheap and simple technique to identify genetic markers from 10 enzyme systems. Detection of allozymes was first based upon the survey of African cassava cultivars and wild accessions, then the genetic interpretation was confirmed on intra and interspecific progenies. Linkage relationships were also studied whenever possible.

In a following paper, these markers are used for the study of genetic diversity within African germplasm.

Materials and methods

This work was done in the Laboratoire de Ressources Génétiques et Amélioration des Plantes Tropicales of ORSTOM[1], in the Ivory Coast. The germplasm collection consisted in 365 *M. esculenta* clones and 109 wild accessions (*M. glaziovii* and spontaneous interspecific hybrides). Thirteen crosses were made by controlled pollination (Table 1). Seeds were dried at 60° C for 14 days before sowing (CIAT, 1981): the germination rates varied from 30% to 90% depending on the progeny. Several *M.*

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Table 1. Intra and interspecific controlled progenies

Female	Male	Progeny size
M. esculenta	*M. esculenta*	
041	041	30
041	043	51
053	043	62
V07*	V22	26
053	V22	41
402*	402*	19
053	041	64
V22	V22	9
053	V17*	42
402*	V17*	14
V07*	V17*	39
V03	V22	10
M. esculenta	*M. glaziovii*	
402*	G-113B	10
	Total=	417

* *M. esculenta* of introgressive origin.

esculenta varieties were known or presumed to come from hybridization with *M. glaziovii:* three of them were involved in the crosses.

We used crude extracts from young unexpanded leaves or pollen. Extraction buffers were taken from Berthou & Trouslot (1977) and Weeden & Gottlieb (1979). Leaves were ground with polyclar, Triton (10%) and extraction buffer, then filtered. For pollen extraction, anthers were taken from male flowers just before they opened, then stored in a desiccation bottle in a cool place for 6 hours, and finally soaked in the buffer (about 3ml for 50mg anthers). Anther fragments were removed after shaking, and the pollen was kept in the buffer at 4° C for 12 hours before electrophoresis.

Ten enzymes were selected. The electrolyte buffers were 0.41M sodium-citrate at pH 6.0 or pH 8.0 depending on the enzyme (Table 2). The gel buffer was 5mM Histidine-HCl, 2.5mM NaCl, adjusted to the corresponding pH value. Starch concentration was 13%; gels were run at 4° C for 5 to 6 hours, and then cut into 3 to 4 slices used for different detection systems. Enzyme activities were determined according to the method given by Second & Trouslot (1980), but for shikimate dehydrogenase, Tanksley & Rick's (1980) procedure was used.

For each of the 10 enzymes, electrophoregram stability was previously tested using 48 *M. esculenta* clones, with young leaves from different cuttings, grown under various physiological or sanitary conditions: bands that did not appear systematically for different extracts from a single clone were discarded for the rest of the study. Genetic analysis was based on the survey of the phenotypes in the collection and in the controlled crosses. Heredity of these markers was tested through progeny analysis. Progenies from a single heterozygous individual crossed with several homozygous genotypes were pooled together. When the chi-square test for segregation homogeneity between families was not significant, offsprings from various crosses showing the same phenotypic combination were pooled.

Linkage between two markers was detected by a chi-square test: for each pair of loci, this test always gave corroborating results among the different progenies. In the case of linkage, the recombination

Table 2. Standard conditions for electrophoresis: enzymes, origin of the extracts and pH value

Enzyme		Extract	pH
Acid phosphatase	ACP	leaves	6.0
Leucine aminopeptidase	AMP	leaves	6.0
Esterase	EST	leaves	6.0
Glutamate oxaloacetate transaminase	GOT	leaves	6.0
Isocitrate dehydrogenase	IDH	leaves	6.0
Malate dehydrogenase	MDH	leaves	8.0
6-phosphogluconate dehydrogenase	PGD	leaves	6.0
Phosphoglucose isomerase	PGI	leaves+ pollen	8.0
Phosphoglucomutase	PGM	leaves	8.0
Shikimate dehydrogenase	SDH	leaves	8.0

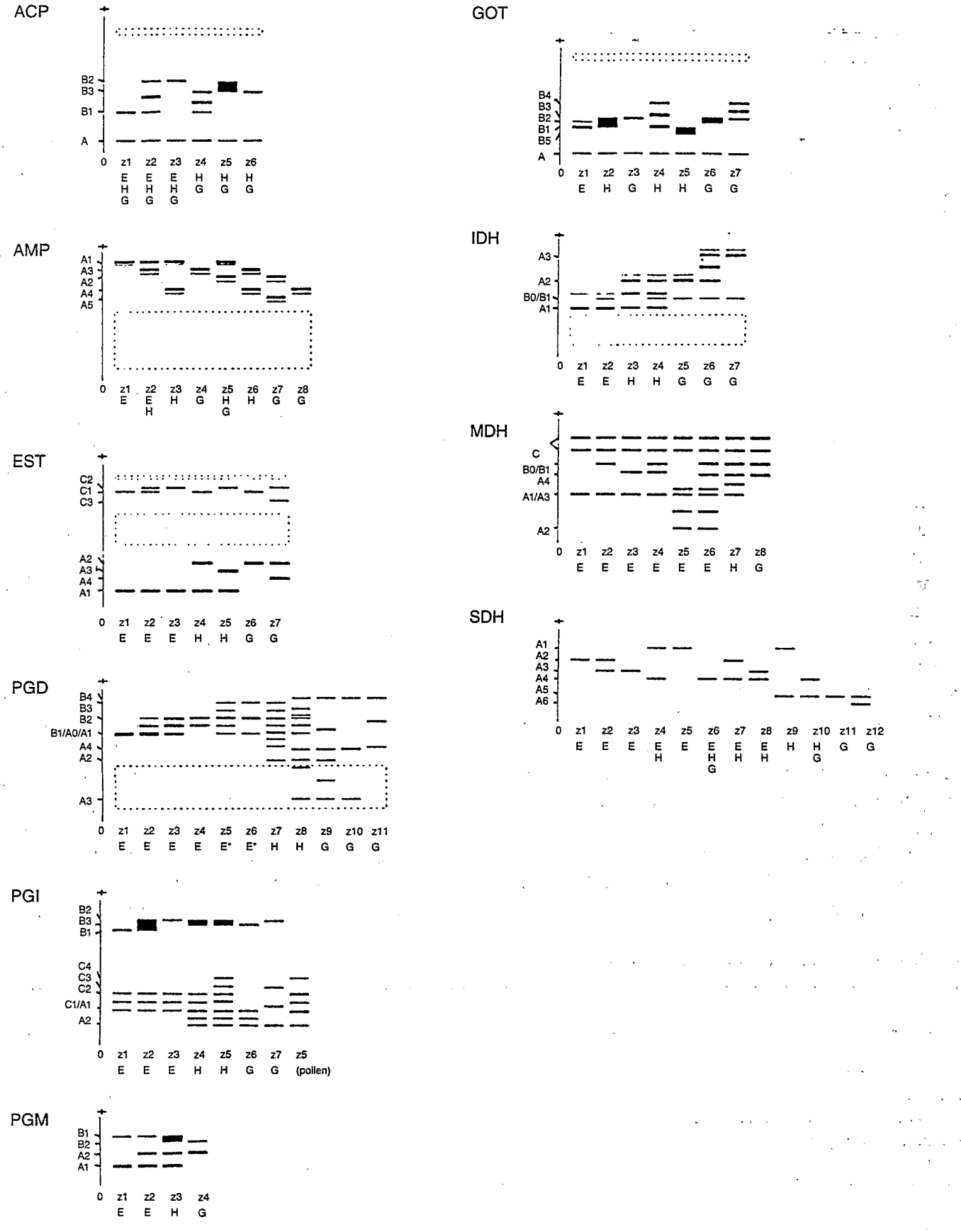

Fig. 1. Main electrophoregrams for *M. esculenta* (E, or E* if of introgressive origin), *M. glaziovii* (G), and their hybrids (H). A code is given for each phenotype (zi), as well as a genetic interpretation (Ai, Bj, ... see text). Dotted lines delimit non interpreted activity.

value and its standard deviation were estimated following the multiple segregation analysis method described by Allard (1956). Homogeneity of the recombination value among the progenies was also tested.

Results

The various phenotypes and their genetic interpretation

Most of the phenotypes found in the collection and in the progenies are presented on Fig. 1. Each presumed locus was labelled with a letter (Est-A, Est-C, Got-B...), followed by different numbers for the various alleles. A classical genetic interpretation (Table 3) was generally supported by the segregations in the controlled progenies (Table 4). The more complex situations for malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD) and phosphoglucose isomerase (PGI) are detailed below.

The *M. esculenta* MDH phenotypes consisted of 3 to 8 bands. This system appeared monomorphic in the *M. glaziovii* samples, with 4 bands. Three migration levels were detected: the A level was interpreted as a dimeric product of a single gene (1 to 3 bands); the B level, a present or absent spot, was considered as the product of a single locus representing a silent recessive allele (this was frequently superimposed on the *M. glaziovii* A4 band); the C level was arbitrarily considered as a single gene product (monomorphic with 2 bands). Several genotypes presented additional bands between the A and the C levels: these could be interpreted as interloci heterodimeric compounds, dimerization occuring between some Mdh-A allozymes (A2 or A3 products) and the Mdh-C product. Segregating progenies showed the existence of a recessive allele, A1, whose product did not form heterodimeric proteins. These hypotheses were consistent with the segregations in the controlled crosses (Table 4).

The PGD phenotypes were rather complex. However, they were stable within each clone, and the relative thickness of each band within a single

Table 3. Genetic interpretation of the electrophoregrams

Enzymes	Presumed loci	No. of bands	Genetic interpretation
ACP	Acp-A	1	monomorphic
	Acp-B	1 or 3	dimeric, 3 codominant alleles
AMP	Amp-A	1 or 2	monomeric, 5 codominant alleles
EST	Est-A	1 or 2	monomeric, 4 codominant alleles
	Est-B	–	not interpreted
	Est-C	1 or 2	monomeric, 3 codominant alleles
	Est-D	–	not interpreted
GOT	Got-A	1	monomorphic
	Got-B	1 or 3	dimeric, 5 codominant alleles
IDH	Idh-A	1 or 3	dimeric, 3 codominant alleles
	Idh-B	1 or 2	1 dominant+1 silent recessive alleles
MDH	Mdh-A	4 to 8	dimeric, 3 codominant+1 recessive alleles
	Mdh-B	(see text)	1 dominant+1 silent recessive alleles
	Mdh-C		monomorphic
PGD	Pgd-A	1 to 9	dimeric, 4 codominant+1 silent recessive alleles
	Pgd-B	(see text)	dimeric, 4 codominant alleles
PGI	Pgi-A	4 to 8	dimeric, 2 codominant alleles
	Pgi-B	+spots	3 codominant alleles
	Pgi-C	(see text)	dimeric, 4 codominant alleles
PGM	Pgm-A	1 or 2	monomeric, 2 codominant alleles
	Pgm-B	1 or 2	monomeric, 2 codominant alleles
SDH	Sdh-A	1 or 2	monomeric, 6 codominant alleles

genotype was repeatable. Phenotypes were defined by 1 to 9 bands. Intraspecific progenies showed the heterozygous origin of the z2 phenotype (with 3 heterogeneous bands), which behaved as a hybrid between z1 (one band) and z3 (3 equally stained bands) (Table 4). Therefore two loci were presumed, Pgd-A and Pgd-B, coding for dimeric isozymes which could form heterodimeric proteins. Alleles

Table 4. Progeny analysis: phenotypes of the parents (*P*) and the offspring (*O*) from various progenies are presented. A genetic interpretation is given for each crosses. For AMP, IDH, MDH, PGD, PGI and SDH, the interspecific progeny is given in the last position

Phenotypes	Genetic interpretation	Chi square	Phenotypes	Genetic interpretation	Chi square
ACP	**Acp-B**		**PGD**	**Pgd-A & Pgd-B**	
P: z2× z2	B1B2× B1B2	ns	*P:* z3× z3	A1A1B2B2×	
O: 12 z1+ 17 z2+ 7 z3				A1A1B2B2	–
AMP	**Amp-A**		*O:* 38 z3		
P: z2× z2	A1A3× A1A3	ns	*P:* z1× z2	B1B1× B1B2	ns
O: 6 z1+ 9 z2+ 4 z4			*O:* 16 z1+ 25 z2		
P: z2× z1	A1A3× A1A1	ns	*P:* z1× z2	B1B1× B1B2	**
O: 6 z1+ 8 z2			*O:* 41 z1+ 21 z2		
P: z2× z8	A1A3× A4A4	ns	*P:* z2× z2	B1B2× B1B2	ns
O: 4 z3+ 6 z6			*O:* 4 z1+ 8 z2+ 7 z3		
EST	**Est-C**		*P:* z2× z3	B1B2× B2B2	ns
P: z1× z2	C1C1× C1C2	ns	*O:* 11 z2+ 15 z3		
O: 16 z1+ 10 z2			*P:* (z1 or z3)× z5	----× B2B3	**
GOT	**Got-B**		*O:* 65 (z2 or z3)+ 30 (z5 or z6)		
P: z2× z2	B1B3× B1B3	ns	*P:* z3× z10	A1A1B2B2×	
O: 3 z1+ 10 z2+ 6 z3				A2A2B4B4	–
IDH	**Idh-A & Idh-B**		*O:* 10 z8		
P: z2× z2	B0B1× B0B1	ns	**PGI**	**Pgi-A, Pgi-B & Pgi-C**	
O: 25 z1+ 51 z2			*P:* z2× z3	B1B2× B2B2	ns
P: z4× z2	B0B1× B0B1	**	*O:* 31 z2+ 31 z3		
O: 13 (z1 or z3)+ 13 (z2 or z4)			*P:* z3× z6	A1A1C2C2×	
P: z1× z4	B0B0× B0B1	ns		A2A2C1C1	–
O: 29 (z1 or z3)+ 27 (z2 or z4)			*O:* 10 z4		
P: z4× z2	A1A1× A1A2	ns	**PGM**	**Pgm-A**	
O: 12 (z1 or z2)+ 15 (z3 or z4)			*P:* z1× z2	A1A1× A1A2	ns
P: z1× z4	A1A1× A1A2	**	*O:* 16 z1+ 10 z2		
O: 37 (z1 or z2)+ 19 (z3 or z4)			**SDH**	**Sdh-A**	
P: z4× z4	A1A2× A1A2	ns	*P:* z2× z2	A2A3× A2A3	ns
O: 10 (z1 or z2)+ 25 (z3 or z4)+ 4 z5			*O:* 23 z1+ 31 z2+ 20 z3		
MDH	**Mdh-A & Mdh-B**		*P:* z2× z1	A2A3× A2A2	ns
P: (z3 or z4)× z6	–A3× A2A3	**	*O:* 19 z1+ 22 z2		
O: 40 z4+ 22 z6			*P:* z1× z7	A2A2× A2A4	ns
P: (z3 or z4)× z4	A1A3× A1A3	ns	*O:* 18 z1+ 18 z7		
O: 36 (z1 or z2)+ 131 (z3 or z4)			*P:* z4× z4	A1A4× A1A4	ns
P: z3× z4	B0B0× B0B1	ns	*O:* 5 z5+ 11 z4+ 3 z6		
O: 78 (z1 or z3)+ 80 (z2 or z4)			*P:* z4× z11	A1A4× A5A5	ns
P: (z2 or z4)× (z2 or z4)	B0B1× B0B1	ns	*O:* 5 z9+ 5 z10		
O: 9 (z1 or z3)+ 33 (z2 or z4)					
P: z2× z8	A1A1× A4A4	–			
O: 10 z7					

– no segregation.
ns non significant.
** significant distortion at the 1% level.

A1 and B1 were superimposed, and Pgd-A locus presented a silent recessive allele, A0, detected in phenotype z4. The genetic interpretation of the wild phenotypes followed the same model; Pgd-A3 homodimeric isozymes, as well as some of their derived heterodimeric proteins, belonged to the slow migration level.

The PGI phenotypes were characterized by two levels of migration corresponding to different cell compartments. The most anodic level consisted of a single band or a diffuse spot delimited by two bands, and was considered as a single gene protein located in the cell plastids; these bands did not appear with the pollen extracts. The slowest migration level was monomorphic within the *M. esculenta* collection, with 3 bands, and polymorphic in the *M. glaziovii* or the interspecific hybrid samples, with 3 to 7 bands. Some of these slow bands were not stained from pollen extracts. Comparing diploid tissues (leaves) with a haploid mixture (pollen grains), from a single genotype, allowed the distinction between intralocus heterodimeric bands (i.e. real heterozygosity, characteristic of the diploid tissues) and interloci heterodimeric bands which remained in the haploid extracts. Thus, the slowest migration level of PGI was considered as composed of two single locus dimeric proteins, able to form heterodimers (Table 4).

Heredity of the markers

For each enzyme, 1 to 7 informative progenies were available: most of the segregations agreed with the hypothesis of disomic inheritance. Distortion was significant for Idh-B in one progeny out of three, in one of two progenies for Mdh-A, and two progenies out of five for Pgd-B. The introgressed variety 'V17' was a common ancestor for the progenies which showed distortion for the Pgd-B locus: in these cases, there was a significant loss of the Pgd-B3 allele, specific to *M. glaziovii.* Two crosses from variety '043' showed significant distortions at the Mdh-A and the Pgd-B loci.

At the Est-A locus, the occurrence of the *M. esculenta* phenotype in the interspecific cross suggested the presence of a silent recessive allele in the wild genome.

Linkage relationships

Three linkage groups were detected. The smallest recombination value was found between the Pgd-B and Idh-A loci ($p = 0.02 \pm 0.019$), among two crosses from the introgressed variety 'V17', which had a heterozygous (interspecific) genotype at these loci. The recombination value between Mdh-B and Pgm-A was $p = 0.15 \pm 0.045$, these loci being independent from the previous group. The Sdh-A locus appeared independent from those two groups, its linkage with the Amp-A locus should be confirmed ($p = 0.31 \pm 0.075$), for it was only expressed through the cohesion between wild electromorphs in the progenies of a double heterozygous introgressive variety. In relation to these 3 linkage groups, the Mdh-A locus appeared independant from the Pgd-B locus, and the Acp-B locus was independant from the Sdh-A locus.

Table 5. Isozyme polymorphism in *M. esculenta* and *M. glaziovii.* Some of the *M. glaziovii* specific electromorphs were occasionally found in cassava varieties of introgressive origin. Silent recessive alleles Est-A0 and Pgd-A0 were presumed in the *M. glaziovii* genome, they should be confirmed

Enzymes	Loci	*M. esculenta* alleles	*M. glaziovii* alleles
ACP	Acp-A	1	1
	Acp-B	1-2	1-2-3
AMP	Amp-A	1-3	2-3-4-5
EST	Est-A	1	2-3-4-(0)
	Est-C	1-2	1-2-3
GOT	Got-A	1	1
	Got-B	1	2-3-4-5
IDH	Idh-A	1	2-3
	Idh-B	0-1	1
MDH	Mdh-A	1-2-3	4
	Mdh-B	0-1	–
	Mdh-C	1	1
PGD	Pgd-A	0-1-2	2-3-4-(0)
	Pgd-B	1-2	2-3-4
PGI	Pgi-A	1	2
	Pgi-B	1-2	2-3
	Pgi-C	2	1-2-3-4
PGM	Pgm-A	1-2	2
	Pgm-B	1	2
SDH	Sdh-A	1-2-3-4	4-5-6

Discussion

Different authors have shown the interest of enzyme electrophoresis for the studies of cassava genetics, although some phenotypes were unstable depending on the origin of the extract and the buffers (Zoundjihekpon & Touré, 1983; Ramirez et al., 1987). In our study, we were able to give a genetic interpretation for most of the phenotypes, after fixing standard conditions for extraction, migration and staining. Seventeen polymorphic loci were identified, with a total of 59 alleles (Table 5). The same genetic model was assumed for *M. esculenta* and *M. glaziovii* species, both showing typical diploid patterns with one or two alleles per locus. A single name was given for electromorphs of the two species that presented identical mobility; however, their relationship should be further investigated, trying to reveal residual variability by other biochemical tests: by the use of specific substrates, inhibitors, or thermosensitivity test (Second & Trouslot, 1980). The Got-B3, Idh-A2, Amp-A2 and Pgd-B3 electromorphs, which appeared specific to *M. glaziovii* were also found in several cassava varieties of introgressive origin: they should be of great help to follow the introgressive process.

We observed typical disomic heredity of the markers. The heterogenity of the germination rate might account for the distortion observed at the Idh-B locus in one progeny. The unexpected segregations for the Pgd-B locus in the crosses involving the 'V17' variety might be symptomatic of local chromosomal disturbance due to the interaction of *M. esculenta* and *M. glaziovii* genomes. Besides, the significant distortions detected for the two independent loci Mdh-A and Pgd-B suggested an abnormal gametic segregation in the variety '043'.

Three linkage groups were detected. The linkage between the Pgd-B and the Idh-A loci in the 'V17' introgressive variety could not be confirmed from pure *M. esculenta* progenies, since Idh-A was monomorphic in such cases. However, very strong cohesion between the Pgd-B3 and the Idh-A2 alleles was noticeable: first because these two electromorphs, which both derived from a *M. glaziovii* ancestor, remained in the 'V17' genotype although it was obtained through repeated backcrossing and selection for resistance to cassava mosaic virus, and second because a reduction of recombination rate has been observed in various interspecific crosses (Bonierbale et al., 1988; Paterson et al., 1988).

Genetic interpretation of the phenotypes included the presence of heterodimeric forms for three enzyme systems. Such interactions between loci coding for MDH have been reported in rice (Second & Trouslot, 1980) and maize (Goodman et al., 1980a). PGD is generally coded by two loci (see Gottlieb, 1982, for review), and the formation of heterodimeric compounds has been mentioned in maize (Goodman et al., 1980b). The duplicated origin of PGI genes was previously mentioned in several genera (Gottlieb, 1977; Second & Trouslot, 1980; Chevreau et al., 1985; Goldring et al., 1985; Martinez Zapater & Oliver, 1985).

The duplication of some loci would support the hypothesis of an allopolyploid origin for *M. esculenta* and *M. glaziovii* genomes, as was first suggested by cytological studies on the cultivated cassava (Magoon et al., 1969). The same chromosome number 2n= 36 was found in wild *Manihot* species (Krishnan et al., 1970; Nassar, 1978; Nassar, 1979; Nassar et al., 1986), indicating that polyploidy was probably prior to *Manihot* diversification. Ploidy modifications were also mentioned in interspecific crosses (Hahn et al., 1990). However, in our study, it did not seem to affect the pure disomic inheritance of the markers.

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