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PCR marker-based analysis of wild and cultivated yams (*Dioscorea* spp.) in Nigeria: genetic relationships and implications for *ex situ* conservation

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Abstract

Reliable characterization of the variation among wild and cultivated yams in Nigeria is essential for improved management and efficient utilization of yam genetic resources. RAPD and double stringency PCR (DS-PCR) analyses were used to investigate genetic relationships and the extent of redundancy among 30 accessions of two cultivated, and 35 accessions of four wild yam species collected from Nigeria. Twenty-five selected random decamer and two microsatellite primers were used individually and in combination to generate DNA profiles for each accession of the six *Dioscorea* species. The number of amplified fragments varied from 7 to 18 fragments per primer/primer combination. Different levels of intraspecific genetic diversity were found, with *Dioscorea rotundata* Poir. being the most variable. Based on identical profiles for the RAPD and DS-PCR primers, 12 duplication groups consisting of a total number of 37 accessions were observed in the present study. An UPGMA analysis grouped the majority of plants according to the species. Cultivated yams belonging to the *D. cayenensis–rotundata* species complex, which were classified into seven morphotypes/varietal groups, could be clearly separated into two major groups corresponding to *D. rotundata* Poir. and *D. cayenensis* Lam. *D. cayenensis* cultivars exhibited a low level of intraspecific variation and were genetically close to the wild species *Dioscorea burkilliana* J. Miège. *D. rotundata* cultivars classified into six varietal groups showed a high degree of DNA polymorphism and were separated into two major groups that appeared most closely related to *Dioscorea praehensilis* Benth. and *Dioscorea liebrechtsiana* de Wild. We propose, based on these results, that cultivars classified into *D. cayenensis* should be considered as a taxon separate from *D. rotundata*. The implications of intraspecific variability for the *ex situ* conservation of wild and cultivated yam germplasm in Nigeria are discussed.

Introduction

Yams (*Dioscorea* species) are important staple food crops in tropical and subtropical regions, particularly in West and Central Africa, where they are widely grown and consumed. Edible yams comprise the domesticated species and their wild relatives. In spite of the economic and cultural importance of edible yams, relatively few studies based on biochemical and molecular markers have been conducted to understand the relationships among the various species and the extent of

genetic similarity within cultivated and wild species of Guinea yams domesticated in West Africa (Hamon and Touré 1990; Terauchi et al. 1992; Dansi et al. 1999; Mignouna and Dansi 2003).

Grouping together of the Guinea yams *Dioscorea cayenensis* Lam. and *Dioscorea rotundata* Poir. in a species complex was proposed by Ayensu and Coursey (1972). However, the taxonomy and evolution of the *D. cayenensis–rotundata* complex remains controversial, partly because of the continuous variation in morphological descriptors observed in the cultivated and wild species in West Africa. We recently called for a revision of the taxonomy of *Dioscorea* species because it is difficult to understand how individuals identified in the wild as *Dioscorea praehensilis* Benth. or *Dioscorea abyssinica* Hochst. ex Kunth can directly become *D. rotundata* or *D. cayenensis* following "domestication" without any genetic change (Mignouna and Dansi 2003). Isozyme analysis of wild yam species from Côte d'Ivoire revealed three groups: annual, semi-perennial and perennial. Some cultivated accessions clustered with annual wild species whereas others cluster clustered with semi-perennial or perennial species (Hamon 1987). Phylogenetic relationships of Guinea yams and some wild relatives were studied using RFLP analysis of the chloroplast and nuclear ribosomal DNA (Terauchi et al. 1992). This study revealed four different classes with *D. rotundata* and *D. cayenensis* being classified in the same chloroplast DNA-defined group as the wild species *D. praehensilis*, *D. abyssinica* and *Dioscorea liebrechtsiana* de Wild. The other three classes identified among the wild species comprised *Dioscorea minutiflora* Engl., *Dioscorea burkilliana* J. Miège and *Dioscorea smilacifolia* de Wild and *Dioscorea togoensis* Knuth. Terauchi et al. (1992) proposed that *D. cayenensis* should be regarded as a variety of *D. rotundata* based on the ribosomal DNA analyses.

The domestication of the wild yam species *D. burkilliana*, *D. praehensilis and D. abyssinica*, a process that is still on-going in parts of West Africa (Dumont and Vernier 2000; Mignouna and Dansi 2003), is believed to have produced the cultivated forms of yam in this region (Dansi et al. 1999). Successful interspecific hybridizations between *D. praehensilis*, *D. abyssinica* and the domesticated species *D. rotundata* have been carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Wild yams are, therefore, important for yam breeding because they act as a reservoir of useful genes for agronomic characteristics such as yield, storability, tolerance to drought and weeds, organoleptic qualities, and tolerance to pests and diseases. Farmers typically use these criteria during the intense selection that accompanies the domestication process. Wild relatives of cultivated yam species are being collected and conserved *ex situ* at IITA. This is a very expensive and labour-intensive approach, generally requiring annual regeneration. The fields are also vulnerable to pests and diseases, made worse by the clonal nature of propagation of these crops and the diverse origins of the material. Reliable characterization of the variation within the wild and cultivated yam species, and the identification of redundancies is an essential step towards better management of wild genetic resources in yam improvement programmes.

Molecular markers can nowadays be applied to assist characterization of plant germplasm and the identification of redundancies (Ford-Lloyd et al. 1997). Among these, random amplified polymorphic DNA (RAPD) (Williams et al. 1990) and double stringency PCR (DS-PCR) markers (Matioli and de Brito 1995) have been applied in studies of genetic diversity and the identification of duplicate accessions in many crops (Verma et al. 1999), including yams (Ramser et al. 1996; Mignouna et al. 2003). The diversity in wild and cultivated yams has been investigated in Benin Republic (Dansi et al. 1999) and Côte d'Ivoire (Hamon and Touré 1990). However, apart from a few studies of cultivated yams in Nigeria using phenotypic (Onyilagha and Lowe 1985) and molecular (Mignouna et al. 2003) markers, little is known about the genetic relationship among the wild and cultivated yams of Nigeria. Furthermore, there is no report on the genetic diversity of wild yam in Nigeria, which is located at the centre of origin and domestication of Guinea yams. The aim of this study was to use RAPD and DS-PCR analyses to determine the genetic relationships among the cultivated (Guinea) yams and their wild relatives in Nigeria, and to make a preliminary assessment of the extent of redundancy within the wild and cultivated yam collection. The implications of our results for *ex situ* conservation of yam germplasm are discussed.

Materials and methods

Plant material and DNA extraction

The plant materials used in this work consisted of 65 accessions belonging to two cultivated and four wild species of the genus *Dioscorea* growing in Nigeria (Table 1). This material was selected from germplasm gathered during a survey conducted in Nigeria to collect the most popular and widely distributed varieties in the country. During the collection of the wild species, some cultivars showing intermediate morphological characteristics between wild and cultivated species were also collected. The cultivars were classified into seven varietal groups based on morphological descriptors (Dansi et al. 1999) (Table 1). Leaf samples of *D. rotundata* and *D. cayenensis* cultivars were collected from the germplasm characterization experimental field at the IITA, Ibadan or in the farmers' fields in the case of some of the wild yam species. Leaf samples of wild species were collected *in situ*, lyophilized and stored prior to DNA extraction.

Total DNA was extracted from leaf samples of the 65 accessions using a modified CTAB procedure (Mignouna et al. 2002a). DNA quality was visually assessed on 1% agarose gel following electrophoresis, and a model TKO100 DNA fluorometer (Hoefer Scientific Instruments, San Francisco, CA) was used to measure DNA concentration according to the manufacturer's instructions.

PCR amplification and RAPD analysis

A total of 25 random decamer primers (Operon Technologies Inc., Alamada, CA): OPA-01, 09, 13; OPB-01, 04; OPC-02, 04, 05, 07, 08, 09, 12, 19; OPE -19; OPF-17; OPG-11, 13, 16, 20; OPH-04, 13; OPI-16, 18 and OPX-01 that gave reproducible and scorable banding patterns were selected for this study, following a preliminary screening of 300 single primers.

Microsatellite sequences $(GATA)_4$, $(GACA)_4$, $(GTG)_5$, $(CA)_8$, $(GGAT)_4$ and $(GT)_8$ used as primers were synthesized by IDT Technology, Inc. (Iowa, USA). Of these, $(GATA)_4$ and $(GACA)_4$ gave the most polymorphic and reproducible patterns when used in combination with individual primers from the 25 random decamer primers. The two microsatellite and 25 random primers were used in DS-PCR, as described by Matioli and de Brito (1995). RAPD–PCR analysis was carried out as described by Mignouna and Dixon (1997). Amplification products were separated on 1.5% agarose gel using TBE buffer, visualized by ethidium bromide staining, and photographed under UV light with Polaroid film type 667. A 1 Kbp DNA ladder (GIBCO BRL, New York, USA) was used as standard molecular size marker.

Statistical analysis

Amplified DNA fragments that were reproducible in PCR reactions using three separate DNA samples of each accession were scored as present (1) or absent (0) for all accessions. Bands of identical size amplified with the same primer were considered to be the same DNA marker. Parsons and Shaw (2001) have shown that this is a reasonable assumption for closely related taxa, and as the present study involves only Guinea yams and their wild relatives, we did not test the assumption directly. Positions of unequivocally scorable bands were transformed into a binary character matrix ("1" for the presence and "0" for the absence of a band at a particular position). Pairwise distance matrices were compiled by the NTSYS-PC 2.0 software packages (Rohlf 1993), using the Jaccard coefficient of similarity (Jaccard 1908). Two separate data sets were constructed for the RAPD and DS-PCR. The correlation between RAPD and DS-PCR genetic dissimilarity data was tested using the matrix correspondence test (Mantel 1967). The final data set comprised the pooled RAPD and DS-PCR binary data sets, and included only polymorphic fragments. A dendrogram was constructed by UPGMA cluster analysis (Sneath and Sokal 1973; Swofford and Olsen 1990).

Results

Of the 300 RAPD primers initially screened, the RAPD patterns of 25 primers were considered to be reproducible and were selected for analysis. The microsatellite primers $(GATA)_4$ and $(GACA)_4$ gave the most polymorphic and reproducible banding patterns, and were selected for

Table 1. Wild and cultivated yam (*Dioscorea* spp.) accessions used in this study.

S/N	Species[a]	IITA acc. number	Local name	Varietal group[b]	Site of collection in Nigeria
Cultivated species					
1	*D. rotundata*	TDr 131	Abi	Baniakpa	Eastern Nigeria
2	*D. rotundata*	TDr 179	Aga	–	Eastern Nigeria
3	*D. rotundata*	TDr 93-2	Pepa	–	Abuja
4	*D. rotundata*	TDr 93-10	Agba	Frou	Obinagu
5	*D. rotundata*	TDr 93-23	Obiaturugo	Norforwu	Obinagu
6	*D. rotundata*	TDr 93-31	Danacha	Sopere	Zaki-Biam
7	*D. rotundata*	TDr 93-44	Pelli	–	Agyaragu
8	*D. rotundata*	TDr 93-47	Erefu	Norforwu	Delta State
9	*D. rotundata*	TDr 747	Unegbe	Kratchi	Delta State
10	*D. rotundata*	TDr 93-8	C'ikakundu	Norforwu	Zaria
11	*D. rotundata*	TDr 93-9	Amula	Norforwu	Zaria
12	*D. rotundata*	–	Okwacha	–	Nibo
13	*D. rotundata*	Sand peper	Akokwa	–	–
14	*D. rotundata*	–	Mbiri	–	–
15	*D. rotundata*	–	Akokwa	Sopere	–
16	*D. rotundata*	–	–	Norforwu	Akokwa
17	*D. rotundata*	–	Iroko	Norforwu	–
18	*D. rot/abys*[c]	–	–	–	Radere Ore
19	*D. cayenensis*	TDc 91/1205	–	Yaobadou	–
20	*D. cayenensis*	TDc 94-1-2	–	Yaobadou	Oji River
21	*D. cayenensis*	TDc 94-2-3	–	Yaobadou	Ugwuoba
22	*D. cayenensis*	TDc 94-4-1	–	Yaobadou	Emuhu
23	*D. cayenensis*	TDc 94-4-2	–	Yaobadou	Emuhu
24	*D. cayenensis*	TDc 94-4-6	–	Yaobadou	Emuhu
25	*D. cayenensis*	TDc 94-10	Igangan	Yaobadou	Ibadan
26	*D. cayenensis*	TDc 95-52	Aganran	Yaobadou	Ajamnogi
27	*D. cayenensis*	TDc 95-65	Jioku	Yaobadou	Ezzaakpu Ogu
28	*D. cayenensis*	TDc 95-66	Ikpen	Yaobadou	Onogholo
29	*D. cayenensis*	TDc 95-152	Npunu-akpukpu	Yaobadou	Ugwu-nani
30	*D. cayenensis*	Yellow yam	–	Yaobadou	Ibadan

S/N	Species	Immediate source	Site of collection in Nigeria
Wild species			
31	*D. abyssinica*	IITA	Bode Sadu
32	*D. abyssinica*	IITA	Bode Sadu
33	*D. abyssinica*	IITA	Bode Sadu
34	*D. abyssinica*	IITA	Mokwa
35	*D. abyssinica*	IITA	Mokwa
36	*D. abyssinica*	IITA	Mokwa
37	*D. abyssinica*	IITA	Mokwa ranch
38	*D. abyssinica*	IITA	Mokwa ranch
39	*D. abyssinica*	IITA	Mokwa ranch
40	*D. praehensilis*	IITA	Omo forest
41	*D. praehensilis*	IITA	Omo forest
42	*D. praehensilis*	IITA	Omo forest
43	*D. praehensilis*	IITA	Omo forest
44	*D. praehensilis*	IITA	Omo junction
45	*D. praehensilis*	IITA	Ifon
46	*D. praehensilis*	IITA	Indanre
47	*D. praehensilis*	IITA	IITA forest
48	*D. praehensilis*	IITA	IITA forest
49	*D. praehensilis*	IITA	IITA forest

Table 1. Continued.

S/N	Species	Immediate source	Site of collection in Nigeria
50	*D. praehensilis*	IITA	Ibadan
51	*D. praehensilis*	IITA	Ibadan
52	*D. praehensilis*	IITA	Okada
53	*D. praehensilis*	IITA	Abgaro
54	*D. burkilliana*	IITA	Ifon
55	*D. burkilliana*	IITA	IITA forest
56	*D. burkilliana*	IITA	IITA forest
57	*D. burkilliana*	IITA	IITA forest
58	*D. burkilliana*	IITA	Oni Gambari
59	*D. burkilliana*	IITA	Oni Gambari
60	*D. burkilliana*	IITA	Oni Gambari
61	*D. burkilliana*	IITA	Oni Gambari
62	*D. liebrechtsiana*	IITA	Omo forest
63	*D. liebrechtsiana*	IITA	Omo forest
64	*D. lieb/praehensilis*	IITA	Ifon
65	*D. praehensilis/lieb*[d]	IITA	Ifon

– = Unknown.
[a] *D. rotundata* Poir., *D. cayenensis* Lam., *D. abyssinica* Hochst. ex Kunth, *D. praehensilis* Benth., *D. burkilliana* J. Miège, *D. liebrechtsiana* de Wild.
[b] According to Dansi et al. 1999.
[c] Intermediate type between *D. abyssinica* and *D. rotundata* (S/N 18).
[d] Intermediate type between *D. liebrechtsiana* and *D. praehensilis* (S/N 65).

DS-PCR analysis. Results revealing polymorphism in plant samples were ignored during the primer selection process, in order to avoid a biased estimation of variability (Clark and Lanigan 1993). The number of RAPD fragments varied from 6 (primer OPH-05) to 18 fragments (primer OPB-01). A total of 237 bands were amplified following RAPD and DS-PCR analyses, out of which nine were monomorphic and were not included in the analysis. Most amplified DNA fragment sizes ranged from 200 to 2500 bp.

The Mantel test showed that the distance matrix calculated using the RAPD data was highly correlated ($r = 0.96$; $P < 0.0001$) with the matrix derived from the DS-PCR analysis. The RAPD and DS-PCR genetic distance data sets were, therefore, pooled and analysed. A graphical presentation of genetic similarity within, and genetic relationships among, the wild and cultivated *Dioscorea* species was obtained by generating a dendrogram from the combined similarity matrix based on Jaccard's coefficient and the UPGMA clustering method (Figure 1). Twelve groups consisting of a total number of 37 accessions were observed to have identical RAPD and DS-PCR profiles (Table 2). We considered accessions belonging to these groups to be duplicates based on their identical DNA banding pattern and the Jaccard similarity coefficient of 1.0 (McGregor et al. 2002). In *D. cayenensis* for instance, accessions TDc 91/1205, TDc 95-52 and TDc 96-65 had identical DNA profile. Similarly, *D. rotundata* accession TDr 93-2 and the landrace cultivar "Sand pepa" displayed identical DNA profiles. The most dramatic case of duplication was that of eight *D. praehensilis* accessions that showed an identical profile for all the primers investigated. Except for one pair of duplicate accessions that was an intermediate type between *D. liebrechtsiana* and *D. praehensilis*, duplication groups consist of individuals belonging to the same species. Also, the majority of identified duplicates appeared to be sampled from the same collection area (Figure 1 and Table 1). More than 75% of the wild yam accessions analysed shared their RAPD and DS-PCR profiles with other accessions, while only 41% of the Guinea yam accessions showed a shared DNA profile.

Cluster analysis allowed an assessment of the genetic relationships among the cultivated yam species and their wild relatives. In general, the accessions clustered according to their respective taxonomic classification. The 12 *D. cayenensis* cultivars were morphologically similar to the varietal group Yaobadou previously described in the yam

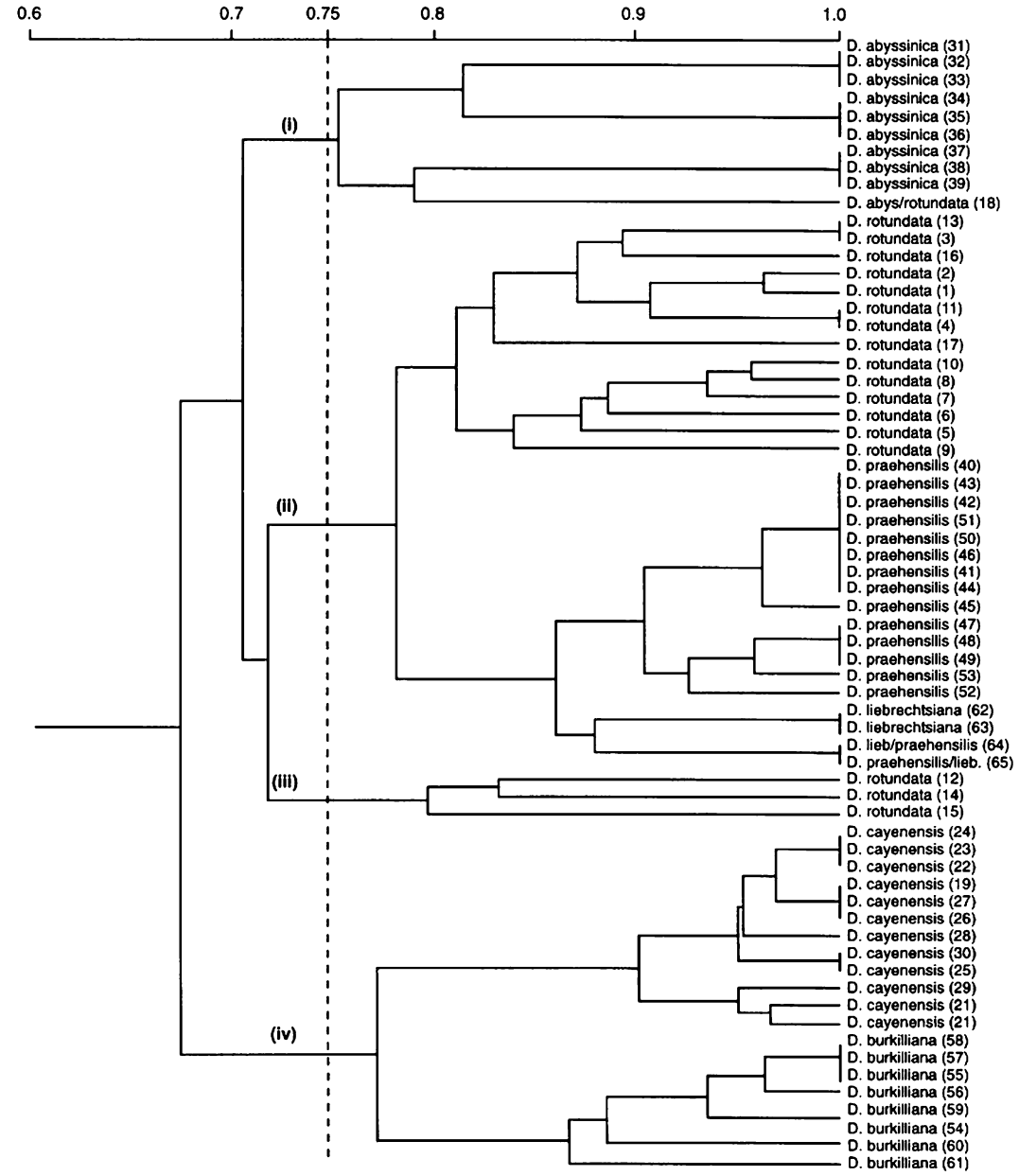

Figure 1. Dendrogram illustrating genetic similarities among 65 accessions of wild and cultivated *Dioscorea* species generated by the UPGMA average linkage cluster analysis (NTSYS) based on a total 237 markers produced by RAPD and DS-PCR analyses. The accessions are identified by the same serial numbers as in Table 1.

germplasm of Côte d' Ivoire (Hamon 1987). These accessions formed a distinct cluster relative to the other yam species. The white yam (*D. rotundata*) cultivars were morphologically classified into six varietal groups among which two (Norforwu and Kratchi) are newly described compared to previous reports on yam germplasm from Côte d'Ivoire (Hamon and Touré 1990). These cultivars groups were reported from yam germplasm of neighbouring Bénin Republic, and Dansi et al. (1999) have

Table 2. Distribution of duplication groups and duplicate accessions among the wild and Guinea yam species used in this study.

Yam species	No. of accessions	No. of duplication groups	No. of duplicate accessions	% of total number of accessions analysed
D. abyssinica Hochst. ex Kunth[1]	9	3	9	100.0
D. burkilliana J. Miège	8	1	3	37.5
D. cayenensis Lam.	12	3	8	66.7
D. liebrechtsiana de Wild[2]	2	1	2	100
D. praehensilis Benth.	14	2	11	78.5
D. rotundata Poir.	17	2	4	23.5
Total[3]	62	12	37	59.6

[1] One unique accession had morphological characteristics that were intermediate between *D. abyssinica* and *D. rotundata*.
[2] One pair of duplicate accessions comprised putative hybrids (intermediate types) between *D. liebrechtsiana* and *D. praehensilis*.
[3] The total does not add up to 65 because three intermediate-type accessions have been excluded.

provided a detailed morphological description of the groups. In this study, the *D. rotundata* cultivars comprised 17 accessions that clustered into two groups. The major group that comprised 14 different genotypes appeared closely related to the wild species *D. praehensilis*, while the remaining three cultivars clustered separately. One genotype, which showed intermediate morphological characteristics between *D. abyssinica* and *D. rotundata*, was closely related to the wild species *D. abyssinica*. Accessions of the wild species *D. abyssinica*, *D. praehensilis* and *D. liebrechtsiana* formed clusters that mostly reflected their taxonomic classification, with the last two species appearing closely related. It was interesting to note that *D. praehensilis* and *liebrechtsiana* accessions were nested within the *D. rotundata* cluster.

Discussion

The genetic relationship among the cultivated *Dioscorea* species and their wild relatives in Nigeria observed by RAPD and DS-PCR analyses was largely in line with the results obtained by Hamon (1987) using morphological and isozyme data, and Terauchi et al. (1992) using RFLP data. *D. cayenensis* was more closely related to *D. burkilliana* than to *D. rotundata*. All the 12 *D. cayenensis* accessions tested formed a clearly distinct cluster and were separated by a large genetic distance (<70% similarity) from *D. rotundata*, contrary to the suggestion by Terauchi et al. (1992) that *D. cayenensis* is a variety of *D. rotundata*. The nesting of *D. praehensilis* and *D. liebrechtsiana* accessions within the *D. rotundata* cluster in the UPGMA tree indicates that *D. rotundata* is more closely related to *D. praehensilis* and *D. liebrechtsiana*. Although only a few *D. liebrechtsiana* accessions were analysed, the similarity observed between this species and *D. praehensilis* lends support to the hypothesis that *D. liebrechtsiana* is the juvenile form of *D. praehensilis* (R. Dumont, pers. comm.).

Establishing the taxonomic identity of germplasm and understanding the systematic relationships among a crop and its wild relatives is vital to the management of genetic resources and the utilization of accessions (Bretting and Widrlechner 1995). The discrepancy between our results and those reported by Terauchi et al. (1992) probably arises from the fact that their RFLP analysis was based on the rDNA gene while we scanned the entire genome using PCR-based markers. The rDNA gene, although useful for inferring phylogenetic relationships, represents only a small fraction of the total genome and there is a risk of recreating gene trees rather than species trees.

Genetic mapping of yams has opened new avenues for selecting markers well distributed throughout the genome (Mignouna et al. 2002b). Future studies using high resolution and well distributed markers will provide independent evidence of lineages and further clarify species-level systematics within *Dioscorea* (Mignouna et al. 2003). Another fact that may underlie some of the discrepancy observed in taxonomic status is that only one plant per accession was analysed in the present study although Guinea yam cultivars tend to be polyclonal (Hamon and Touré 1990). For instance, cases have been reported of individuals with

identical morphotype but different genotype based on isozyme (Mignouna and Dansi 2003) and molecular (Mignouna et al. 2003) markers. Discrepancies may, therefore, have arisen as a result of difficulties in determining the taxonomic status of germplasm on the basis of morphology. In this study, individuals were observed that morphologically seemed to be intermediates between *D. abyssinica* and *D. rotundata*, and between *D. praehensilis* and *liebrechtsiana*. If these accessions indeed represent interspecific hybrids, then efforts should be made towards ensuring the purity of accessions prior to their use in taxonomic studies.

The usefulness of wild *Dioscorea* species as a reservoir of useful genes depends on the number of original/unique accessions, the diversity of collection site habitats, and the range of responses to biotic and abiotic stresses (Berger et al. 2003). Therefore, duplications are a nuisance because they do not contribute to the diversity in the collection, but nevertheless demand capacity for storage and maintenance. Based on identical profiles for the RAPD and DS-PCR primers, 12 duplication groups consisting of a total number of 37 accessions were observed in the present study. For the majority of accessions, passport data supported the duplicate status, as plants from identified duplicates appeared to be collected from the same location (Table 1, Figure 1). McGregor et al. (2002) made a similar observation in an AFLP analysis of wild potato germplasm, and questioned whether accessions should have completely identical AFLP profiles in order to be considered redundant. They showed that, in spite of reproducibility tests, it is possible to consider duplicates as unique accessions based on artefact bands caused by methodological inconsistencies. This problem is further compounded by intra-accession variability that can be expected when analyzing large sample sizes, especially in cross-fertilizing species like *Dioscorea*. These problems may be circumvented by including a tolerance level for polymorphisms in the analysis as a basis for separating duplicate from unique accessions (Arens et al. 1998; McGregor et al. 2002). Such analyses were not considered appropriate in this study due to the small sample sizes used but will be applied in future studies with larger sample sizes.

Dioscorea burkilliana, *D. abyssinica* and *D. praehensilis* are believed to have produced the cultivated forms in Benin Republic (Mignouna and Dansi 2003). The high diversity in the traditional yam landraces in Nigeria, especially within *D. rotundata*, is attributable to the availability of wild yams with cropping potential, different selection pressures, successive domestication, culture-derived modifications and somatic mutations. Due to increasing collection sizes and decreased resources, the yam gene bank is constrained to identify and remove redundancies in its wild and cultivated yam collection to ensure increased efficiency in *ex situ* conservation. Rationalization of the wild and cultivated yam germplasm in Nigeria will require further insight in intra-accession variation.

It was rather surprising that 8 of the 14 *D. praehensilis* accessions included in this study showed an identical profile for all the primers investigated. Unlike the situation in Bénin Republic (Mignouna and Dansi 2003), little is known about the distribution of genetic diversity in the natural areas where wild relatives of cultivated yam occur in Nigeria. Such information is useful to develop sampling strategies that will maximize the probability of collecting genetically distinct samples (McGregor et al. 2002). The finding that the majority of identified duplicates appeared to be sampled from the same collection area suggests that collections have to be made at sites separated by larger geographic distances in order to maximize the diversity in the collection.

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References

Arens P., Coops H., Jansen J. and Vosman B. 1998. Molecular genetic analysis of black poplar (*Populus nigra* L.) along the Dutch rivers. Mol. Ecol. 7: 11–18.

Ayensu E.S. and Coursey D.G. 1972. Guinea yams. The botany, ethnobotany, use and possible future of yams in West Africa. Econ. Bot. 26: 301–318.

Berger J., Abbo S.A. and Turner N.C. 2003. Ecogeography of annual wild *Cicer* species: the poor state of the world collection. Crop Sci. 43: 1076–1090.

Bretting P.K. and Widrlechner M.P. 1995. Genetic markers and plant genetic resource management. Plant Breed. Rev. 31: 11–86.

Clark A.G. and Lanigan C.M.S. 1993. Prospects for estimating nucleotide divergence with RAPDs. Mol. Biol. Evol. 10: 1096–1111.

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

Dumont R. and Vernier P. 2000. Domestication of yams (*Dioscorea cayenensis-rotundata* complex) within the Bariba ethnic group in Benin. Outlook Agric. 29: 137–142.

Ford-Lloyd B.V., Jackson M.T. and Newbury H.J. 1997. Molecular markers and the management of genetic resources in seed gene banks: a case study in rice. In: Caloow J.A., Ford-Lloyd B.V. and Newbury H.J. (eds), Biotechnology in Agriculture Series No. 19, CAB International, New York, pp. 103–118.

Hamon P. 1987. Structure, origine génétique des ignames cultivées du complexe *Dioscorea cayenesis-rotundata* et domestication des ignames en Afrique de l'Ouest. Thèse de Doctorat ès-Sciences, Université Paris XI, Centre d'Orsay, 203 p.

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

Jaccard P. 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaudoise de Sci. Nat. 44: 223–270.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 29: 209–220.

Matioli S.R. and de Brito R.A. 1995. Obtaining genetic markers by using double-stringency PCR with microsatellites and arbitrary primers. Biotechniques 19: 752–758.

McGregor C.E., van Treuren R. Hoekstra and van Hintum Th.J.L. 2002. Analysis of the wild potato germplasm of the series Acaulia with AFLPs: implications for *ex situ* conservation. Theor. Appl. Genet. 104: 146–156.

Mignouna H.D., Abang M.M. and Fagbemi S.A. 2003. A comparative assessment of molecular marker assays (AFLP, RAPD and SSR) for white yam (*Dioscorea rotundata* Poir.) germplasm characterisation. Ann. Appl. Biol. 142: 269–276.

Mignouna H.D., Abang M.M., Onasanya A., Agindotan B. and Asiedu R. 2002a. Identification and potential use of RAPD markers linked to *Yam mosaic virus* resistance in white yam (*Dioscorea rotundata* Poir.). Ann. Appl. Biol. 140: 163–169.

Mignouna H.D. and Dansi A. 2003. Yam (*Dioscorea* spp.) domestication by the Nago and Fon ethnic groups in Benin. Genet. Resour. Crop Evol. 50: 519–528.

Mignouna H.D. and Dixon A.G.O. 1997. Genetic relationships among sources of resistance to ACMD in African cassava landraces using RAPD markers. African J. Root Tuber Crops 2(1 and 2): 28–32.

Mignouna H.D., Mank R.A., Ellis T.H.N., Bosh van den N., Asiedu R., Ng S.Y.C. and Peleman J. 2002b. A genetic linkage map of Guinea yam (*Dioscorea rotundata* Poir.) based on AFLP markers. Theor. Appl. Genet. 105: 716–725.

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

Parsons Y.M. and Shaw K.L. 2001. Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. Mol. Ecol. 10: 1765–1772.

Ramser J., Lopez-Peralta C., Wetzel R., Weising K. and Kahl G. 1996. Genomic variation and relationships in aerial yam (*Dioscorea bulbifera* L.) detected by random amplified polymorphic DNA. Genome 39: 17–25.

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

Sneath P.H.A. and Sokal R.R. 1973. Numerical Taxonomy. Freeman, San Francisco.

Swofford D.L. and Olsen G.J. 1990. Phylogenetic reconstruction. In: Hillis D.M. and Moritz C. (eds), Molecular systematics, Sinauer Associates, Sunderland, pp. 411–501.

Terauchi R., Chikaleke V., Thottappilly G. and Hahn S.K. 1992. Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear ribosomal DNA. Theor. Appl. Genet. 83: 743–751.

Verma S.K., Khanna V. and Singh N. 1999. Random amplified polymorphic DNA analysis of Indian scented basmati rice (*Oryza sativa* L.) germplasm for identification of variability and duplicate accessions, if any. Electrophoresis 20: 1786–1789.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531–6535.