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First controlled progenies checked by isozymic markers in cultivated yams *Dioscorea cayenensis-rotundata*

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Abstract As tested progeny have never been obtained, breeding studies on African yams (*Dioscorea cayenensis-rotundata*) are scarce. We report here the first progenies checked by isoenzyme markers. This was made possible by the choice of well-known genitors [one male (cv Zrezrou) and three females (cvs 'Sopéré', 'Dahomey' and 'C 20')] and special hybridization conditions. Six enzymatic systems [esterase (EST), isocitrate dehydrogenase (ICD), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SDH), and phosphoglucoisomerase (PGI)] were used to check the progenies and detect outbreeding. Despite the small number of progeny, it was possible to provide information on the genetics of the isoenzymatic systems.

Key words Yams · Controlled progenies · Isozymes Genetic analysis

Introduction

During the last decade, genetic mapping of a large number of crops has yielded saturated linkage maps (Bernatzky and Tanksley 1986; Helentjaris et al. 1986; Tanksley et al. 1989, Hulbert et al. 1990). However, no genetic analysis has yet been reported for yams, a tropical tuber crop. There are two possible reasons for this: first, the genetic complexity of *Dioscorea cayenensis-rotundata* (the main African cultivated species), and second, its particular floral biology.

The first problem is to ensure that crosses do not correspond to interspecific hybridization. The *D. cayenensis-rotundata* complex comprises two clusters (A and B), which differ in morphological and isoenzymatic traits. Some authors (Hamon 1988–91; Hamon and Touré 1988–91; 1990 a, b; Hamon et al. 1992 b) consider the cultivars of cluster A to be related to the wild annuals *D. abyssinica* and/or *D. praehensilis*. Cluster B is more complex; all of its forms are related to perennial or semi-perennial wild African species. It includes one cultivar of hybrid origin between *D. praehensilis* and *D. burkilliana*, one closely related to *D. mangenotiana*, and three of unknown origin. Terauchi et al. (1992) reported a similar hypothetical scheme but they considered the species *D. rotundata* and *D. cayenensis* instead of the two clusters. More wild species are thus involved as putative progenitors, but the wild origin of cultivars included in *D. cayenensis* is not indicated.

Polyploidy is another complicating factor. Within the genus *Dioscorea*, polyploidy has been reported by Miège (1952, 1954), Essad (1984), Zoundjihekpon et al. (1990) and Hamon et al. (1992 a). Cultivars of cluster A are tetraploids like their putative wild progenitors, while all cultivars of cluster B, except for one octoploid, are hexaploids.

The second problem is that cultivated yams are only vegetatively propagated. Consequently, in some varieties flowering is irregular or absent (Sadik and Okereke 1975; Touré and Ahoussou 1982; Akoroda 1983; Hamon 1987; Zoundjihekpon 1993).

Yams are dioecious plants with entomophilous pollination. Thrips (*Larothrips dentipes*) are mainly involved, but other species such as *Acantolepis* sp., *Chirothrips* sp. and *Haplothrips* sp. may also be implicated. Flowers, especially male, are small (Zoundjihekpon 1993), difficult to handle, and often have sticky pollen. Male and female flowering are reported to be asynchronous (Akoroda 1983). Ayensu and Coursey (1972) consider the failure of crosses to be mainly due to the lack of flowering overlap.

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Taking all these considerations into account, we have used isolated hybridization plots to produce checked seeds for the first time. We report the results obtained in such plots and elucidate the inheritance of some isoenzyme loci.

Materials and methods

The male (Zrézrou) and three female ('Sopéré', 'Dahomey', 'C 20') varieties (all annuals, i.e., cluster A) chosen are tetraploids according to Zoundjihekpon et al. (1990) and Zoundjihekpon (1993). Morphological identification was made according to Hamon et al. (1986) and Zoundjihekpon (1993).

In the hybridization plot, males and females were alternated in rows. On each side, 3 rows of male plants bordered the plot. Ten plants per row were planted on knolls 1 m apart, the rows were 1.5 m apart (42 rows per plot). In order to test different crosses, two experiments were carried out in southern Ivory Coast (wet tropical climate with two rainy seasons). One plot was located near the National University of the Ivory Coast, at the "Centre National de Floristique" (CNF); the other at Azaguié, 50 km north-west of Abidjan. Yams were staked, with at least one male and one female on each stake.

Starch gel electrophoresis was performed on young adult leaves according to Hamon and Touré (1982, 1990a), as modified by Zoundjihekpon (1993). Six enzyme systems, namely esterase (EST), isocitrate dehydrogenase (ICD), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SDH), and phosphoglucoisomerase (PGI), were investigated.

After the harvest in December, fruits were dried for 1–2 months, and then seeds were stored at room temperature for 3–4 months. In June, the seeds were put on wet plate dishes. Germination was observed after 2–8 weeks. Plantlets were staked and those producing tubers were transferred to the field the second year.

Results

Flowering data

In southern Ivory Coast, yam flowering is erratic and ranges from 22.7% to 81.7% (Table 1). Nevertheless, in our plots only a few plants produced enough seeds (from 183 to 872) for genetic analysis. Unfortunately, seed germination was low: 11.8–31.7%. The numbers of plants available for the study were 22, 34, 37 and 63 from 'Sopéré (89)', 'Sopéré (91)', 'Dahomey' and 'C20', respectively.

Genetic analyses

Although limited in size (22), the progeny 'Zrézrou' × 'Sopéré (89)' obtained at the CNF was interesting (Table 2):

– in four enzyme systems – ICD, PGI, SDH and EST – one locus was obvious and 2 loci (only 1 polymorphic) were present for MDH and PGD;
– PGI was monomorphic and displayed only one band;
– ICD was a dimeric protein while SDH was monomeric like PGD. EST and MDH were monomeric, but with one secondary isoenzyme;
– for the four polymorphic loci (ICD, MDH A, SDH and EST), 2 codominant alleles were found. Monogenic ratios expected for diploids species 1-0 or 1-2-1 were observed;
– for PGD, the simplest hypothesis implies 3 alleles including 1 null. The observed ratio is more likely that for 2 codominant alleles and 1 recessive in tetraploids;
– the genotype of genitors indicates 50% heterozygosity.

Open hybridization

The patterns of the three progenies ('Zrézrou' × 'Sopéré (91)'–'Zrézrou'×'Dahomey'–'Zrézrou'× 'C 20') obtained at Azaguié confirmed the isoenzymatic structure previously described (Table 3). A new, three-band pattern for PGI indicates that the corresponding enzyme is dimeric. Pollen contamination was confirmed proved by the presence of A2A2 genotypes in ICD for the three crosses. It is worth noting that small progenies could reveal outcrossing. For the other enzyme systems, contamination led to distorted segregations. Wild male genitors seemed to be genetically similar to cultivated tested genitors as among the 14 alleles assessed, only 1 new allele was noted.

Heterozygosity among females was 50% ['Sopéré (89)'], 28.5% ['Sopéré (91)'], 14.3% ('Dahomey') and 12.5% ('C20').

Table 1 Flowering and fructifying data obtained in different crosses involving the male genitor 'Zrézrou' and two plot hybridization locations

Plot location	Variety	Total number of plants	Flowering plants (%)	Fructified plants (%)	Number of seeds produced	Germinative seeds (%)	Plantlets
	Females						
C.N.F.	Sopéré (89)	88	22.7	40	337	13.3	22
Azaguié	Sopéré (91)	25	36	33	183	31.7	34
	Dahomey	24	41.6	60	674	11.8	37
	C20	20	55	72.7	872	23.3	63
	Males						
C.N.F.	Zrézrou	426	81.7	–	–	–	–
Azaguié	Zrézrou	152	40	–	–	–	–

Table 2 Genetic analysis of six enzyme systems in the 'Zrézrou' × 'Sopéré (89)' cross. The expected ratios were calculated from hypotheses based on diploid or tetraploid species. For PGD, the diploid hypothesis implies B1 and B2, B2 and B0 codominants, but B0 dominant over B1

Enzyme system		Parental genotypes Male	Female		Offspring				
ICD		———			———				
					———				
		———			———				
		A1A1	A2A2		A1A2	Expected,	observed		
Est		———	———		———	———			
		———	———		———	———	———		
		———	———			———	———		
		A1A2	A1A2		A1A1	A1A2	A2A2		
				Expected	0.25	0.5	0.25		
				Observed	9	19	8		
PGI		———	———		———				
		A1A1	A1A1		A1A1	Expected,	observed		
SDH		———	———		———	———			
		———	———			———	———		
		A1A2	A1A2		A1A1	A1A2	A2A2		
				Expected	0.25	0.5	0.25		
				Observed	7	7	8		Chi-square NS (5%)
MDH		———	———		———	———			
	Locus A	———	———		———	———			
		———	———			———	———		
		———	———			———	———		
	Locus B	———	———		———	———	———		
		A1A2	A1A2		A1A1	A1A2	A2A2		
				Expected	0.25	0.5	0.25		
				Observed	6	11	5		
PGD	Locus A	———	———		———	———	———	———	
	Locus B	———					———	———	
		———	———			———	———		
		B1B2	B2B0	Expected	0.25	0.5	0.25	0	
				Observed	1	9	8	0	Chi-square S (5%)
		B0B0	B0B0	Expected	0.027	0.472	0.444	0.055	
		B1B2	B2B2	Observed	1	9	8	0	

Table 3 Patterns and ratios observed for six enzymes systems in three progenies obtained at Azaguié

Enzyme system	Parental genotypes		Offspring			Parental genotypes		Offspring			Parental genotypes		Offspring		
	Male Zrézrou	Female Sopéré (91)				Male Zrézrou	Female Dahomey				Male Zrézrou	Female C20			
ICD	—		—			—	—	—	—		—	—	—	—	
			—				—		—			—		—	
		—	—	—			—		—	—		—		—	—
			18	6				6	21	5			8	19	24
MDH	—	—	—	—		—	—		—		—	—		—	—
	—	—	—	—		—	—		—		—	—		—	—
	—	—		—	—	—					—				—
	—	—		—	—	—					—				—
	—	—	—	—	—	—	—		—		—	—		—	—
			8	12	3				37					61	2
PGD	—	—	—	—		—	—		—		—	—		—	—
	—			—		—					—				—
	—	—	—	—		—					—				—
			4	16					37					59	2
PGI	—	—	—	—		—	—	—	—		—	—	—	—	
				—					—					—	
				—					—					—	
			31	3				31	3				54	3	
SDH	—	—		—		—			—						
	—	—	—	—		—	—	—	—						
			17	6				28	9						
EST											—	—	—	—	
											—	—	—	—	
											—			—	
													18	14	

Discussion

In yams, the genetic structures of ICD, SDH, and PGI isoenzymes are identical to those commonly observed in other plant species (Lanaud 1987; Pasteur et al. 1987; Glaszmann et al. 1988; Ollitrault et al. 1989; Rajora 1989; Sié 1991). PGD and MDH structures differ, and are monomeric in yams. There are two possible explanations for this: (1) genetic systems most probably evolve from simpler to more complex structures, and (2) according to Burkill (1960) and Terauchi et al. (1991), yams are very ancient plants. Asian and African *D. bulbifera* are the same species, and separated at the beginning of Tertiary Pliocene (about 10 million years ago).

Although isoenzymes provide good markers for checking progenies, the small size of the progeny used here did not allow the analysis of genetic linkages. An increase in this particular progeny is therefore a priority.

Heterozygosity in genitors ranged from 12.5 to 50% and was too low considering yam dioecy. Indeed, dioecy implies strict allogamy and then marked heterozygosity. One possible explanation is that the natural sex-ratio disequilibrium is in favor of the male and perennation of tubers. If so, half-sib progenies could be common.

Uncontrolled pollination was observed in three out of four cases. How is this possible? Where is the foreign pollen coming from? Is the interpollination distance large? What recommendations can be formulated to increase controlled progenies in yams? The plants of the controlled cross ['Zrézrou' × 'Sopéré (89)'] were at the CNF (Abidjan), and neither wild nor cultivated yams were found in the surroundings. In contrast, the three other crossing experiments were performed at Azaguié (50 km north-east of Abidjan), where several cultivated and wild yams were found about 20 m away. These were identified by the morphological criteria of Hamon et al. (1986) and Hamon (1988) to be three wild species, namely, *D. praehensilis*, *D. minutiflora* and *D. mangenotiana*, two varieties belonging to the *D. cayenensis-rotundata* complex ('Yaobadou' and 'Kangba'), and several varieties of *D. alata*. Generally, the absence of flowering in *D. alata* of the Ivory Coast can be eliminated. 'Yaobadou' and 'Kangba' have specific PGI patterns (Table 4) that were not observed in the offspring, so they can also be excluded. In *D. minutiflora* and *D. mangenotiana*, ICD exhibits only slower bands which were not found in the three progenies. Faster bands are only found for *D. praehensilis* (Hamon 1988 and Hamon and Touré 1991). We can conclude that *D. praehensilis* is certainly the foreign pollen donor. This result is in good agreement with the wild origins of the female genitors proposed by Hamon et al. (1992 b), and is a case of natural introgression between wild and cultivated yams.

The minimal interpollination distance is at least 20 m. The highest outcrossing rate (up to 75%) indicates that wild pollen is more efficient than cultivated pollen. Many non-exclusive reasons could explain these rates:

(1) pollen fertility is higher in wild yams than in cultivated ones;

Table 4 Expected progenies for the PGI system in female × 'Yaobadou' or 'Kangba' crosses. Patterns for male varieties are those previously established by Hamon et al. (1990 a)

Cross	Expected progenies					
Female × Yaobadou	—	×	—	⟶	—	
					100%	
			—			—
			—			—
Female × Kangba	—	×	—	⟶	—	—
					1/2	1/2

(2) wild yams produced more pollen than cultivated yams. This is correlated with the loss of flowering in cultivated yams often observed in field collections (Touré and Ahoussou 1982). Another explanation could be better maturity overlapping of wild male and cultivated female flowers; (3) Zoundjihekpon (1993) has shown that mature female flowers remain open and receptive for 3 days, but some varieties cannot produce offspring because of unsynchronized male and female flower maturation.

In conclusion, we show that controlled progenies of *D. cayenensis-rotundata* can be produced by hybridization plots if the genitors are well chosen. Site location is very important and implies both that the environment is well controlled and that specific markers to check progenies are available. We report the first progeny checked by genetic markers. Between 183 and 872 seeds were obtained, but mortality was considerable in the first propagation steps (Table 2). Efforts must now be concentrated on defining optimal methods for seed germination and for safeguarding plantlets, such as in vitro embryo rescue and greenhouse assistance. This could be partly achieved by improving technical practices and increasing financial and human resources. Despite this substantial seed loss, small progenies can be obtained each year because yams are perennial (via the tuber) and an adequate harvest can be collected from the same plant annually.

This work is fundamental for a knowledge of yams and for future developments, such as genome mapping by means of molecular markers. This could be very helpful in the search for quantitative trait loci for important agronomic traits and in marker-assisted selection.

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