Genetic Resources and Crop Evolution **47:** 371–383, 2000.

Using isozyme polymorphism to assess genetic variation within cultivated yams (*Dioscorea cayenensis/Dioscorea rotundata* complex) of the Republic of Benin

A. Dansi[1,5], H.D. Mignouna[2], J. Zoundjihékpon[3], A. Sangare[4], R. Asiedu[2] & N. Ahoussou[4]
[1]*International Institute of Tropical Agriculture (IITA), 08 BP 0932 Cotonou, Benin*
[2]*International Institute of Tropical Agriculture (IITA), PMB 5320 Ibadan, Nigeria*
[3]*Wordwide Fund for Nature (WWF), 08 BP 1776 Abidjan 08, Côte d'Ivoire*
[4]*UFR Biosciences, Faculté des Sciences et Techniques, 22 BP 582 Abidjan 22, Côte d'Ivoire*
[5]*Laboratoire de Génétique, UNB/FAST, BP 526 Cotonou, Bénin*

Received 1 July 1999; accepted in revised form 29 September 1999

Key words: core collection, cultivar groups, *Dioscorea cayenensis*, *Dioscorea rotundata*, genetic relatedness, Guinea yam, isozyme markers, morphotype, yam identification

Abstract

Four hundred and sixty-seven accessions of cultivated Guinea yam (*Dioscorea cayenensis/Dioscorea rotundata* complex) collected from different localities of Benin Republic were analysed to study isoenzymatic variability in seven enzyme systems: aspartate aminotransferase (AAT), esterase (EST), glucose-6-phosphate dehydrogenase (G6PDH), isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), and shikimate dehydrogenase (SKDH) using starch gel electrophoresis. Polymorphism was observed in all of the enzyme systems and a total of 62 electromorphs of different frequency and variability patterns were recorded. Different combinations of banding patterns of these systems led to identification of 227 different cultivars within the 467 accessions analysed. For an old and vegetatively propagated crop (with a considerable number of vernacular names) such as yam, and for which a high rate of duplication is expected, the 227 cultivars were found to be good enough to be considered as the adequate number of accessions representing the diversity in the germplasm analysed. Cluster analysis (UPGMA) produced a most likely division of the 467 accessions into two groups corresponding to *D. rotundata* Poir. and *D. cayenensis* Lam., supporting the concept that the two forms of guinea yam represent different genetic entities. The different clusters formed within the white yams (*D. rotundata*) did not exactly conform to the known cultivar groups. Additional polymorphic enzymes are needed for an accurate isozyme-based genetic discrimination of most of the cultivar groups.

Introduction

Tropical root and tuber crops occupy a pre-eminent position as food crops, next only to cereals and grain legumes, and they also form the subsidiary staple of over 20% of world population. Among the tropical tuber crops, cultivated guinea yam (*Dioscorea cayenensis/Dioscorea rotundata* complex) is one of the most important, especially in the so-called 'yam belt' of West Africa. Because of its important contribution to food security, yam has become an important target for breeding new cultivars with novel or improved characteristics. However, one of the prerequisites to this important task is better knowledge of the existing traditional cultivars held by farmers.

In order to access the diversity within this species complex (*D. cayenensis–D. rotundata)* in Benin Republic, systems of classification and identification based on morphological characters were recently used (Dansi et al., 1998, 1999). Although these methods are effective, they present practical drawbacks due to the effect of environmental fluctuations on expression of some morphological traits. The use of biochemical markers such as isozymes overcomes these prob-

lems since they are little affected by the environment and can easily be detected in a variety of tissues by relatively simple, rapid and inexpensive procedures.

During the last decade, isozymes have been used extensively in many crop breeding programs as genetic markers for identifying cultivars (Torres & Bergh, 1980; Nielsen, 1985; Weeden & Lamb, 1985; Degani et al., 1995), in marker-assisted selection (Manganaris et al., 1994), for confirming hybridity (Anderson et al., 1991) and for performing many other aspects of plant breeding, such as selecting donor and recipient parents and monitoring backcross progeny (Tanksley & Orton, 1983). With yams, only few attempts have been reported up to now (Hamon & Touré, 1990a,b).

The objectives of the present study were to identify polymorphic isozyme banding patterns, to use combinations of these patterns to assess genetic variation within the different yam cultivar groups identified based on morphological traits (Dansi et al., 1999), to distinguish cultivars, and to examine the relationships among yam cultivars.

Materials and methods

Materials

The germplasm studied consisted of 467 accessions of yams belonging to the *D. cayenensis–D. rotundata* complex which were collected in 1996 and 1997 from different localities of Benin and maintained as a field collection at the International Institute of Tropical Agriculture (IITA) in Benin Republic (Dansi et al., 1997).

Methods

Starch gels (14%) were prepared with hydrolysed starch (Sigma, USA) in heated gel buffer (0.085 M Tris and 0.048 M Histidine, pH 8), degassed with a tap aspirator, poured into an acrylic gel mould in which electrode strips had been sealed with masking tape. Occasional air bubbles were quickly removed with forceps. Cast gels were allowed to cool for approximately 30 min at room temperature, covered with a plastic film to prevent dehydration and left to set in position overnight at room temperature (25 °C).

Enzymes were extracted by crushing pieces of fresh leaves in a small amount of extraction buffer (Hamon & Touré, 1990a). A small spatula tip of insoluble PVP was added during the homogenisation to improve zymograms (Kephart, 1990). Filter paper wicks (7 × 6 mm, Whatman no. 3) were dipped into the leaf extracts. The wicks were then removed, lightly blotted and loaded into a transverse cut in the gels. Wicks dipped in bromophenol blue dye solution were also inserted to visualise the migration of the front. A sample with known, distinctive banding pattern was repeated three times across the gel to serve as reference.

The electrophoresis was conducted at 4 °C for 4 h using an electrode buffer of 0.153 M Tris and 0.04 M citric acid (pH 8). After electrophoresis, gels were sliced horizontally and stained for the appropriate enzymes. In order to detect the presence of background staining before routine use of enzyme staining procedures, the staining was carried out with and without substrate for each enzyme system on replicate slabs.

The enzymes assayed were aspartate aminotransferase (AAT; EC 2.6.1.1), esterase (EST; EC 3.1.1.-), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), isocitrate dehydrogenase (IDH; EC 1.1.1.41), phosphoglucomutase (PGM; EC 5.4.2.2), phosphoglucoisomerase (PGI; EC 5.3.1.3), shikimate dehydrogenase (SKDH; EC 1.1.1.25). Gels of PGI, SKDH, ICD and EST were stained according to Hamon & Touré (1990a). For AAT and PGM, methods used are those described by Tanksley & Orton (1983). To improve clarity of the AAT gels, 1% (w/v) soluble polyvinylpyrrolidone (PVP 40) was added to its staining solution according to Jongedijk et al. (1990).

Statistical methods

Each band was treated as a unit character, and the accession was scored for the presence or absence of a band and coded as 1 or 0, respectively. Using this methodology, 55 variables were created and a binary matrix was compiled. Pairwise distances between samples were computed by the NTSYS-pc 1.8 software package (Rohlf, 1993) using the simple matching coefficient of similarity (Gower, 1985). Dendrograms were created by UPGMA cluster analysis (Sneath & Sokal, 1973; Swofford & Olsen, 1990).

Results and discussion

Isozyme systems

Polymorphisms were observed in all the seven systems analysed. The enzyme systems used provided adequate resolution to even allow scoring more than

Table 1. Frequency of the isozyme phenotypes observed among the 467 analysed yam accessions. Numbers in the table represent the total number of accessions for which the corresponding phenotype has been recorded for each of the enzyme systems

Phenotypes	AAT	PGM	IDH	SKDH	PGI	PGD	EST
A	126	268	304	142	402	276	127
B	27	12	07	198	53	07	261
C	245	10	30	31	02	107	68
D	33	87	02	50	04	33	07
E	02	63	102	07	06	28	03
F	02	06	02	10	–	05	01
G	05	06	05	10	–	01	–
H	14	05	01	12	–	09	–
I	06	05	01	07	–	01	–
J	02	05	03	–	–	–	–
K	05	–	05	–	–	–	
L	–	–	05	–	–	–	–

one zone of activity for some of them. All observed zymogram patterns are illustrated in Figure 1. The zones of activity were numbered, as well as the bands, according to their proximity to the anodal end.

SKDH activity was detected in a single region of the zymogram and nine phenotypes were revealed, three homozygous and six heterozygous (Fig. 1a). SKDH is monomeric in yam (Zoundjihekpon et al., 1994). The banding patterns observed for this enzyme support its monomeric structure. The *F* phenotype is consistent with a polyploid individual and is found with cultivars known as hexaploid (MAKPAWA) or octoploid (ALAKISSA).

In the *PGD* zymogram, two polymorphic zones of activity *(PGD-I, PGD-II)* were detected (Figure 1a). The most anodal *(PGD-I)* was resolved as either single bands or double bands. *PGD-II* exhibited three phenotypes of single-, double- or five-banded pattern. PGD has been shown to be monomeric in yam (Zoundjihekpon et al., 1994). Effect of a null allele (lack of staining activity) is found in the phenotypes *C*, *F*, *G* and *I*. Phenotype *H* is the one of a polyploid individual and is found with the hexaploid cultivars of the cultivar group BARIDJO.

For *EST*, a single region of activity was found with six phenotypes, two homozygous and four heterozygous (Figure 1a). In yams, *EST* is also monomeric but with one secondary isozyme (Zoundjihekpon et al., 1994). Phenotypes exhibited by this system are consistent with a monomeric enzyme. Phenotypes D, E and F indicate polyploid individuals, and were found only with ALAKISSA (octoploid) and MAKPAWA (hexaploid).

IDH was resolved as one zone of activity with four single-banded phenotypes, three double-banded phenotypes and five triple-banded phenotypes (Figure 1b). As in many other plant species (Weeden & Weeden, 1989; Kephart, 1990), IDH is dimeric in yam (Zoundjihekpon et al., 1994) and phenotypes observed are also consistent with a dimeric structure of the enzyme. Either the allelic dominance effect or the presence of a null allele would explain the three double-banded phenotypes observed.

For *PGI*, a single region of activity was found with five phenotypes, two homozygous and three heterozygous (Figure 1b). PGI is also dimeric in yam (Zoundjihekpon et al., 1994) and phenotypes observed are consistent with a dimeric structure of the enzyme.

Two polymorphic zones of activity (AAT-I and AAT-II) were found in the AAT zymogram. AAT-I exhibits five phenotypes, three with double bands and the other with three regularly spaced bands. The slower migration zone (AAT-II) has five phenotypes: three with a single band and the other with two bands. Based on the zymogram, AAT in yam, like in many other plants (Gottlieb, 1982; Kephart, 1990), is likely controlled by two loci. Since no controlled crosses have been made from known parents for evaluating segregating populations, the hypothesis of two loci must, however, be considered preliminary.

In gels stained for *PGM*, two zones of activity were also observed. The fastest migration zone (PGM-I) had three phenotypes and was resolved either as a single band or two bands. The lower migration zone (PGM-II) was also polymorphic with single-banded and triple-banded patterns. PGM in plants is typically controlled by two loci (Gottlieb, 1982; Kephart, 1990) and known to be monomeric (Weeden & Weeden, 1989; Kephart, 1990). Considering that the quaternary structure of enzymes in plant species has remained highly conserved throughout biochemical evolution, especially for the enzymes catalysing steps in primary metabolism (Weeden & Weeden, 1989; Gottlieb, 1982), we can assume that the three-banded patterns observed in PGM-II reflect the polyploid nature of yam (tetraploid, hexaploid or octoploid), and that PGM conforms to a monomeric system. This, however, needs confirmation by progeny analysis.

As far as the frequency distribution of the different patterns of a given isozyme system within the germplasm is concerned, Table 1 and Figure 2 clearly show

Table 2. The cultivar groups, their morphological diversity and the number of isozyme phenotypes identified within them

Cultivar groups*	NA	NM*	Morphological diversity*		NGI
			Shoot	Tuber	
AGOGO	14	04	Heterogeneous	Heterogeneous	08
AHIMON	34	02	Homogeneous	Heterogeneous	19
ALAKISSA	08	03	Homogeneous	Heterogeneous	03
ANTAWORÒROU	06	03	Heterogeneous	Heterogeneous	03
BANIOURE	30	04	Homogeneous	Heterogeneous	13
BARIDJO	09	03	Homogeneous	Heterogeneous	01
DIKPIRI	02	01	Homogeneous	Homogeneous	01
DOUBA YESSIROU	10	02	Homogeneous	Heterogeneous	05
GNALABO	10	03	Heterogeneous	Heterogeneous	04
GNIDOU	28	01	Homogeneous	Homogeneous	05
KOKOROGBANOU	88	14	Homogeneous	Heterogeneous	28
KPANHOURA	06	02	Homogeneous	Homogeneous	01
KPONAN	07	01	Homogeneous	Heterogeneous	05
KRATCHI	18	02	Homogeneous	Heterogeneous	08
MAKPAWA	03	02	Heterogeneous	Heterogeneous	02
MONDJI	60	11	Heterogeneous	Heterogeneous	29
MOROKOROU	23	02	Heterogeneous	Heterogeneous	08
NONFORWOU	13	04	Homogeneous	Heterogeneous	09
NOUALAYE	18	01	Homogeneous	Homogeneous	07
OURTCHOUA	12	03	Heterogeneous	Heterogeneous	06
PORCHEHBIM	06	02	Homogeneous	Heterogeneous	01
SOUSSOU	42	11	Heterogeneous	Heterogeneous	24
TABANE	13	05	Homogeneous	Heterogeneous	01
TAM SAM	01	01	Homogeneous	Homogeneous	01
TERKOKONOU	04	01	Homogeneous	Homogeneous	01
TOGNIBO	02	01	Homogeneous	Homogeneous	01
Total	467	90			194

Abbreviations: NA, number of accessions analysed; NM, number of morphotypes; NGI, number of genotypes identified; *from Dansi et al., 1999.

that, according to the systems, only two, three or four of the identified patterns are well represented and the others are more or less rare. Similar results were obtained on Guinea yam germplasm in Cote d'Ivoire by Hamon & Touré (1990a).

Genetic diversity within the cultivar groups

During the morphological analysis, the different accessions of the germplasm were classified into 26 cultivar groups, among which some are homogeneous and others heterogeneous. With the systems used, considerable genetic diversity was detected within many of the cultivar groups. The data recorded allows us to classify the 26 cultivar groups into four categories:

Cultivar groups morphologically and genetically homogeneous

There are five groups: DIKPIRI, KPLANHOURA, TAM SAM, TERKOKONOU and TOGNIBO. Only one isozyme phenotype is identified in each of these groups (Table 3). One is therefore tempted to believe that each of them is constituted of a unique cultivar.

Cultivar groups morphologically homogeneous but genetically heterogeneous

Two cultivar groups, GNIDOU and NOUALAYE, are classified in this category. With the markers used, five and seven clones have been respectively detected within GNIDOU and NOUALAYE. With GNIDOU, accessions collected under different names, although morphologically identical, appeared as different clones. Hence, the cultivars Dagui-dagui,

Table 3. Distribution and frequency of the different isozyme phenotypes within the 26 cultivar groups. Capital letters (A–L) refer to the different phenotypes in Figure 1 while numbers in brackets correspond to the number of accessions in which these phenotypes have been detected

Cultivar groups	AAT	PGM	IDH	SKDH	PGI	PGD	EST
AGOGO	A [10]	A [09]	A [14]	A [01]	A [14]	A [05]	B [14]
	C [04]	E [05]		B [13]		C [05]	
						D [02]	
						E [02]	
AHIMON	A [26]	A [12]	A [25]	A [05]	A [34]	A [07]	A [08]
	B [01]	B [02]	E [04]	B [29]		C [05]	B [26]
	C [07]	D [18]	G [05]			D [08]	
		G [02]				E [10]	
						F [04]	
ALAKISSA	C [01]	**C** [07]	**B** [07]	C[01]	A [04]	A [01]	**D** [07]
	H [07]	D [01]	**F** [01]	F [07]	C [04]	**B** [07]	**E** [01]
ANTAWOROROU	C [06]	A [05]	A [06]	E [06]	A [06]	A [06]	A [02]
		D [01]					B [04]
BANIOURE	A [29]	A [01]	A [30]	C [30]	A [27]	A [18]	A [09]
	C [01]	D [03]			D [03]	D [03]	B [19]
		E [20]				E [08]	C [02]
		F [06]				F [01]	
BARIDJO	D [09]	A [09]	A [09]	A [09]	A [09]	**H** [09]	A [09]
DIKPIRI	A [02]	D [02]	A [02]	B [02]	B [02]	C [02]	A [02]
DOUBA YESSIROU	A [01]	A [06]	E [10]	B [07]	A [08]	A [10]	A [02]
	C [05]	B [01[		D [03]	C [02]		B [08]
	D [04]	D [03]					
GNALABO	A [02]	A [10]	A [04]	A [08]	A [10]	D [10]	B [10]
	C [08]		C [01]	B [01]			
			E [05]	D [01]			
GNIDOU	C [28]	A [27]	A [01]	B [02]	A [28]	A [27]	B [28]
		D [01]	E [27]	D [26]		C [01]	
KOKOROGBANOU	A [13]	A [27]	A [86]	B [60]	A [71]	A [87]	A [60]
	C [73]	D [43]	E [02]	G [09]	B [17]	C [01]	C [28]
	H [02]	E [18]		H [12]			
				I [07]			
KPANHOURA	A [06]	E [06]	C [06]	A [06]	**E** [06]	A [06]	B [06]
KPONAN	A [02]	A [07]	A [01]	A [07]	A [02]	C [06]	A [03]
	C [01]		C [05]		B [05]	E [01]	B [01]
	D [04]		K [01]				C [03]
KRATCHI	A [02]	A [18]	A [17]	B [17]	A [18]	C [09]	B [14]
	D [13]		E [01]	D [01]		D [07]	C [04]
	H [03]					E [02]	
MAKPAWA	**F** [02]	**C** [03]	**D** [02]	F [03]	A [03]	A [01]	**E** [02]
	G [01]		**F** [01]			E [02]	**F** [01]
MONDJI	A [04]	A [46]	A [21]	A [47]	A [60]	A [43]	A [10]
	B [02]	D [09]	C [05]	B [05]		C [14]	B [50]
	C [43]	E [05]	E [20]	D [07]		E [02]	
	D [02]		**H** [01]	G [01]		**G** [01]	
	I [02]		**I** [01]				
	J [02]		**J** [03]				
	K [05]		K [05]				
			L [04]				

Table 3. Continued

Cultivar groups	AAT	PGM	IDH	SKDH	PGI	PGD	EST
MOROKOROU	C [19] I [04]	A [04] D [01] E [03] **H** [05] *I* [05] *H* [05]	A [01] C [03] E [19]	A [19] B [04]	A [23]	C [22] E [01]	B [23]
NONFORWOU	A [01] C [11] D [01]	A [04] D [05] E [04]	A [11] E [02]	A [09] B [01] D [02] E [01]	B [13]	A [04] C [09]	A [03] C [10]
NOUALAYE	B [18]	A [18]	A [17] E [01]	A [01] B [14] D [03]	A [18]	A [12] C [02] D [03] *I* [101]	C [18]
OURTCHOUA	B [06] C [06]	A [05] B [06] E [01]	A [12]	A [01] B [10] B [01]	A [10] B [02]	A [06] C [06]	A [01] B [08] C [03]
PORCHEHBIM	A [06]	A [06]	A [06]	B [06]	A [06]	A [06]	B [06]
SOUSSOU	A [08] C [28] G [04] H [02]	A [38] B [03] E [01]	A [27] C [08] E [07]	A [29] B [07] D [06]	A [31] B [10] D [01]	A [23] C [19]	A [04] B [38]
TABANE	A [13]	A [13]	A [13]	B [13]	A [13]	A [13]	A [13]
TAM SAM	A [01]	A [01]	A [01]	B [01]	A [01]	A [01]	A [01]
TERKOKONOU	C [04]	G [04]	E [04]	B [04]	A [04]	C [04]	B [04]
TOGNIBO	**E** [02]	A [02]	C [02]	B [02]	A[02]	C [02]	B [02]

Doyesserou and Idjitedetedeka collected from different locations of the country and classified in this group, differ from each other and are different from the cultivar named GNIDOU, constituted itself of two genotypes. For the two groups, the results obtained perfectly support the observations of farmers, who, during the collecting survey, reported to us the existence of several clones within each of these groups. In fact, apart from the morphological traits, farmers also used to distinguish yam cultivars based on their cooking qualities and their agronomic traits (the tuber's time of maturity, storage aptitude, number of tubers per mound, interaction with the soil types, etc.).

Cultivar groups morphologically heterogeneous but genetically homogeneous

BARIDJO, PORCHEHBIM and TABANE are the three cultivar groups falling into this class, and for which only one genotype is identified. The results obtained indicate that the different morphotypes of each of these groups are very close genetically. Results also support the different hypothesis formulated on the evolution of the groups PORCHEHBIM and TABANE. In fact, based on the morphological observation and the farmers' explanation, it was hypothesised that cultivars considered as different within each of these groups, would be identical and derived one from another by either somatic mutation (case of TABANE) or shape fixation (case of PORCHEHBIM) further to many years of vegetative multiplication (Dansi et al., 1999).

Cultivar groups morphologically and genetically heterogeneous

Sixteen cultivar groups fall into this category (Table 3). These are AGOGO, **AHIMON**, ALAKISSA, ANTAWOROROU, **BANIOURE**, DOUMA, YESSIROU, GNALABO, **KOKOROGBANOU**, KPONAN, KRATCHI, MAKPAWA, MOROKOROU, MONDJI, NONFORWOU, OURTCHOUA and **SOUSSOU**.

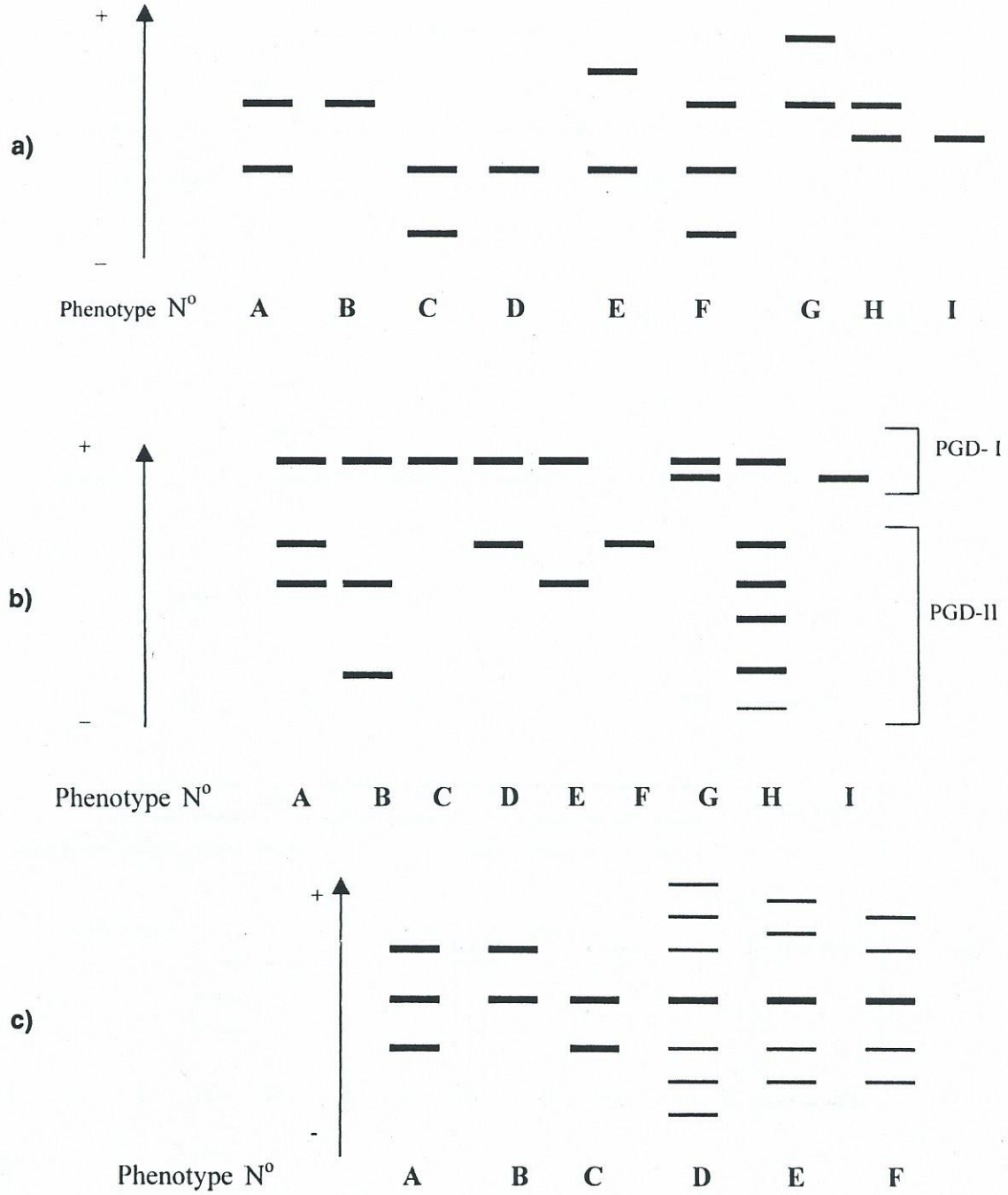

Figure 1a. Diagrammatic representations and designated phenotypes of the different isozyme patterns detected within the germplasm. (a) Shikimate dehydrogenase (SKDH); (b) 6-phosphogluconate dehydrogenase (6-PGD); (c) esterase (EST).

Groups covering wide geographical zones (printed in bold above) and for which more accessions have also been collected, are likely the most genetically diverse (Table 2). Apart from the cultivar group KOKOROGBANOU, results obtained for all the above-cited cultivar groups, considering the analysis at the morphotype level, are satisfactory and often reflect farmers' indications. In fact, within KOKOROGBANOU, data recorded for the morphotype Kinkerekou were contrary to farmers' considerations. For farmers, many of the cultivars assigned to this morphotype are very different although morphological similar. Unfortunately, only two genotypes have been detected within the 25 accessions classified in this morphotype. Giving priority to farmers' opinions because of their good knowledge of their yam

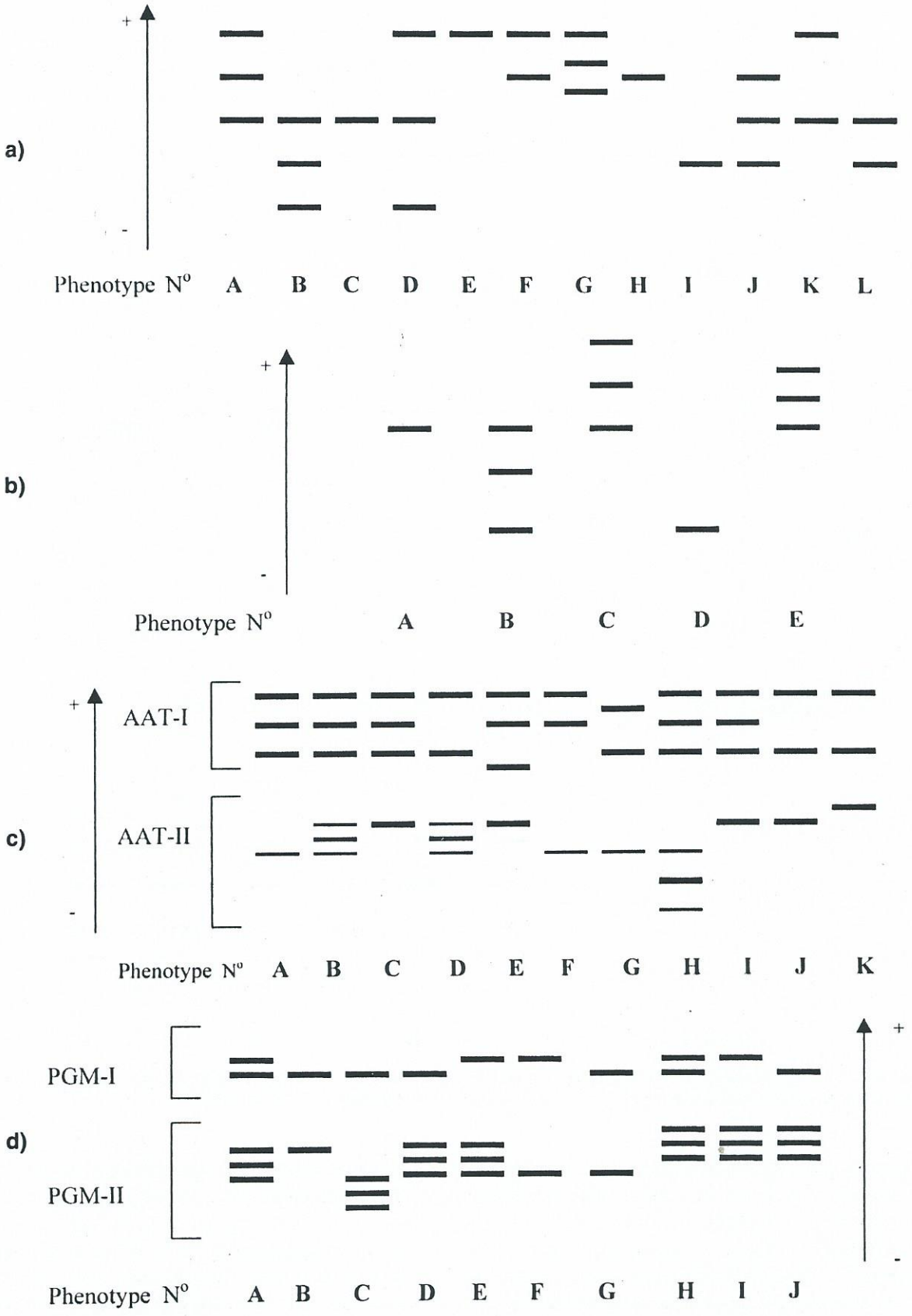

Figure 1b. Diagrammatic representations and designated phenotypes of the different isozyme patterns detected within the germplasm. (a) Isocitrate dehydrogenase (IDH); (b) phosphoglucose isomerase (PGI); (c) aspartate aminotransferase (AAT); (d) phosphoglucomutase (PGM).

Table 4. Number of clones detected within the different morphotypes

Morphotype	NA	NC	Morphotype	NA	NC	Morphotype	NA	NC
Agangan	03	03	Gnawounkoko	02	01	Oroutanai	21	07
Agogo	05	05	Gnidou	28	05	Orou Yinsingué	03	02
Ahimon	32	18	GnifôKpado	02	01	Ossoukpana	09	06
Akpazin	08	06	GuiéNa	01	01	Ourtchoua	04	01
Alakissa	03	01	Guirissa	03	01	Ouwonpèotina	02	01
Ala N'Kojèwoué	07	05	Hounbonon	01	01	Piédjè	02	01
Androki	01	01	Ihdonou	01	01	Porchèhhim	03	01
Ankpoloman	02	01	Issou Agatou	01	01	Singou	12	06
Antawororou	03	01	Kagourou	04	01	Soagona	04	03
Assaboné	01	01	Kangni	02	02	Sobasson	06	04
Baniakpa	03	01	Kéé	02	01	Sogodo	02	01
Baniouré Bagarou	08	05	Kinkérékou	25	02	Soussouka	25	15
Baniouré Montoguè	03	01	Kokoné	03	02	Soussounin	01	01
Baniouré Oloukobi	18	08	Kokouma	04	03	Soussou Souanbou	04	02
Baridjo	04	01	Kologo	04	02	Tabané	04	01
Boki	01	01	Kouragouroko	01	01	Tam-Sam	01	01
Bonakpo	02	01	Kpanhoura	06	01	Terlounto	01	01
Brizi	10	01	Kpirou Kpika	01	01	Terkokonou	04	01
Danwari	10	04	Kponan	05	05	Tognibo	02	01
DéBa	06	03	Kratchi	16	06	Walassi	01	01
Djatouba	04	03	Laboko	02	02	Wamai	01	01
Djikpiri	02	01	Makpawa	01	01	Wolouchahabim	03	01
Djiladja	01	01	Marétassou	01	01	Wossou	03	02
Dikpiri	02	01	Monji	11	08	Yahou	01	01
Douba Yéssirou	08	04	Nonforwou	07	04	Yaka	03	01
Doundoua	02	01	Nindouin	03	02	Yakarango	03	03
Effourou	01	01	Morokorou	19	05	Yoblè	01	01
Fêni	01	01	Noualaye	18	08	Youbè	01	01
Gbèra	01	01	Ofêgui	03	01	Youèyouèdota	01	01
Gnalabo	08	03	Omoya	01	01	Yorou Tassou	01	01

Abbreviations: NA, number of accessions analysed; NC, number of clones detected.

different morphotypes (within or between cultivar groups) have shown the same electrophoretic patterns for all the systems used. For this reason, a better classification of the cultivars should take into account the results of the morphological analysis by counting the number of genotypes detected within each of the 90 morphotypes of the germplasm (Dansi et al., 1998, 1999). This combination of the morphological and isozymic analysis leads to a total of 227 different cultivars out of the 467 accessions analysed (Table 4). In terms of genetic resources conservation, the result obtained is quite satisfactory and these 227 cultivars will help identifying the minimum number of accessions of the germplasm representing the maximum of diversity known as core collection (Brown, 1989; Hintum, 1995; Noirot et al., 1995).

Cultivar and cultivar group identification

Some isozyme patterns (printed in bold in Table 3) characterise some cultivar groups or are found only within them. As reported by Hamon & Touré (1990a) in Côte d'Ivoire's yam germplasm, the IDH, PGD and SKDH isozyme patterns having slower migration bands were found only within the cultivar groups ALAKISSA and MAKPAWA of perennial origin, known as *D. cayenensis*. Alakissa and Makpawa in Benin, respectively, correspond to Yaobadou and Kangba in Côte d'Ivoire. Patterns having slow bands in PGM (PGM-C) were also found only with the same two cultivar groups (Figure 1b; Table 3). Moreover, they all display a complex pattern of five or seven bands for EST that is not found in any of the *D.*

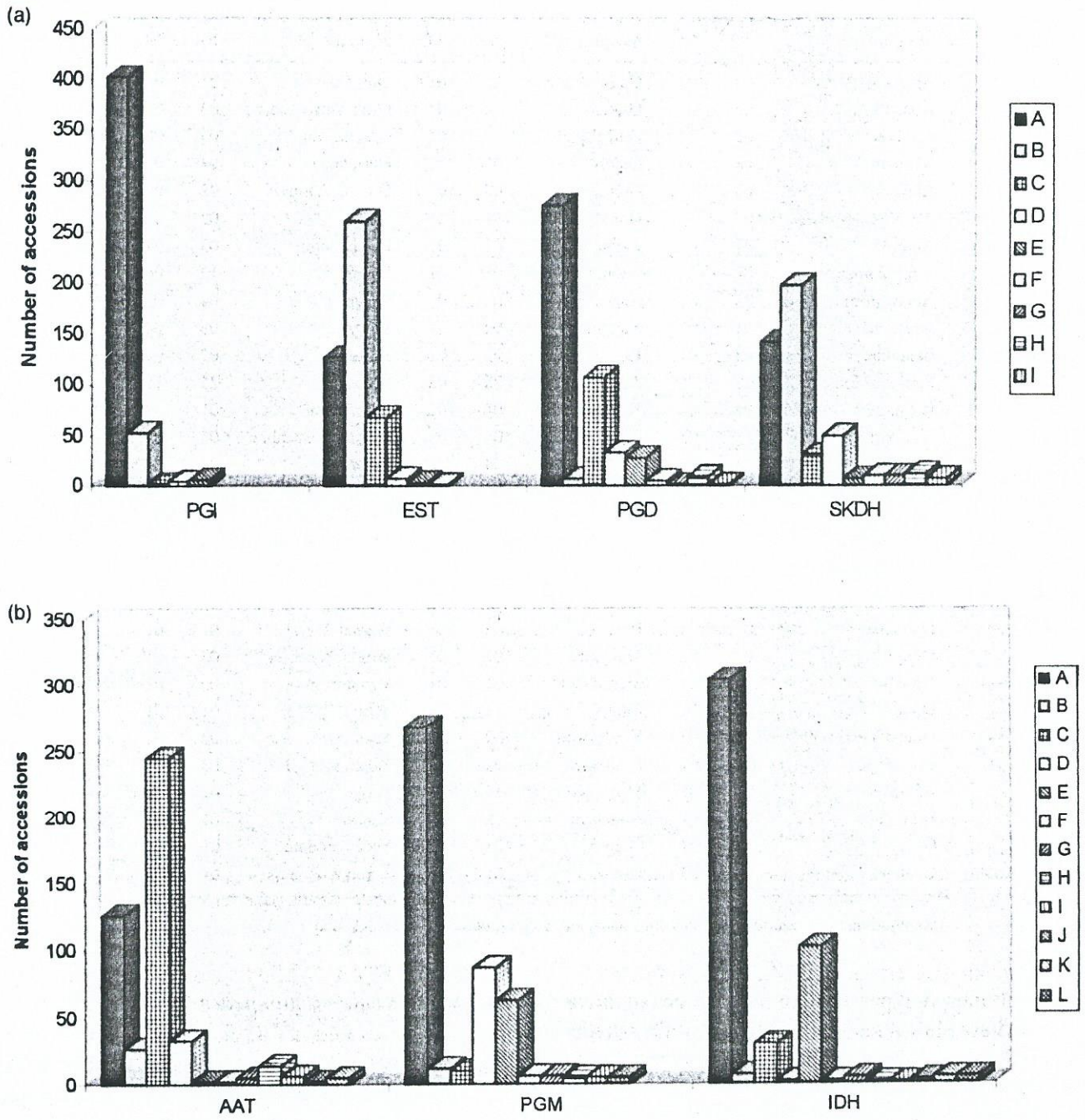

Figure 2. Frequency of the different isozyme systems' phenotypes.

cultivars, it is assumed that the markers used are ineffective in screening the different genotypes within Kinkerekou. The use of molecular (DNA) markers such as RAPD and AFLP would be desirable for the separation of the cultivars of this morphotype.

By comparing morphological and isozymic classification, perfect coincidence between the two was obtained for only two (ANTAWORÒROU and MAKPAWA) of the above-cited groups. In fact, phenotypes identified in each of these groups exactly correspond to the different morphotypes defined in each of them.

In total, while only 90 morphotypes were constituted based on morphological data, isozyme markers allowed the identification of 194 genotypes considering the data recorded by cultivar groups (Table 2). In the present analysis, some cultivars belonging to

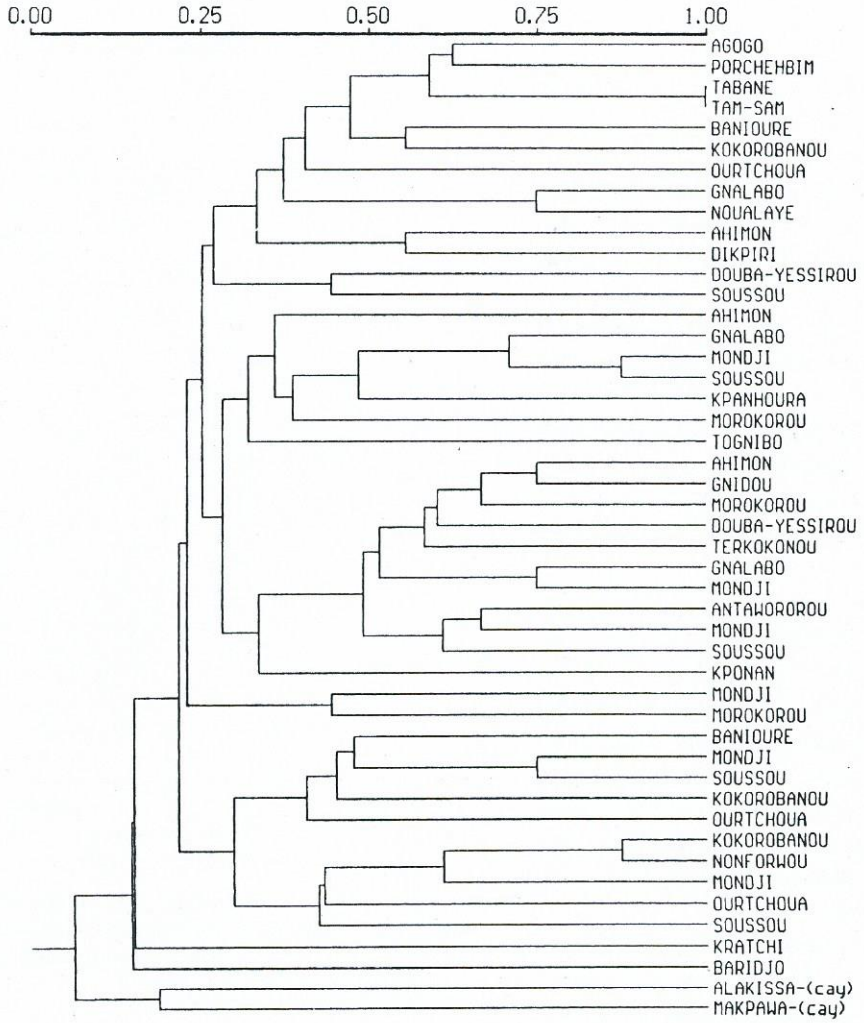

Figure 3. Dendrogram of 467 accessions of Benin Republic's Guinea yam (*D. cayenensis–D. rotundata* complex) generated by UPGMA cluster analysis based on isozyme data, using simple matching coefficient of similarity. Only the cultivar groups are shown; 'cay': *D. cayenensis*.

rotundata groups. Hence PGM-C, EST-E and EST-F can be considered as three other markers in separating *D. rotundata* from *D. cayenensis*. It has been shown in Côte d'Ivoire yam germplasm that a specific PGD marker (PGD-H) characterises the cultivar group BANIAKPA considered as a hybrid between *D. rotundata* and *D. cayenensis* (Hamon & Touré, 1990a). The same result is obtained for this cultivar group (represented in almost all the countries of the African yam belt) named BARIDJO in Benin.

A few cultivars can be identified within their groups based upon a given isozyme pattern. Hence, AAT-C isolates Kokouma from Morokorou within MOROKOROU, Soagona from all the other cultivars of the group AGOGO, Walassi from all the Banioure in the cultivar group BANIOURE. Within the group NONFORWOU, PGM-E characterises the morphotype Djatouba.

The method of *electrophoretic identity or ID* (combination, in a given order, of isozyme phenotypic differences across the enzymatic systems) as defined by Hamon & Touré (1990a) was also used for cultivar identification. For example, there is a given electrophoretic ID for each of the cultivars Gnalabo, Assaboné, Agada, Ounonyahoun and Terlounto classified into three morphotypes within the group GNALABO. The list of all the cultivars analysed as well as their electrophoretic IDs is available on request from the authors.

Relationship among cultivars

The species concept of Guinea yam is rather controversial. Different authors consider Guinea yam to be represented either by one species, two species, or even a species complex (Martin & Rhodes, 1978; Miège, 1982a,b; Onyilagha & Lowe, 1985; Hamon & Touré, 1990a,b; Hamon et al., 1992; Terauchi et al., 1992; Asemota et al., 1996).

In the present study the dendrogram obtained clearly separates the *D. rotundata* (white yam) and the *D. cayenensis* (yellow yam) accessions (Figure 3). This clear partition into two groups is consistent with the concept that the two forms of Guinea yam represent different genetic entities which may be treated as two separate taxa, supporting the view of Onyilagha & Lowe (1985).

Within the class of the white yam (*Dioscorea rotundata*), the cluster analysis isolates the cultivar group Baridjo from the remaining groups (Figure 3). A similar result has been already reported on Côte d'Ivoire's yam germplasm by Hamon & Touré (1990a,b) for that same cultivar group (named BANIAKPA in Cote d'Ivoire) represented in almost all countries of the yam belt and considered (by the same authors) as intermediate between *Dioscorea cayenensis* and *D. rotundata*.

Within the 'true *D. rotundata*' and at 80% of similarity, 13 cultivar groups (AGOGO, ANTAWORORO, DIKPIRI, GNIDOU, KPANHOURA, KPONAN, KRATCHI, NOUALAYE, PORCHEHBIM, TABANE, TAM SAM, TERKOKONOU, TOGNIBO) seem distinct on the basis of isozyme phenotypes. The accessions of the remaining *D. rotundata* groups are distributed to two, three or four of the different clusters formed (Figure 3). Additional polymorphic enzymes are needed for an accurate isozyme-based genetic discrimination of most of the cultivar groups.

The cultivar groups TAM SAM and TABANE (of *D. rotundata*) are identical and cluster together (Figure 3). This result supports our hypothesis on the origin of cultivar Tam Sam considered, based upon morphological data and farmer's comments, as derived from Tabané further to a chloroplastic mutation (Dansi et al., 1999).

Conclusions

Overall, these isozyme studies have allowed the identification of 227 cultivars out of the 467 accessions analysed. The results are encouraging but they do reveal a need for improved means of discriminating genetic differences among Guinea yam cultivars. In fact, the cultivars of the cultivar group TABANE, although morphologically different, have an identical isozyme genotype and could not be uniquely identified. This result simply manifests a reality that morphological variation in yam may not be well reflected in isozymic variation of the seven enzymes examined here. The dendrogram constructed based on the morphological data (Dansi et al., 1999) is included for direct comparison with that derived from the isozyme data (Figure 4). Additional polymorphic enzymes and DNA markers such as randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) are needed to accurately discriminate cultivars in Tabane and to assess more fully genetic variation within the cultivated yams. This will be important in germplasm management and maintenance and in the development of new cultivars.

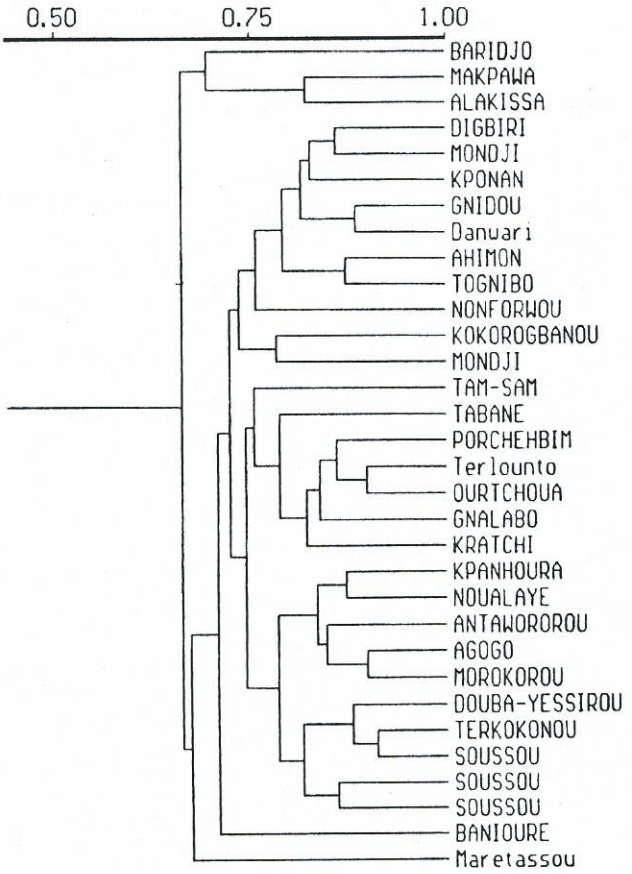

Figure 4. UPGMA dendrogram of Benin Republic's yams (*Dioscorea cayenensis–Dioscorea rotundata* complex) based on morphological data using simple matching coefficient of similarity (Dansi et al., 1999).

Acknowledgements

We thank the International Foundation for Science (IFS) and the Gatsby Charitable Foundation (UK) for financial support. The third author (J.Z.) acknowledges support from AUPELF-UREF. We wish to thank J.-L. Noyer (CIRAD Montpellier) and Dr O. Daïnou (National University of Benin) for technical advice. We express our sincere thanks to E. Fassoundé (IITA, Cotonou) for his technical assistance throughout the study and M. Odishiwo (IITA, Ibadan) for helping with the NTSYS computer program. We are also grateful to all the farmers we met for sharing their material and fruitful discussions while collecting and classifying the yams.

References

Anderson, C.M., W.S. Castle & G.A. Moore, 1991. Isozymic identification of zygotic seedlings in 'Swingle Citumelo' (*Citrus paradisi* × *Poncitrus trifoliata*) nursery and field population. J. Am. Soc. Hort. Sci. 116: 322–326.

Asemota, H.N., J. Ramser, C. Lopez-Peralta, K. Weising & G. Kahl, 1996. Genetic variation and cultivar identification of Jamaican yam germplasm by random amplified polymorphic DNA analysis. Euphytica 92: 341–351.

Brown, A.H.D., 1989. Core collections: a practical approach to genetic resources management. Genome 31: 818–824.

Dansi, A., J. Zoundjihekpon, H.D. Mignouna & F.M. Quin, 1997. Collecte des ignames cultivées du complexe *Dioscorea cayenensis–rotundata* au Benin. Plant Genet. Res. Newslett. 112: 81–85.

Dansi, A., H.D. Mignouna, J. Zoundjihekpon, A. Sangare, R. Asiedu & F.M. Quin, 1998. Varietal identification key of the cultivated yams (*Dioscorea cayenensis–Dioscorea rotundata* complex) of Benin Republic. Plant Genet. Res. Newslett. 116: 18–25.

Dansi, A., H.D. Mignouna, J. Zoundjihekpon, A. Sangare, R. Asiedu & F.M. Quin, 1999. Morphological diversity, cultivar groups and possible descent in the cultivated yams (*Dioscorea cayenensis–Dioscorea rotundata* complex) of Benin Republic. Genet. Resour. Crop Evol. 46: 371–388.

Degani, C., A. Beiles, R. El-Batsri, M. Goren & S. Gazit, 1995. Identifying lychee cultivars by isozyme analysis. J. Am. Soc. Hort. Sci. 120: 307–312.

Gottlieb, D., 1982. Conservation and duplication of isozymes in plants. Science 216: 373–380.

Gower, J.C., 1985. Measure of similarity, dissimilarity and distance. In: Kotz, S. & N.L. Johnson (Eds.), Encyclopedia of Statistical Sciences, Vol. 5. pp. 297–405. Wiley, New York, NY.

Hamon, P. & B. Touré, 1990a. Characterisation of traditional yam varieties belonging to the *Dioscorea cayenensis–rotundata* complex by their isozymic patterns. Euphytica 46: 101–107.

Hamon, P. & B. Touré, 1990b. The classification of the cultivated yams (*Dioscorea cayenensis–rotundata* complex) of West Africa. Euphytica 47: 179–187.

Hamon, P., J.P. Brizard, J. Zoundjihekpon, C. Duperray & A. Borgel, 1992. Etude des index d'ADN de huit espèces d'ignames (*Dioscorea* spec.) par cytométrie en flux. Can. J. Bot. 70: 996–1000.

Hintum, Th.J.L. van, 1995. Hierarchical approaches to the analysis of genetic diversity in crop plants. In: Hodgkin, T., A.H.D. Brown, Th.J.L. van Hintum & E.A.V. Morales (Eds.), Core Collections of Plant Genetic Resources, pp. 23–34. John Wiley and Sons, UK.

Jongedijk, E., J.M.A.S.H. Van der Wolk & L.C.J.M. Suurs, 1990. Analysis of glutamate oxaloacetate transaminase (GOT) isozyme variants in diploid tuberous *Solanum*; Inheritance and linkage relationships to ds1 (desynapsis), Y (tuber flesh colour), cr (crumpled) and yc (yellow cotyledon). Euphytica 45: 155–167.

Kephart, S.R., 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. Am. J. Bot. 77: 693–712.

Manganaris, A.G., F.H. Alston, N.F. Weeden, H.S. Aldwinckle, H.L. Gustafson & S.K. Brown, 1994. Isozyme locus Pgm-1 is tightly linked to a gene (V_f) for scab resistance in apple. J. Am. Soc. Hort. Sci. 119: 1286–1288.

Martin, F.W. & A.M. Rhodes, 1978. The relationship of *Dioscorea cayenensis* and *D. rotundata*. Trop. Agric. (Trinidad) 55: 193–206.

Miège, J, 1982a. Notes sur les especes *Dioscorea cayenensis* Lamk. et *Dioscorea rotundata* Poir. In: J. Miège & S. N. Lyonga (Eds.), Yams (Ignames), pp. 367–375. Oxford University Press, Oxford.

Miège, J., 1982b. Etude chimiotaxonomique de dix cultivars de Côte d'Ivoire relevant du complexe *Dioscorea cayenensis–rotundata*. In: J. Miège & S.N. Lyonga (Eds.), Yams (Ignames), pp. 185–196. Oxford University Press, Oxford.

Nielsen, G., 1985. The use of isozymes as probes to identify and label plant varieties and cultivars. In: M. C. Rattazzi, J. G. Scandalios & G. S. Whitt (Eds.), Isozymes: Current Topics in Biological and Medical Research, Vol. 12, pp. 1–32. Alan R. Liss, New York, NY.

Noirot, M., S. Hamon & F. Anthony, 1995. The principal component scoring: a new method of constituting a core collection using quantitative data. Genet. Resour. Crop Evol. 41: 1–6.

Onyilagha, J.C. & J. Lowe, 1985. Studies on the relationship of *Dioscorea cayenensis* and *Dioscorea rotundata* cultivars. Euphytica 35: 733–739.

Rohlf, F.J., 1993. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Exeter, New York.

Sneath, P.H.A. & R.O. Sokal, 1973. Numerical taxonomy. Freeman, San Francisco, CA.

Swofford, D.L. & G.J. Olsen., 1990. Phylogeny reconstruction. In: Hillis, D.M. & C. Moritz (Eds.), Molecular Systematics, pp. 411–501. Sinauer Associates, Sunderland, MA.

Tanksley, S.D. & T.J. Orton, 1983. Isozyme in Plant Genetics and Breeding. Elsevier, Amsterdam.

Terauchi, R., V.A. Chikalake, G. Thottapilly & S.K. Hahn, 1992. Origin and phylogeny of guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear DNA. Theor. Appl. Genet. 83: 747–751.

Torres, A.M. & B.O. Bergh, 1980. Fruit and leaf isozymes as genetic markers in avocado. J. Am. Soc. Hort. Sci. 105: 614–619.

Weeden, N.F. & R.C. Lamb, 1985. Identification of apple cultivars by isozyme phenotypes. J. Am. Soc. Hort. Sci. 110: 509–515.

Wendel, J.F. & N.F. Weeden, 1989. Visualization and interpretation of plant isozymes, In: Soltis, D.E. & P.S. Soltis (Eds.), Isozymes in Plant Biology, pp. 5–45. Dioscorides Press, Portland, OR.

Zoundjiékpon, J., S. Hamon, B. Tio-Touré & P. Hamon, 1994. First controlled progenies checked by isozymic markers in cultivated yams *Dioscorea cayenensis–rotundata*. Theor. Appl. Genet. 88: 1011–1016.