African Journal of Biotechnology Vol. 8 (1), pp. 26-36, 5 January, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Genetic variability in yam cultivars from the Guinea-Sudan zone of Benin assessed by random amplified polymorphic DNA

A. Zannou[1,2,3]*, E. Agbicodo[4], J. Zoundjihékpon[5], P. C. Struik[3], A. Ahanchédé[1], D. K. Kossou[1] and A. Sanni[4]

[1]Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, 01 BP 526, Cotonou, Bénin.
[2]Technology and Agrarian Development Group, Wageningen University, Wageningen, The Netherlands.
[3]Centre for Crop Systems Analysis, Wageningen University, Wageningen, The Netherlands.
[4]Laboratoire de Biochimie et de Biologie Moléculaire, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 01 BP 526, Cotonou, Bénin.
[5]Laboratoire d'Ecologie Appliquée, Faculté des Sciences Agronomiques, Université d'Abomey – Calavi, 01 BP 526, Cotonou, Bénin.

Accepted 6 November, 2008

Yam (*Dioscorea* spp.) is an important food and cash crop in the Guinea-Sudan zone of Benin. The genetic diversity of about 70 cultivars of *Dioscorea cayenensis/Dioscorea rotundata* (Guinea yam) and about 20 cultivars of *Dioscorea alata* (water yam) was analysed using random amplified polymorphic DNA (RAPD). The amplified bands revealed high polymorphism. These polymorphic DNA fragments were used to construct dendrograms, clustering all accessions into 18 groups: 12 for *D. cayenensis/D. rotundata* and six for *D. alata*. The analysis of molecular variance revealed highly significant variation among species, among groups within species, and among cultivars within groups. The study showed that the genetic diversity changed along a spatial gradient. In general, there was a tendency that most of the varieties from the north-east and north-west of the zone investigated appeared to be distinctive from the ones of the centre. However, few varieties were distributed randomly and did not reflect any specific relation to their zone of collection. The current study suggests that the Guinea-Sudan zone of Benin has a large gene pool of yam varieties. Yam farmers may have played a significant role in the enrichment and the maintenance of the genetic diversity of yam.

Key words: *Dioscorea alata*, *Dioscorea cayenensis/D. rotundata,* farmers' varieties, gene pool, genetic diversity, RAPD.

INTRODUCTION

Yam represents an important component of West-African agriculture and contributes to the food security of large parts of the populations of West-Africa, particularly that of Benin. In addition to its economic and nutritional values, yam also plays a significant role in the cultural life in Benin (Zannou et al., 2004, 2007), elsewhere in Africa (Ayensu and Coursey, 1972; Coursey, 1976b; Dounias, 2001) and Tropical America and Asia (Lebot et al., 2005).

Yam belongs to the genus *Dioscorea* of the family Dioscoreaceae. The genus contains some 600 species with more than 10 species cultivated for food and for pharmaceutical use (Coursey, 1976a; Aké Assi, 1998). Six species are important staples: white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), yellow yam (*Dioscorea cayenensis*), trifoliate yam (*Dioscorea dumetorum*), aerial yam (*Dioscorea bulbifera*) and Chinese yam (*Dioscorea esculenta*) (Ng and Ng, 1994).

*Corresponding author. E-mail: afiozannou@yahoo.com. Tel: +229.97.44.92.55.

Abbreviations: **AMOVA,** analysis of molecular variance**; C,** centre; **CTAB,** cetyl trimethyl ammonium bromide; **NE,** North East; **NW,** North West; **UPGMA,** unweighted pair-group method using arithmetic averages.

Different species were brought into cultivation independently in three regions of the world: West Africa (*D. rotundata*, *D. cayenensis* and *D. dumetorum*), South East Asia (*D. alata* and *D. esculenta*), and Tropical America (*D. trifida*).

When we analysed (together with farmers) the agronomic and physiological constraints to develop adaptive technology for farmers, it became obvious that it was necessary to really understand the genetic traits of yam (Zannou et al., 2004). Conversely, other studies have shown the necessity to put more emphasis on farm management of genetic resources (Zoundjihékpon et al., 1997; Pardey et al., 1999; Jarvis et al., 2000).

A characterization only based on morphological or agronomic traits hides important genetic information. Apart from morphological traits (Dansi et al., 1998, 1999), isozymic techniques (Dansi et al., 2000a; Mignouna et al., 2002; Mignouna and Dansi, 2003), flow cytometry (Dansi et al., 2000b) and molecular techniques provide opportunities to obtain high amplification of genetic traits for the development of genetic maps, variety identification and for the analysis of important morphological and agronomic traits (Fatokun et al., 1997; Dansi et al., 2000c; Tostain et al., 2002; Tostain et al., 2003; Dumont et al., 2005). Molecular markers showing a high level of polymorphism on plant materials include micro-satellites (Sonnante et al., 1994; Akkaya et al., 1995), RAPDs (Williams et al., 1990; Williams et al., 1993; Dansi et al., 2000c), and AFLP (Vos et al., 1995; Tostain et al., 2002; Tostain et al., 2003; Kiambi et al., 2005). RAPD (Random Amplified Polymorphic DNA) markers have been shown to be useful in assessing intra-specific or inter-specific genetic variability in many crop plant species (Liu and Furnier 1993; Haley et al., 1994; Katsiotis et al., 2003; Ravi et al., 2003).

The present study is based on RAPD techniques, aims at characterising the different varieties of yam in Benin and is intended to contribute to a better knowledge of the genetic diversity and a better use of the genetic potential of the crop.

MATERIALS AND METHODS

Plant material

Tubers of 70 cultivars of the *D. cayenensis/D. rotundata* complex and 20 cultivars of *D. alata* were collected from farmers throughout the transitional Guinea-Sudan zone of Benin and were subsequently planted (Table 1). Samples of young fresh leaves of each of these cultivars were taken for DNA extraction.

DNA isolation

The collected fresh leaves were frozen in liquid nitrogen. Leaves were ground with a mortar and pestle. DNA was isolated according to the cetyl trimethyl ammonium bromide (CTAB) protocol by Rogers and Bendich (1985) with slight modifications as described below. Up to 200 mg of ground leaf tissue was transferred to 2 ml eppendorf tubes, mixed with 500 µl of 2 × CTAB extraction buffer and incubated in a 65°C water bath with frequent agitation by hand for 90 min. The tubes were removed from the water bath and allowed to cool at room temperature before 500 µl of phenol was added and mixed thoroughly. The mixture was centrifuged at 12,000 rpm for 10 min and the upper supernatant phase collected in a new tube. A second extraction was performed with 500 µl of a mixture of 24% of phenol/chloroform and 1% of isoamyl alcohol (v/v). After centrifugation, the supernatant was treated with RNase and the last extraction was performed with chloroform isoamyl alcohol. The upper phase was transferred into a new tube and DNA was precipitated with equal volumes of 2–propanol and Na-acetate. The DNA pellet was washed with 70% ethanol and dried for 5 min in a heating bloc at 60°C. The resulting DNA pellet was dissolved in 100 µl of distilled and sterilized water (Sigma). DNA quality was tested, using 1.5% agarose gel electrophoresis, and its concentration was determined with a UV spectrophotometer. Part of the DNA was then diluted to 25 ng/µl for PCR amplification.

PCR amplification

PCR reactions were performed in 25 µl volume in a mixture containing 1.7 mM $MgCl_2$, 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0), 0.1 mM of each dNTPs, 0.1 µM of random decamer primer, 50 ng of DNA and 1 unit of Taq DNA polymerase. The PCR amplification process was conducted in either T3 Thermocycler Biometra or Eppendorf Mastercycler. For each amplification process, an initial heat denaturation of DNA at 94°C for 3 min was followed by 45 cycles consisting of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. A final incubation for 10 min at 72°C was performed and the amplification products analysed on 2% agarose gel in Tris-borate buffer at 150 volts for 1 h. The agarose gel was stained in ethidium bromide, visualized under UV and photographed using digital camera Canon ISUS 3030. The ladder from SIGMA was used as standard molecular weight size marker.

The 12 primers used in this research were obtained from Invitrogen Life Technologies (Table 2). These primers were identified by Dansi et al. (2000c) as the best for genetic diversity characterization of yam.

Preliminary PCR amplification trials were performed on four cultivars arbitrarily selected in order to standardize the DNA amplification conditions on yam. These cultivars included two of *D. cayenensis*/*D. rotundata* (Gangni and Laboko) and two of *D. alata* (Djekin and Sankou kergba).

Different concentrations of $MgCl_2$, DNA, dNTPs, and Taq DNA polymerase were tested to obtain the most reproducible and reliable DNA amplification profiles. Optimal conditions which revealed clear and reproducible amplification fragments were used in the study as earlier described.

Selection of the most informative primers

PCR amplification was performed on 14 yam accessions (12 accessions of the *D. cayenensis* /*D. rotundata* complex and two of *D. alata*). The 12 primers were used individually in order to select the primers that showed most important polymorphic amplification fragments. Six out of 12 primers (OPW-2, OPW-5, OPW-6, OPW-8, OPW-16, and OPQ-4) revealed important polymorphic bands on the 14 yam cultivars screened and these were then selected for the whole study.

Molecular data analysis

After electrophoresis separation, amplified DNA fragments detected in each cultivar were scored for presence (1) or absence (0) of a particular DNA fragment at a particular position. A data matrix was

Table 1. List of yam cultivars used for RAPD analysis and origins of collection in the transitional Guinea-Sudan zone of Bénin.

Code	Species and varieties names	Village	Region	Code	Species and varieties names	Village	Region
D. cayenensis/D. rotundata							
1	Adigbili	Yagbo	C	54	Kaagourou	Sontou	NE
3	Aguida	Kaboua	C	55	Kokorogbarou	Ouroumonsi	NE
4	Ahimon	Yagbo	C	57	Moroko	Kpébié	NE
5	Ala N'kodjéwé	Yagbo	C	58	Morokorou	Kpébié	NE
6	Alakitcha	Ouoghi	C	59	Oroubessi	Sirarou	NE
7	Anago	Yagbo	C	60	Sika	Sakagbansi	NE
8	Assibo	Ouoghi	C	61	Singo	Sonnoumon	NE
10	Bodi	Aklampa	C	62	Wabè	Alfakpara	NE
11	Dègbo	Assanté	C	63	Wobo	Sakagbansi	NE
12	Djilaadja	Okounfo	C	64	Yakassougo	Suya/Sandiro	NE
13	Dodo	Ouèdèmè	C	65	Yontémé	Marégourou	NE
14	Effourou	Yagbo	C	39	Alassoura	Alédjo-Kpatago	NW
15	Efour	Ouoghi	C	66	Assana	Ouassa	NW
16	Enanwaï	Okounfo	C	67	Bakanon	Alfakpara	NW
17	Gangni	Ouèdèmè	C	68	Héléba	Foubéa	NW
18	Gnanlabo	Kpataba	C	69	Itolo	Foubéa	NW
19	Gnidou	Yagbo	C	70	Koutounou	Alfakpara	NW
20	Gogan	Assanté	C	71	Kpagnina	Alédjo-Kpatago	NW
21	Idoun	Pira	C	72	Kpakara	Foubéa	NW
22	Ilèkè	Kaboua	C	73	Lorie	Alédjo-Kpatago	NW
23	Kabilatonan	Yagbo	C	74	Noudoss	Ouassa	NW
24	Kanatonan	Assanté	C	75	Noukpam	Foubéa	NW
26	Kokoro	Yagbo	C	76	Papetè	Foubéa	NW
27	Kokoro Djougou	Ouoghi	C	77	Younouan	Alédjo-Kpatago	NW
28	Kokouman	Kaboua	C				
29	Kpakala	Ouoghi	C		***D. alata***		
30	Kpakra	Ouoghi	C	2	APK Florido	Ouoghi	C
31	Laboko	Ouèdèmè	C	4	Djekin	Aklampa	C
32	Laboko Parakou	Ouèdèmè	C	6	Florido	Yagbo	C
33	Mafobo	Kpakpaza	C	8	Kèègbè	Kaboua	C
35	Mondji	Ouoghi	C	9	Kpakata	Kaboua	C
36	Ofègui	Kaboua	C	12	Louelougan	Yagbo	C
37	Okoguin	Kaboua	C	13	Ogbo	Koko	C
38	Adani	Ginagourou	NE	14	Ogbo otcho adjana	Akpassi	C
40	Angogo	Sonoumon	NE	22	Sonouko	Yagbo	C
42	Baniwouré Bakarou	Suya	NE	24	Tchoko la vipère	Kaboua	C
43	Baniwouré Yantékpéron	Suya	NE	25	Tifiou	Okounfo	C
44	Boniyakpa	Marégourou	NE	15	Sankou arisso	Kpébié	NE
45	Danwaré	Biro	NE	16	Sankou Gankou	Sonri	NE
46	Dibiri	Sontou	NE	17	Sankou Garkou	Sandiro	NE
47	Dourokonou	Suya	NE	18	Sankou Kergba	Sontou	NE
48	Doudouwourou	Sontou	NE	19	Sankou souan	Ouroumonsi	NE
50	Youbakatanou	Sirarou	NE	20	Sankou Wa	Marégourou	NE
51	Gbarao	Sakabansi	NE	21	Sankourou	Ouénou	NE
52	Gonni	Ouénou	NE	11	Kpatagnan Pénin	Ouassa	NW
53	Ibérégbesse	Marégourou	NE	26	Toufou	Foubéa	NW

C = centre; NE = North-East; NW = North-West.

Table 2. List and sequence of the 10-base nucleotide primers used for the RAPD analysis.

Selected primers		Not selected primers	
Primer code	**Nucleotide sequence**	**Primer code**	**Nucleotide sequence**
OPW-2	5'-ACCCCGCCAA-3'	OPW-1	5'-CTCAGTGTCC-3'
OPW-5	5'-GGCGGATAAG-3'	OPW-12	5'-TGGGCAGAAG-3'
OPW-6	5'-AGGCCCGATG-3'	OPW-14	5'-CTGCTGAGCA-3'
OPW-8	5'-GACTGCCTCT-3'	OPW-15	5'-ACACCGGAAC-3'
OPW-16	5'-CAGCCTACCA-3'	OPW-17	5'-GTCCTGGGTT-3'
OPQ-4	5'-AGTGCGCTGA-3'	OPW-18	5'-TTCAGGGCAC-3'

then prepared for different analyses. To assess genetic diversity, a pair-wise similarity matrix was generated using the Nei – Li similarity index ($S = 2N_{AB}/(N_A+N_B)$); (Nei and Li, 1979), where N_{AB} is the number of RAPD fragments shared by two genotypes or cultivars (A and B); N_A and N_B are the total number of RAPD fragments analysed in each genotype (Levi et al., 2001). A dendrogram was then constructed based on the similarity matrix data using the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) cluster analysis of NTSYSpc-2.02j (Numeral Taxonomy and Statistical Analysis; Rohlf, 1998). The genetic variation between cultivars was investigated by an Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992). The total molecular variance (σ_T^2) was partitioned into a variance component due to differences among species (σ_{CT}^2), a variance component due to differences among groups within species (σ_{SC}^2), and to differences among cultivars within groups within species (σ_{ST}^2).

In a natural selection system, the allele fluctuation which occurs within groups of individuals tends to create or increase the genetic differentiation among groups by increasing homozygosity and decreasing heterozygosity (Conner and Hartl, 2004). It is the same pattern produced by inbreeding within groups. As reported by Conner and Hartl (2004), the geneticist Sewal Wright used this similarity between the fluctuation frequency within groups and inbreeding to create the F-Statistics, which provide an integrated view of genetic variation at three levels: within groups, among groups, and the total variation. As differentiation increases, so does the variance in allele frequency among groups, so the fixation index increases (Conner and Hartl, 2004). It is called fixation index because it increases as more groups become fixed for one allele (or close to fixation with the frequency tending to 0 or 1). To analyse the genetic structure, the fixation index is a measure that is more and more used (Weir and Cockerham, 1984; Excoffier et al., 1992; Weir, 1996; Schneider et al., 2000; Excoffier, 2001; Rousset, 2001; Dugoujon et al., 2004; Kiambi et al., 2005; Excoffier et al., 2006). This index, also called Wright's (1969) fixation index, was calculated for polymorphic loci and notated F_{ST}. F_{ST} is considered as the standardized variance of allele frequencies among cultivars (Excoffier, 2001).

The AMOVA was performed based on a pair-wise squared Euclidean distance matrix using Arlequin ver 3.01 software (Excoffier et al., 2006). The different patterns of gene-pool differentiation are presented using the bi-plot of the multivariate analysis component of the statistical package GenStat 8.11 (2005).

RESULTS

Specificity of the primers

Figure 1 shows DNA polymorphism detected in the 14 accessions screened using primers OPW-8 and OPW-16. The primer OPW-8 revealed 5 different bands in size range between 400 bp and 1600 bp, while the primer OPW-16 distinguished four different amplified fragments in size range between 200 bp and 1000 bp. From lanes 1 to 7, PCR profiles showed polymorphic patterns with different band sizes as revealed by the primer OPW-16: lane 1: 0 band, lane 2: 2, lane 3: 1, lane 4: 2, lane 5: 2, lane 6: 4, and lane 7: 2 bands. However, PCR amplification profiles were similar in some of the accessions as revealed by this primer OPW-16 showing two amplified bands in size of 420 and 520 bp in lane 10: Dourokonou, lane 11: Sika, lane 12: Yakassougo. While OPW-16 was unable to distinguish these accessions, the primer OPW-8 distinguished them by detecting polymorphic bands, lanes 10 and 11 showed two bands at different size and lane 11 showed three bands. Similarly, the four other primers used in the study also detected DNA polymorphisms. Hence, these six primers were used to characterise genetic diversity of the 90 cultivated germplasm accessions investigated (Table 2).

Genetic diversity and cluster analysis

Important genetic diversity was detected in the yam species investigated in this study. However, the diversity was larger in the *D. cayenensis*/*D. rotundata* complex compared to the *D. alata* species. Considering the results from all the six primers, a total of 67 amplified DNA bands were generated. The size of the amplified bands ranged from 100 to 2000 bp. The number of RAPD marker loci detected was 10 for the primer OPW-16, 11 for the primers OPW-5, OPW-8 and OPQ-4, and 12 for the primers OPW-2 and OPW-6. None of the primers considered individually was able to distinguish all the accessions. However, when other primers were used, accessions which were showing the same DNA fingerprint based on a particular primer could be differentiated from each another.

Also while the following bands 250 bp (OPW-2), 350 bp (OPW-5), 1200 and 1500 bp (OPW6), 150, 250 and 300 (OPW-16), 700 and 1000 (OPQ-4) were present in *D. cayenensis*/*D. rotundata* species, they were absent in *D. alata* species. Conversely, while the bands 450 (OPW-6) and 200 bp (OPW-8) were present in *D. alata*, they were absent in *D. cayenensis/D. rotundata* species.

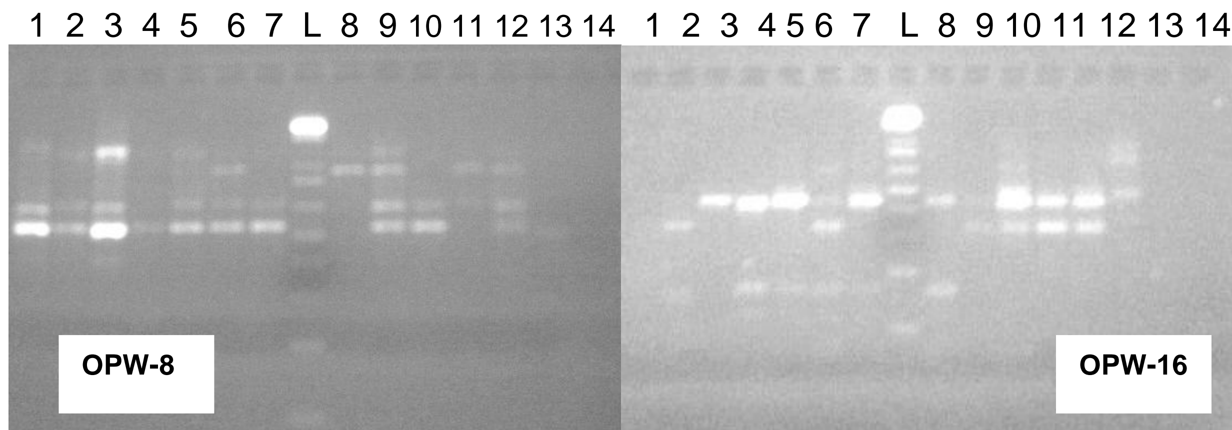


Figure 1. Gel electrophoresis of PCR products. Samples 1 to 12 represent accessions of *D. cayenensis/D. rotundata* (DCR) 1: Ala-N'kodjéwé, 2: Alakitcha, 3: Djilaadja, 4: Efour, 5: Gangni, 6: Laboko, 7: Mafobo, 8: Angogo, 9: Boniyakpa, 10: Dourokonou, 11: Sika, 12: Yakassougo and samples 13 and 14 represent accessions of *D. alata* (DAL), 13: Djekin, 14: Sankou kergba. L: molecular marker (100 bp).

Based on the presence or absence of DNA fragments, the estimates of the similarities among cultivars were calculated and used to construct the dendrograms for all cultivars together and separately by species. All cultivars from the yam species were partitioned into 18 groups based on the main class clusters generated at the level of 72% of similarity coefficient (Figures 2 and 3). The cultivars of the *D. cayenensis/D. rotundata* complex composed the groups between 1 and 12, while the 20 cultivars of *D. alata* belonged to the groups between 13 and 18. In the dendrogram of the *D. cayenensis/D. rotundata* complex (Figure 2), the accessions were clustered into 12 groups. The groups 1, 5, 8 and 9 containing 7, 2, 1 and 5 cultivars, respectively, consisted of only accessions collected from the central part of the transitional zone of Benin. There were also groups (7, 10 and 11) containing only germplasm originating from the North-East. The largest group of the dendrogram contained 14 cultivars of which 12 were collected from central Benin and two from the North-East part of this zone.

In the dendrogram constructed with *D. alata* cultivars only (Figure 3), the 20 accessions were clustered into six groups. Two groups contained the largest number of cultivars: group 15 consisted of nine accessions, group 16 contained six. Group 13 consisted of two accessions only. Some groups even consisted of only one accession (groups 14, 17, 18).

Frequencies of the amplified bands as revealed by the six primers

The 67 amplified DNA fragments frequency as revealed by the six primers is shown in Figure 4. Most primers showed fragments of high frequencies. However, some of the fragments had very low frequencies. The frequencies of amplified bands 450, 1200, 1500 bp (OPW-6) and 200, 1000, 1200 bp (OPQ-4) were very low, and ranged between 0.00 and 0.10. The frequency of the fragment in size of 200 bp was very low in five different primers (OPW-2, OPW-5, OPW-8, OPW-16, and OPQ-4). Some of the amplified bands were revealed by more than two primers and some were specific to a particular primer. None of the amplified bands was simultaneously revealed by the six primers. However, in general, all amplified DNA fragments revealed by the six primers had high frequencies. This result showed that all six primers were reliable in assessing the genetic variation in the yam cultivars analysed.

Molecular genetic differentiation

The components of the molecular variance (AMOVA) are summarized in Table 3. The total molecular variation was partitioned to variation between species, between varietal groups within species and between individuals within varietal groups. AMOVA showed that 12.7% of the molecular variance resulted from the variance among species. Most of the variation (52.7%) was due to the variation among individuals within varietal groups and variation among varietal groups (34.6%). AMOVA revealed that significant individual and varietal group differences existed. The genetic variability of the cultivars investigated by analysis of molecular variance from different levels (species and group) revealed significant genetic differentiation when considering the F-Statistics' values. The results showed highly significant variation among species with respect to all cultivars ($F_{CT} = 0.127$; p = 0.0009), among groups within species ($F_{SC} = 0.396$; p<0.0001), and among cultivars ($F_{ST} = 0.473$; p<0.0001). This genetic differentiation index value ($F_{ST} = 0.473$) indicates a very large genetic differentiation among the cultivars.

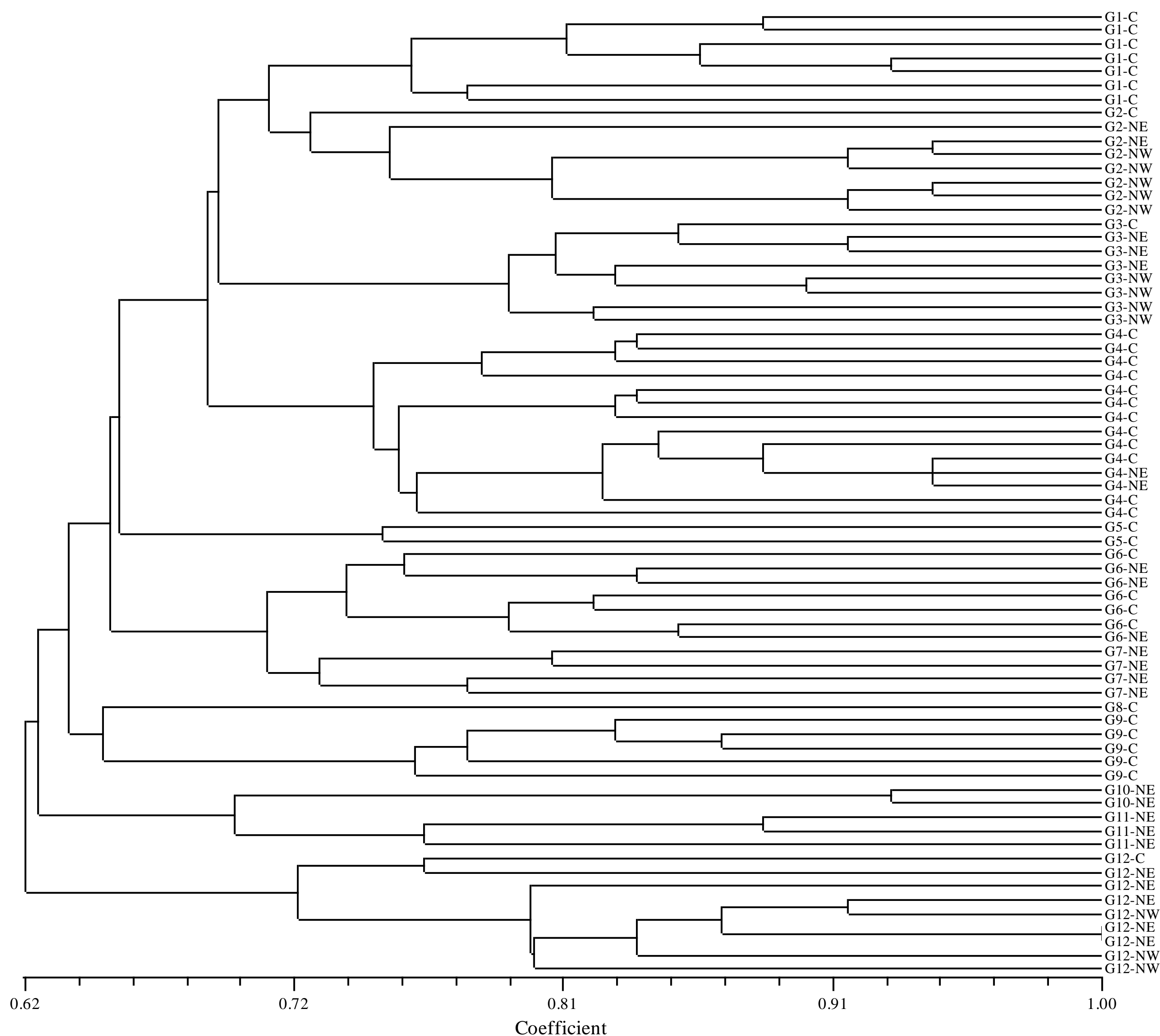


Figure 2. Dendrogram of *D. cayenensis/D. rotundata* accessions based on coefficient of similarity matrix. The codes correspond to the groups and the origin of collections (Table 1).

Table 3. Analysis of molecular variance of *D. cayenensis/D. rotundata* and *D. alata* cultivars

Source of variation	Degree of freedom	Sum of squares	Variance components[1]	Percentage of variance	F-Statistics[2]
Among species	1	91.253	$\sigma_{CT}^2 = 1.715$	12.72	F_{CT}: 0.127***
Among groups within species	16	467.001	$\sigma_{SC}^2 = 4.664$	34.60	F_{SC}: 0.396***
Among cultivars within groups	72	511.335	$\sigma_{ST}^2 = 7.102$	52.68	F_{ST}: 0.473***
Total	89	1069.589	$\sigma_T^2 = 13.480$		

[1] (σ_T^2) = the total molecular variance is partitioned into a variance component due to differences among species (σ_{CT}^2), a variance component due to differences among groups (σ_{SC}^2), and to differences among cultivars (σ_{ST}^2).
[2] The allele frequency variation index (F-statistics) for the three variance components are F_{CT}, F_{SC}, F_{ST}, respectively.
*** These three F-statistics are highly significant (p<0.001).

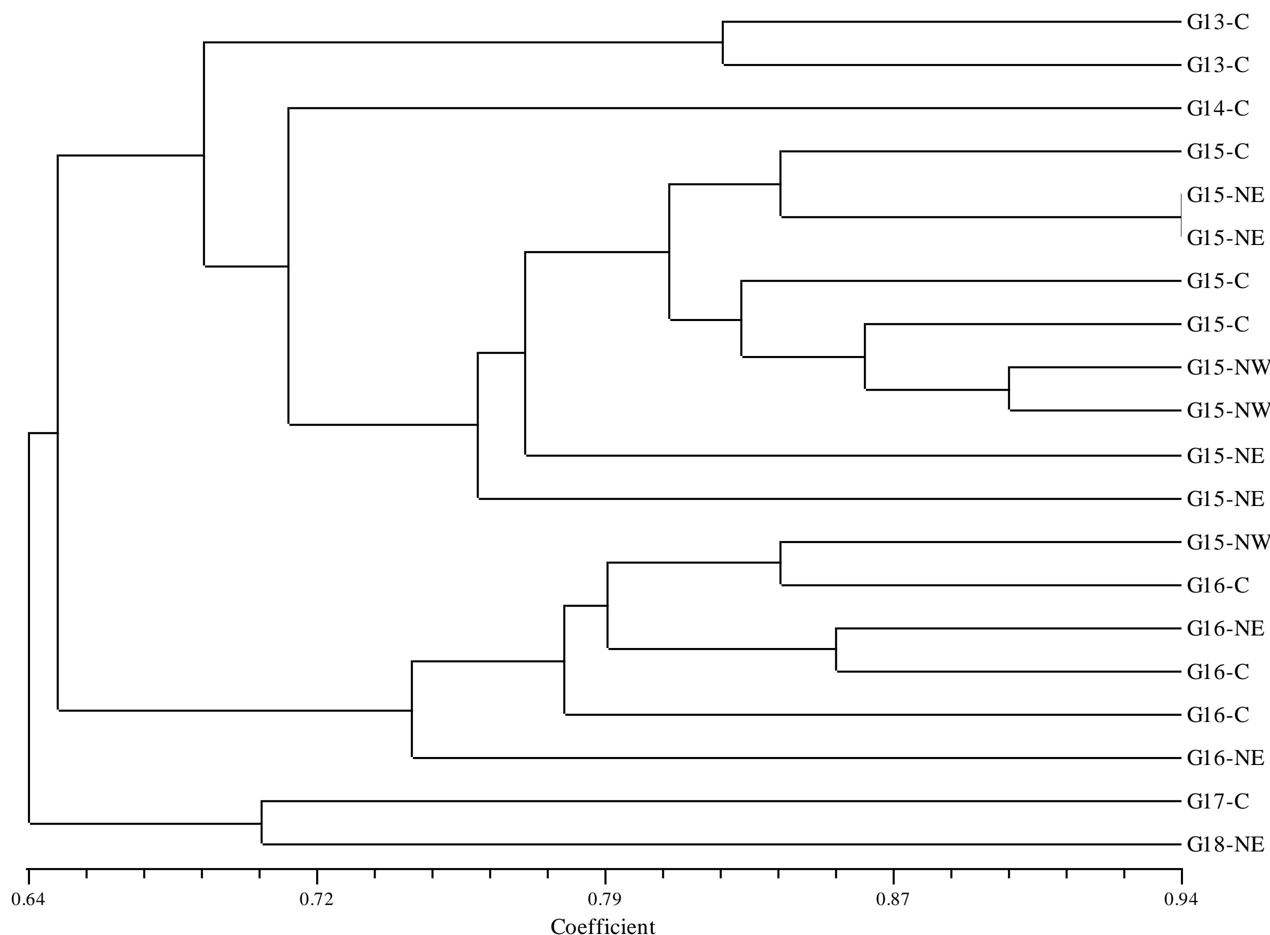


Figure 3. Dendrogram of *D. alata* accessions based on coefficient of similarity matrix. The codes correspond to the groups and the origins of collection (Table 1).

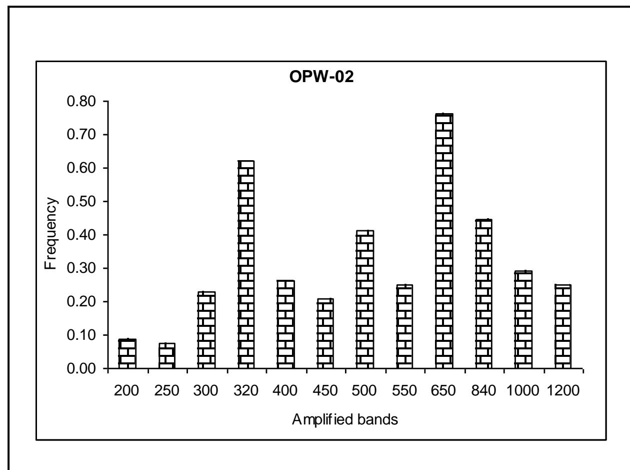
Combining the information provided by all molecular markers, most of the varieties from the North-East and North-West of the area investigated appeared to be distinctive from the ones of the centre as shown along the two axes for the *D. cayenensis/D. rotundata* (Figure 5) and for *D. alata* cultivars (Figure 6).

DISCUSSION

In the present study, where RAPD analysis was performed to evaluate the genetic diversity in 70 cultivars of the *D. cayenensis/D. rotundata* complex and 20 of *D. alata* collected throughout the transitional Guinea-Sudan zone of Benin, the genetic variation was higher in the *D. cayenensis/D. rotundata* complex, compared to *D. alata.* This result is in agreement with that of Dansi et al. (2000c) who reported important diversity in 23 accessions of the *D. cayenensis/D. rotundata* complex using RAPD analysis. This result may suggest that cultivars of the *Dioscorea* species analysed were originally generated by different ancestors of yam in the past. This is also possible since genetic diversity of wild yams is structured geographically (Tostain et al., 2003). All accessions of *D. alata* were separated from those of the *D. cayenensis/D. rotundata* complex.

From the analysis of the molecular variance, the average fixation index value (Wright's F_{ST} = 0.473) is above the reference value (0.25) of great differentiation revealed by Wright (1978), Hartl (1987) and Kiambi et al. (2005). This value suggests a very large genetic differentiation among yam cultivars in Benin. The value of Wright's F_{ST} (0.473) is higher than the reported average for animal- or insects pollinated out-crossing seed plants (Hamrick, 1989) or for out-crossing cultivated seed plants (Hamrick and Godt, 1997) (F_{ST} mean values = 0.187 and 0.234, respectively (Montes-Hernandez and Eguiarte, 2002)). This means that farmers' selection and domestication strategies played significant roles in the enlargement of the genetic diversity of the cultivars investigated.

The study showed that the genetic diversity changed along a spatial gradient. In general there was a tendency that most of the varieties from the North-East and North-



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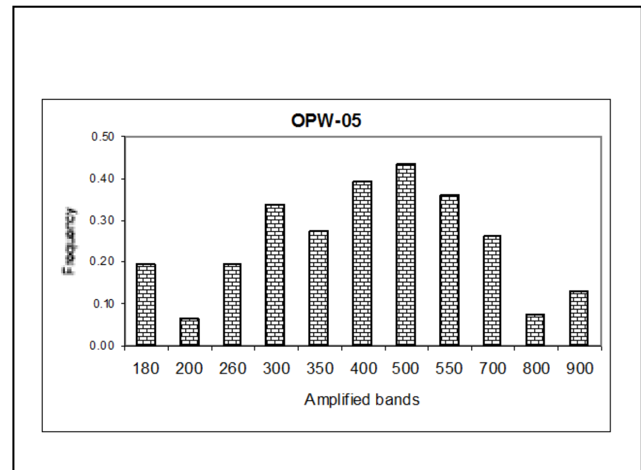

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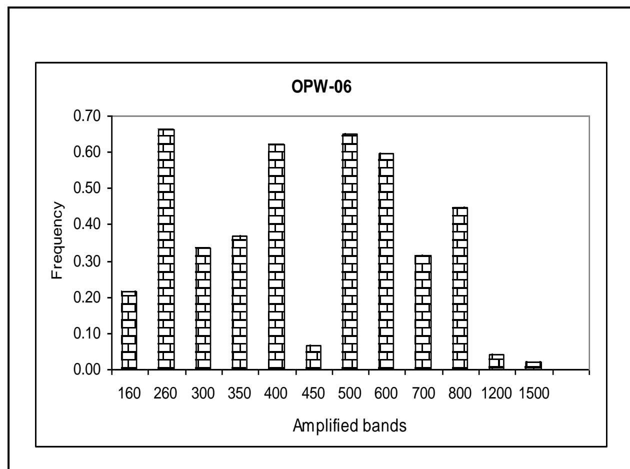

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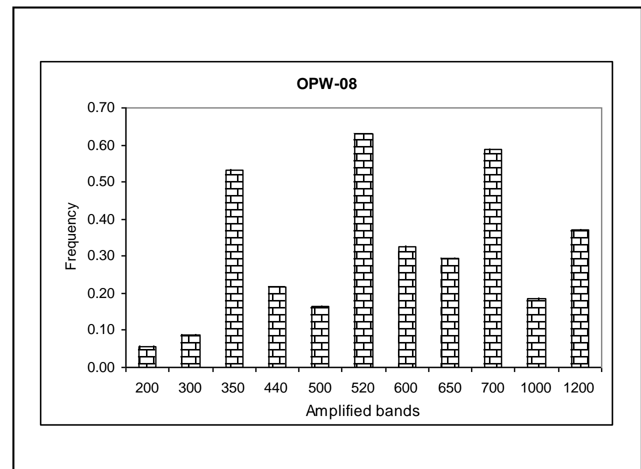

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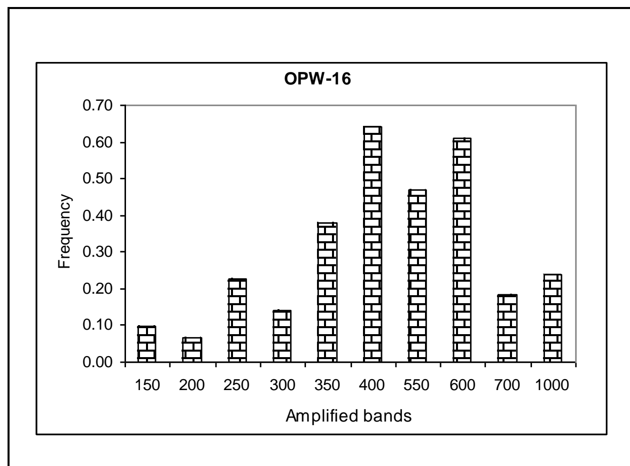
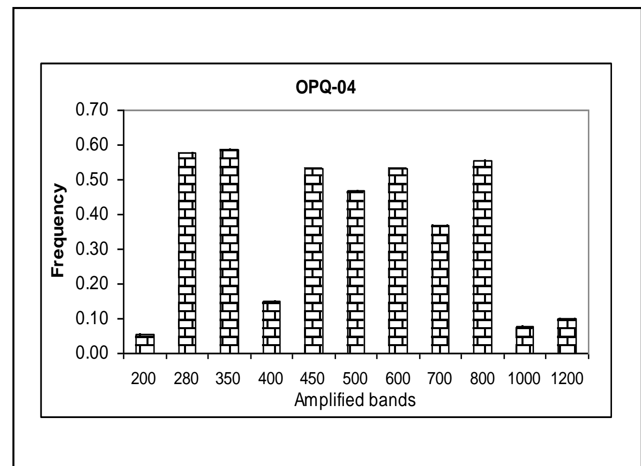


e)



f)

Figure 4. Amplified DNA fragments frequencies as revealed by the six primers.

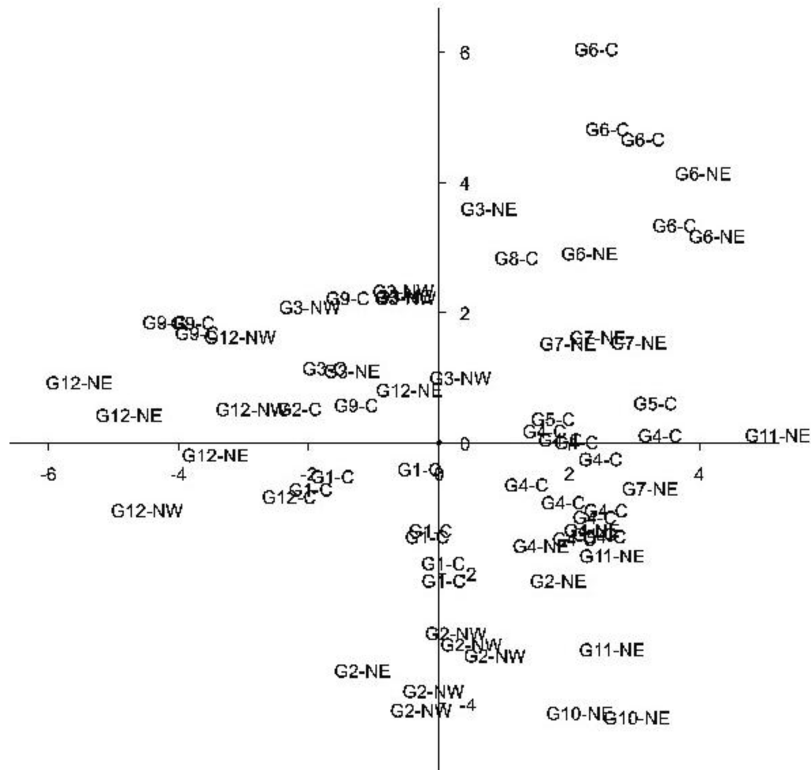


Figure 5. Pattern of gene-pool revealed by the molecular markers on *D. cayenensis/D. rotundata* cultivars.

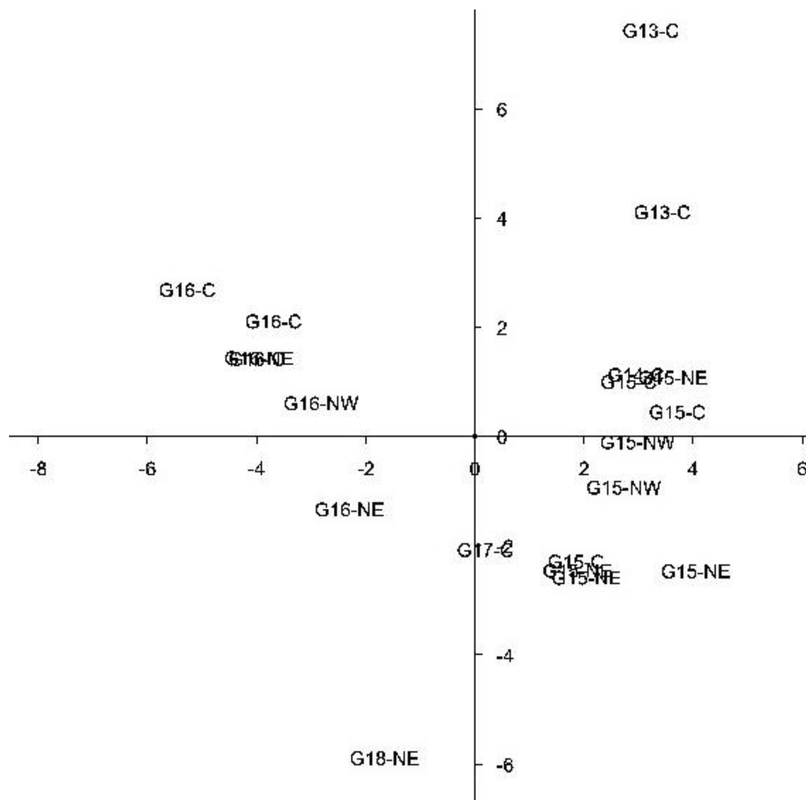


Figure 6. Pattern of gene-pool revealed by the molecular markers on *D. alata* cultivars.

West of the zone investigated appeared to be distinctive from the ones of the centre according to the dendrograms. However, few varieties were distributed randomly and did not reflect any specific relation to their zone of collection. The current study suggests that the Guinea Sudan zone of Benin is a very large gene-pool of yam varieties. Yam farmers in Benin with their continuous activity of domestication may have played a significant role in the enrichment and the maintenance of the genetic diversity.

The large genetic differentiation among cultivars suggests that each cultivar is distinctive and owns distinc-tive traits as confirmed by the level of polymor-phism. Additionally, this study shows the presence of important genetic variability among the Benin yam germ-plasm which can be used to broaden the genetic bases of the crop for better use of its genetic potential. For germplasm management, it is important, in addition to morphological characterisation, to reveal the extent of genetic diversity present in a collection, using other means such as molecular marker techniques.

ACKNOWLEDGEMENTS

We are grateful to the research team of Prof. Ambaliou Sanni, head of the Laboratoire de Biochimie et de Biologie Moléculaire, Faculté des Sciences et Techniques, Université d'Abomey-Calavi in Benin where this research was successful conducted, and to Dr. Alexandre Dansi for his advices during the whole research. We are indebted to the Interdisciplinary Research and Education Fund/Convergence of Sciences project of Wageningen University, Wageningen, The Netherlands, for financial support.

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