Biochemical Systematics and Ecology 38 (2010) 320–334

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic diversity of *Dioscorea dumetorum* (Kunth) Pax using Amplified Fragment Length Polymorphisms (AFLP) and cpDNA

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ARTICLE INFO

Article history:
Received 13 July 2009
Accepted 23 January 2010

Keywords:
Dioscorea dumetorum
Amplified Fragment Length Polymorphisms (AFLP)
Chloroplast DNA
Genetic diversity
Africa

ABSTRACT

We have utilized Amplified Fragment Length Polymorphisms (AFLP) in conjunction with chloroplast DNA (cpDNA) sequence data to study the genetic diversity in 53 accessions of *Dioscorea dumetorum* from six countries in West and Central Africa. Our results provide a comparison of the two marker systems with regards to their applicability to differentiate intraspecific genotypes and the grouping of the accessions based on localities of collection. A total of 1052 AFLP fragments (of which 94.1% were polymorphic) produced from twelve primer combinations indicate a relatively high level of polymorphism among the accessions. Three major genetic groups that do not strictly follow a geographic distribution pattern were identified using Neighbour-joining and the principal coordinate (PCo) analyses. Accessions from Togo showed higher numbers of private fragments and the highest percentage polymorphism (59.4%). The detection of highest genetic diversity in accessions from Nigeria and Togo and their relationship to other accessions suggest that these countries are the centre of origin and diversity of *D. dumetorum*. The moderately high genetic diversity (average of 61%) is suggesting great influence on the *D. dumetorum* germplasms through exchange and transfer of cultivars among local farmers in the subregion. In contrast, DNA sequence data from the *psb*A-*trn*H and the *rpo*B-*trn*C chloroplast regions revealed no variation among accessions from the different localities and clearly differentiated by AFLP patterns. The results demonstrate the usefulness of the AFLP marker in generating high polymorphism in the *D. dumetorum* accessions from West and Central Africa and hence may be used for agronomic purposes.

1. Introduction

Dioscorea dumetorum (Kunth) Pax is a monocotyledonous tuber crop of the family Dioscoreaceae reported to be one of the eight most important species commonly grown and consumed in West Africa (Agbor-Egbe and Treche, 1995; Afoakwa and Sefa-Dedeh, 2001; Medoua Nama, 2005). *Dioscorea* species are distributed mainly in Southeast Asia, Africa, Central America, South America, and other tropical or subtropical regions, while a few occur in Europe and North America (Gao et al., 2008; Onwueme and Sinha, 1991). In Africa, the species are found wild between 15°N and 15°S, and are cultivated in West Africa from Côte d'Ivoire through Ghana, Togo, Bénin, Nigeria, Cameroon, Gabon, Central African Republic, and the western part of the Democratic Republic of Congo (FAO, 2002) but especially Nigeria. *D. dumetorum* is one of four species native to Africa (Coursey, 1967). Tubers of *D. dumetorum* are rich in protein (9.6%), fairly balanced in essential amino acids (chemical score of

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0305-1978/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2010.01.012

0.94) and contain easily digestible starch (Mbome Lape and Treche, 1994). *D. dumetorum*, referred to as the 'trifoliate yam' or 'cluster yam', belongs to the section *Lasiophyton* and is widely cultivated throughout West Africa for consumption as food. It is not as regularly eaten as a main meal compared to *Dioscorea rotundata* Poir and *Dioscorea cayenensis* Lam but it is a popular snack in big towns where its boiled tubers are hawked. Tubers of some of the wild forms of the species are highly toxic because of the presence of alkaloids (Bevan et al., 1956; Trèche and Guion, 1980; Hladik and Dounias, 1996; Millogo-Rasolodimby, 2000, 2001). The use of *D. dumetorum* as famine food is therefore recommended with caution. The tubers are carefully prepared by soaking for several days in running or salt water and boiling over night (Meduoa et al., 2007). The tubers are not only used for human consumption but also for pharmaceutical purposes. The pharmaceutical effect of certain species of *Dioscorea* is caused by its poisonous alkaloids, which are also the reason why the tubers need to be cooked before they can be consumed. The most important species in this category is the African *D. dumetorum* often known as 'bitter yam', which contains the alkaloid dihydrodioscorine (Bevan and Hirst, 1958). *Dioscorea* species also contain steroidal sapogenins (Martin, 1969, 1979), likely to occur in *D. dumetorum* but hitherto not reported. The steroidal sapogenins occur within the plant as water soluble glycosides or saponins. Iwu et al. (1990) detected a novel bio-active compound dioscoretine in *D. dumetorum*, which is acceptable pharmaceutically and which can be used advantageously as a hypoglycemic agent. It is useful in veterinary and human medicine for therapeutic treatment of diabetes mellitus. Additionally, dioscoretine can be used as a hypoglycemic agent to reduce the blood glucose level in situations of acute stress such as experienced by patients with hyperthermia, trauma, sepsis and burns and those undergoing general anesthesia.

In the practice of folk medicine in West Africa, *D. dumetorum* has been used by herbalists and traditional medical practitioners for the treatment of diabetes and as a topical anesthetic while others have used it as an arrow poison and as bait for monkeys (Corley et al., 1985). It is one of the only two species in which high level of resistance to the two major nematode parasites of yams has been reported (Kwoseh, 2000). *D. dumetorum* can easily be distinguished from other species in the genus by its trifoliate leaves thus its name 'trifoliate yam'. It has large globular or lobed tubers that are not found deeply in the ground and so are easy to harvest (Devineau et al., 2008). Farmers involved in domestication of wild yam species have been reported to have developed renewed interest in the domestication of wild yam species such as *D. dumetorum* claiming that their domestication appears to be an active way for improving accessions of yam already in cultivation. Wild yam accessions have been observed to be useful in mitigating genetic erosion due to predominant vegetative propagation of cultivated yams (Trèche, 1996; Dumont and Vernier, 2000; Vernier et al., 2003; Mignouna and Dansi, 2003). The apparent gene flow between the wild and cultivated species makes the former important in conservation strategies for the crop (Dansi et al., 1999, 2000; Scarcelli et al., 2006a, b).

Yams play unique roles in the nutrition, economy, as well as social and religious festivities of Africa and most importantly in Nigeria, the world's largest yam producing country (Ayensu and Coursey, 1972; FAO, 2002). However, in spite of the significance of *D. dumetorum* as both a food crop and a wild yam species, it has received little or no attention with regard to quantitative and qualitative improvement. From studies of the cytology of some *Dioscorea* species, Baquar (1980) reported that all the four accessions of *D. dumetorum* studied possessed 40 somatic chromosomes. Four different chromosome numbers: 36, 40, 45 and 54 were reported earlier by Miège (1954) in *D. dumetorum*. It was observed that this species posed the greatest difficulty with regard to fixation and staining of chromosomes. All the chromosomes, except three pairs, were comparatively small (0.5–1.0 μm) with the largest pair being 1.5 μm in length. Until recently, little information was available on the genetics of yams (Martin, 1966; Zoundjihékpon et al., 1994) especially that of *D. dumetorum*. The available information on other species of yam includes the first linkage map of *Dioscorea* using the diploid wild yam species *Dioscorea tokoro* (Terauchi and Kahl, 1999), genetic linkage maps of white Guinea yam *D. rotundata* and water yam *Dioscorea alata* L. (Mignouna et al., 2002a, b), and analysis of diversity of Amplified Fragment Length Polymorphism (AFLP) markers in water yam (*D. alata*) (Egesi et al., 2006). Such studies have not been conducted on *D. dumetorum*. In view of the importance of *D. dumetorum* as a staple food in Africa, it is therefore necessary to study the genetic diversity using appropriate molecular tools.

Various molecular markers have been applied recently to plant genetic resource management and marker assisted selection (Bretting and Widrlechner, 1995; Mohan et al., 1997), based on Restriction Fragment Length Polymorphism (RFLP) and PCR-based markers, such as Random Amplified Polymorphic DNAs (RAPD), Amplified Fragment Length Polymorphisms (AFLP) and microsatellites. RAPD markers have been used to study the genetic diversity of plants including vegetatively propagated species such as yams (Muzac-Tucker and Ahmad, 1995; Asemota et al., 1996; Dansi et al., 2000). Although RAPD and its derivatives are useful molecular markers that can discriminate yam germplasm, the number of polymorphic markers per PCR assay is still low and the reliability and repeatability of RAPD markers are being questioned (Ramser et al., 1997; Bahieldin et al., 2006). Therefore, it is imperative that a more robust, polymorphic and reliable molecular marker technique be used to study the genetic diversity of yam. A more advanced method of DNA fingerprinting is the Amplified Fragment Length Polymorphism (AFLP)-technique. The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA that does not require prior sequence information (Vos et al., 1995). The usefulness of AFLP for the analysis of genetic relationships and intraspecific genetic diversity has been demonstrated in several studies (Mignouna et al., 1999; Ude et al., 2002a; Tremetsberger et al., 2003; Egesi et al., 2006; Albach, 2007; Meudt and Clarke, 2007). One other extensively used marker is chloroplast DNA sequence data. The use of cpDNA has been well documented in many studies (Vekeman et al., 1998; Graham and Olmstead, 2000; Ogihara et al., 2002; Katayama and Uematsu, 2005; Dane and Lang, 2004; Yukawa et al., 2005; Dane and Liu, 2007; Albach, 2007). The objective of the present study was to investigate the usefulness and comparative effectiveness of AFLP and cpDNA in differentiating among 53

accessions of *D. dumetorum* representing the broad variation of cultivar of this species in Africa and in assessing the intra-specific relationships.

2. Material and methods

2.1. Plant materials

A total of 53 accessions of *D. dumetorum* germplasm collected from various countries of West and Central Africa (Benin, Congo, Gabon, Ghana, Nigeria and Togo), and planted in the experimental field of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were included in the present study (Table 1). Three other *Dioscorea* species: *Dioscorea japonica* Thunb. (China-Japan), *Dioscorea bulbifera* L. (Southeast Asia) and *D. tokoro* Makino ex Miyabe (China-Japan) from *Dioscorea* accessions in the botanical garden of the Johannes Gutenberg-university Mainz were chosen as outgroup species

Table 1
Accession numbers, local names and localities of the investigated *Dioscorea dumetorum* accessions.

Accession no	Local name	Country
TDd3804	–	Benin
TDd3829	KLIPM-36	Benin
TDd3848	LEIFE	Benin
TDd3908	AKUCHA	Benin
TDd3935	–	Benin
TDd3717	–	Congo
TDd3114	PS-89-123	Gabon
TDd3101	ALAYANYO-WUDIDI	Ghana
TDd3109	NYA	Ghana
TDd3098	FA 26	Nigeria
TDd3112	D. DOMENTORUM	Nigeria
TDd3686	–	Nigeria
TDd3687	–	Nigeria
TDd3771	–	Nigeria
TDd3779	AMOLA	Nigeria
TDd3790	PEEBA	Nigeria
TDd3909	–	Nigeria
TDd3947	–	Nigeria
TDd04-146	–	Nigeria
TDd08-13-13	Esuru	Nigeria
TDd08-14-25	Esuru	Nigeria
TDd08-36-19	Esuru	Nigeria
TDd08-37-9	Esuru	Nigeria
TDd08-38-79	Esuru	Nigeria
TDd3093	VOTE 333	Togo
TDd3095	KOUTE 281	Togo
TDd3097	VOTEDRE 340	Togo
TDd3100	DOKOUTE 33	Togo
TDd3102	VOTEHE 341	Togo
TDd3104	AGBOTE 93	Togo
TDd3106	AGBOTE	Togo
TDd3107	AGBOTE 104	Togo
TDd3110	AGBOTA 457	Togo
TDd3111	VOTE 293	Togo
TDd05-2	–	Togo
TDd05-3	–	Togo
TDd05-5	–	Togo
TDd05-6	–	Togo
TDd05-7	–	Togo
TDd05-8	–	Togo
TDd05-9	–	Togo
TDd05-10	–	Togo
TDd05-12	–	Togo
TDd05-16	–	Togo
TDd05-17	–	Togo
TDd05-20	–	Togo
TDd05-23	–	Togo
TDd05-24	–	Togo
TDd05-25	–	Togo
TDd05-26	–	Togo
TDd05-27	–	Togo
TDd4088	DEKOUTE	Togo
TDd4118	N'Kafo	Togo

since they belong to the closely related sections *Enantiophyllum*, *Opsophyton* and *Stenophora* of *Dioscorea* respectively (Coursey, 1967; Burkill, 1960).

2.2. DNA isolation

DNA was isolated from ca. 20 mg of tissue from 2 to 3 month old silica gel dried leaves using the DNeasy™ plant minikit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. The quality of the extracted DNA was checked on a 0.8% TBE-agarose gels and the concentration was measured spectrophotometrically with a GeneQuant RNA/DNA calculator (Pharmacia, Cambridge, UK).

2.3. AFLP fingerprinting

The AFLP analysis was performed using the protocol described by Vos et al. (1995) with a little modification. A total of 250 ng genomic DNA was digested with EcoRI (Promega, Madison, Wisconsin, USA) and MseI (New England Biolabs, Beverly, Massachusetts, USA) at 37 °C for 4 h and incubated at 70 °C for 15 min. The DNA fragments were ligated to EcoRI and MseI adapters at 15 °C over night (ca. 15 h). The reaction mix (final volume 10 μL) contained 0.95 μL 10X T4 DNA ligase buffer (Promega), 0.1 μL BSA (10 mg/mL, New England Biolabs), 0.95 μL 0.5 M NaCl, 0.08 μL 10 U MseI (New England Biolabs), 0.10 μL 20 U EcoRI (Promega), 0.07 μL T4 DNA ligase (Promega), 0.47 μL 50 pmol MseI adapters (genXpress, Wiener Neudorf, Austria), 0.47 μL 5 pmol EcoRI adapters (genXpress). Preselective amplification by PCR was conducted in a 10 μL volume reaction in a thermal cycler (Gene Amp PCR System 9700, PE Applied Biosystems, Foster City, California, USA). The reaction mix contained 5 μL Promega kit PCR master mix, 0.52 μL MO2: 5′-GAT GAG TCC TGA GTA AC-3′ (50 ng/μL), 0.53 μL EO1: 5′-TG CGT ACC AAT TCA-3′ (50 ng/μL), 1.45 μL PCR-grade water and 2.5 μL diluted product (10X) of restriction/ligation. The selective amplification was performed with twelve EcoRI/MseI (three +2/+3 and nine +3/+3, see Table 2) primer combinations. The selective PCR reaction mix contained 1.667 μL NEB Taq 10X PCR Buffer (Sigma), 0.055 μL NEB Taq, 0.167 μL 20 mM dNTPs (Applied Biosystems), 0.83 μL 50 mM $MgCl_2$, 0.200 μL of EcoRI (50 ng/μL), 0.280 μL MseI (50 ng/μL), and PCR-grade water using 5 μL of 1:10 diluted product from preselective PCR as template. The incubation of the restriction–ligation reactions as well as the two consecutive PCR reactions (preselective and selective amplifications) were performed on a Biometra® T3 or a PTC 100™ MJ Research thermocycler. The fluorescence-labelled products of selective amplification were separated as a multiplex of three primer combinations each together with an internal size standard (GeneScan™-500 [ROX] on an automated DNA Sequencer (ABI 3130*xl* Genetic Analyzer, Applied Biosystem, Foster City, California, USA). Fragments were analyzed using the GeneMarker Genotyping software version 1.51 (SoftGeneticsLLC, State College Pennsylvania, USA). AFLP products were automatically scored as either present (1), or absent (0) and manual control. Ambiguous fragments were scored as (?). Only AFLP fragments within the readable region 70–500 bp in length were included in the analysis.

2.4. Amplification and sequencing

Five primer combinations consisting of: *psb*A-*trn*H, *ndh*F-*rpl*32, *rp*S16-*trn*K, *rpo*B-*trn*C and *trn*S–*trn*S (GCU) were used in PCR reactions with four of the 53 *D. dumetorum* accessions (Shaw et al., 2005). Accessions: TDd 3829 (from Benin), TDd 3112 (from Nigeria), TDd 3097, TDd 05-20 (from Togo) were selected to represent the most divergent genotypes based on the AFLP

Table 2
Primers, fluorescent dye labels and degree of polymorphismfor 12 primer combinations used.

AFLP primer combination	Dye colour	NTF[a]	NMF[b]	NPF[c]	% Polymorphism
EcoRI-AA MseI-CGT	HEX	86	0	86	100
EcoRI-AT MseI-CAG	6-FAM	147	3	144	98
EcoRI-AT MseI-CCC	NED	105	3	102	97.1
EcoRI-AAC MseI-CAG	6-FAM	105	6	99	94.3
EcoRI-AAG MseI-CAA	NED	73	7	66	90.4
EcoRI-AAG MseI-CAC	HEX	97	7	90	92.8
EcoRI-ACG MseI-CAA	HEX	96	5	91	94.8
EcoRI-ACG MseI-CAT	NED	83	0	83	100
EcoRI-ACG MseI-CCT	NED	56	9	47	83.9
EcoRI-ACT MseI-CGC	HEX	29	2	27	93.1
EcoRI-ACT MseI-CTG	FAM	95	12	83	87.4
EcoRI-AGC MseI-CTA	FAM	80	2	78	97.5
Total	–	1052	56	996	94.7
Minimum		29	0	27	83.9
Maximum		147	12	144	100
Average		87.7	4.7	83	94.1

[a] Total number of fragments generated (NTF).
[b] Number of monomorphic fragments (NMF).
[c] Number of polymorphic fragments (NPF).

analysis. Reactions were performed in a total volume of 25 μL, using 0.6 μL 25 mM $MgCl_2$, 2.5 μL NEB Taq 10X PCR Buffer (Sigma), 0.25 μL 20 mM dNTPs (Applied Biosystems), 0.2 μL NEB *Taq* DNA polymerase, 2.0 μL BSA (10 mg/mL, New England Biolabs), 16.45 μL PCR-grade water and 1.0 μL of each primer using 1 μL of DNA as template. Thermal cycling program consisted of 34 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 1 min, and extension at 72 °C for 90 s. Reactions were terminated with a final extension of 4 min at 72 °C. Amplified double-stranded PCR products were checked on a 0.8% TBE-agarose gel for amplification. The products were purified using QIA quick PCR purification and gel extraction kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocols. The same primers used for PCR amplification were also used for the cycle sequencing reactions (10 μL) carried out using the Big Dye Terminator Ready Reaction kit according to the manufacturer's instructions (Applied Biosystems Inc., Foster City CA, USA). Sequencing was done on an ABI 3130XL automated sequencer (Applied Biosystems, Inc.). Assembled sequences were manually edited using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Michigan, USA).

2.5. Data analysis-AFLP

The data matrix was analyzed phenetically with the Neighbour-joining algorithm using Nei-Li (1979)-distances in TreeCon (Van de Peer and De Wachter, 1994) and parsimony in PAUP4.10b (Swofford, 2002). Internal branch support was examined by bootstrapping (1000 replicates). Pairwise similarity coefficients were calculated according to the equation SAB = 2NAB l (NA + NB), where NA and NB are the numbers of scored fragments from individuals A and B, respectively, and NAB is the number of fragments shared by both (Nei and Li, 1979; Wetton et al., 1987). Genetic distance was then calculated as DAB = 1 − SAB, and the result was multiplied by 100. Principal coordinate analyses (PCoA) were calculated and plotted with Nei and Li (1979)-distances using r4.0 (Casgrain and Legendre, 2001) for all the accessions as a whole and based on three politically defined subgroups (Benin, Nigeria and Togo) via covariance matrix with data standardization (Orloci, 1978). For better interpretation of the data, calculations such as number of fragments per population, percentage of polymorphic loci per population, number of fragments that are unique to a single population (private fragment), expected heterozygosity (HE), unbiased expected heterozygosity (UHE) were calculated with GeneAlex 6.1 (Peakal and Smouse, 2006). Utilizing the genetic distances calculated from Euclidean similarity index, the analysis of molecular variance (AMOVA) procedure (Excoffier et al., 1992) was also applied to estimate variance component for the AFLP phenotypes using GenAlex 6.1. This was carried out with accessions from only three countries (Benin, Nigeria and Togo) because the three other countries had too few accessions represented in the set of genotypes. Individual variation was partitioned within and between countries. The variance components were extracted and tested using non-parametric permutation procedures. Variation between countries was partitioned into pairwise distances between them to examine the regional contribution to total molecular diversity (Excoffier and Smouse, 1994).

In order to further strengthen the information on phylogeographic structure of the accessions, data were analyzed with the individual-based clustering method in BAPS 3.2 2 (Corander et al., 2003, 2006). The Bayesian method, assuming Hardy–Weinberg equilibrium and linkage equilibrium, reveals population structure by clustering individuals into panmictic groups. It weights information across the genome, taking into account possible variation in the degree of empirical uncertainty about parameters at different loci. This has been found to be highly relevant, for instance, when the amount of missing observations varies largely across the loci. Both the number of populations in the sample and their allele frequencies are estimated by the program as unknown parameters. Identification of panmictic populations can provide a preliminary indication of relevant evolutionary units (Dawson and Belkhir, 2001). The method does not make use of geographical information for detecting clusters. The procedure was run 10 times each for $K = 2$–15 as the assumed maximum number of populations present in the sample (K was set arbitrarily and not dependent on the number of clusters sought in the sample). Instead, the program considers all values equal to or smaller than K to be a plausible number of clusters.

2.6. Data analysis-sequence data

No further analysis was carried out on the nucleotide base sequences obtained from the selected accessions due to lack of variation.

2.7. Morphological character optimization

To assess congruency between molecular and morphological characters in characterizing the *D. dumetorum* accessions, 35 morphological characters and states (see Table 3) were optimized onto the tree generated from the AFLP data set using MacClade 4.1 (Maddison and Maddison, 2001). Characters and states were obtained from modification of the field characters used by the IITA for passport characterization of *D. dumetorum* germplasms.

3. Results and discussion

Amplification was unsuccessful given inappropriate universal primers for *ndh*F-*rpl*32, *rp*S16-*trn*K and *trn*S–*trn*S (GCU), whereas nucleotide sequence data were obtained from *psb*A-*trn*H and *rpo*B-*trn*C although no variation was observed. The sequence data were submitted to the GenBank with the following accession numbers: *Dioscorea dumetoum psb*A-*trn*H spacer:

Table 3
Field characters used for morphological characterization of *D. dumetorum* accessions.

S/N	Characters	States	
		1	2
1	Days to emergence	12–27 Days	28–42 Days
2	Stem length (cm)	9–189	190–368
3	Stem height eight weeks after planting (m)	1	2–3
4	Internode number	5–12	13–19
5	Internode length (cm)	5–13	14–21
6	Petiole length (cm)	>5	<5
7	Spine length	Long	Intermediate/short
8	Leaf number at 20 days after emergence	8–16	17–31
9	Number of tubers per seedbed	2–5	1
10	Tuber length (cm)	21–40	6–20
11	Tuber width (cm)	11–35	36–58
12	Leaf length (cm)	7–12	13–17
13	Leaf width (cm)	5–10	11–20
14	Leaf tip (mm)	<2	2–5
15	Adult stem colour	Green/light green	Purple
16	Leaf type	Shallow/deeply lobed	Serrate/compound
17	Leaf colour	Light green/green	Dark green
18	Onset of leafing	Early	Late
19	Leaf base	Obtuse	Acute/emarginate
20	Leaf density	Dense	Intermediate
21	Waxiness	Present	Absent
22	Adult wings	Present	Absent
23	Spine shape	Curved down	Curved up/straight up
24	Coloured spot at the base of spine	Present	Absent
25	Vigour of entire plant	High	Low-intermediate
26	Twining habit	Yes	No/not provided
27	Twining direction	Clockwise	Anticlockwise
28	Corm	Present	Absent
29	Corm ability to separate from tuber	Yes	No
30	Tuber skin colour	Light/dark brown	Greyish
31	Tuber skin thickness	Thick	Thin
32	Tuber shape	Irregular	Cylindrical
33	Uniformity of tuber	Very uniform/medium	Not uniform
34	Tendency of tuber to branch	Highly branching	Branched/little branching
35	Place where tuber branched	Lower third part	Upper third part

GQ166693; *D. dumetorum rpo*B-*trn*C spacer: GQ166694; *D. japonica psb*A-*trn*H spacer: GQ166695; *D. japonica rpo*B-*trn*C spacer: GQ166696.

From the twelve selective primer combinations (Table 2) and 66 individuals (including ten replicate samples) used in the study, 1052 fragments ranging from 70 to 492 bp were scored, of which 996 (94.7%) were polymorphic and 56 (5.3%) were monomorphic. The total number of fragments (monomorphic and polymorphic) for each primer combination ranged from 27 to 147 with an average of 87.7 (Table 2). The percentage of polymorphic fragments varied from 83.9% to 100% with an average of 94.1% per primer combination. The error rate in the AFLP data set was calculated as the ratio between observed number of phenotypic differences and total number of phenotypic comparisons (Bonin et al., 2004) and amounts to 1.7%. The highest number of fragments was obtained from the primer pair EcoRI – AT MseI-CAG (147 fragments, of which 144 were polymorphic), while the lowest was found with the combination EcoRI – ACT MseI-CGC (29 fragments, of which 27 were polymorphic). The maximum average number of fragments per individual per primer pair was found in TDd 05-25 (32.3), while the minimum was obtained in TDd 3102 (10.5). The accessions from Togo showed the highest number of fragments, private fragments (fpr), as well as the highest percentage of polymorphic loci compared to those from other countries. Observed and expected heterozygosties ranged from 0.069 to 0.149, and 0.078 to 0.152 respectively (Table 4). The values observed for the similarity index for all accessions ranged from 16% to 57%, with an average of 39% (values not shown), while the genetic distance ranged from 43.2% to 83.4% and an average of 61.0%. The minimum genetic distance of 43.2% was found between two Nigerian species TDd 3909 and TDd 08-14-25, while the maximum of 83.4% was between the accessions TDd 3848 from the Republic of Benin and TDd 05-24 from Togo. The Neighbour-joining analysis included 27 constant, 174 variable but

Table 4
AFLP fragment pattern over twelve primer combinations used for three of the populations investigated.

Population	N	fpop	%Ppop	fpr	HE	UHE
Benin	5	429	20.44	8	0.069 ± 0.005	0.078 ± 0.005
Nigeria	15	642	51.33	56	0.142 ± 0.006	0.148 ± 0.006
Togo	29	701	59.41	108	0.149 ± 0.006	0.152 ± 0.006

Abbreviations: N, number of individuals investigated; fpop, number of fragments per population; %Ppop, percentage of polymorphic loci per population; fpr, number of private fragments; HE, expected heterozygosity (±standard deviation); UHE, unbiased expected heterozygosity (±standard deviation).

parsimony-uninformative characters and 851 parsimony-informative characters. The result of the NJ-analysis (Fig. 1) splits the samples in three major clusters with some subgroups and one outlier (TDd 3102). Of the three *Dioscorea* species (*D. bulbifera*, *D. tokoro* and *D. japonica*) used as outgroup *D. bulbifera* maintains the closest link to the *D. dumetorum* ingroup accessions. Bootstrap values provided good support (>50%) for the monophyly of each of the groups including the subgroups. Cluster one consists of seven accessions of which four: TDd 3095, TDd 3111, TDd 3100, and TDd 05-6 are from Togo and three: TDd 08-37-9, TDd 08-14-25 and TDd 08-13-13 from Nigeria. The second cluster, which is the most genetically diverse group, is composed of 19 accessions from five out of the six countries. Four of the accessions from Benin, five from Togo, eight from Nigeria and one each from Congo and Ghana were found in the group. The third cluster is composed mostly of Togo accessions (19), with four from Nigeria and only one accession each from Benin, Gabon and Ghana. The PCoA for all accessions using Euclidean similarity indices clearly separates *D. dumetorum* accessions into three groups and shows that more than 50% of the variance among the accessions was expressed by co-ordinates one to four; the first coordinate explaining 27.5% of the total

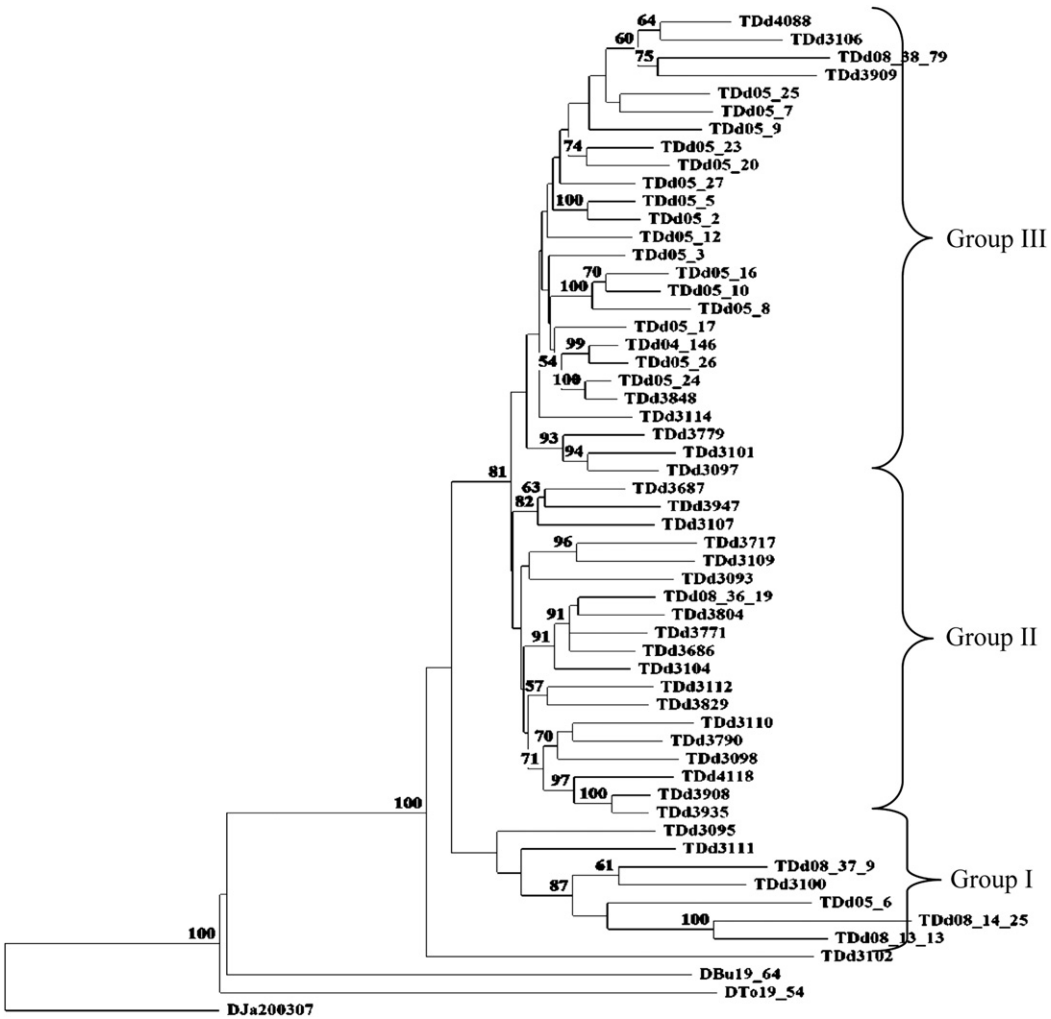

Fig. 1. Neighbour-joining tree relating 53 *D. dumetorum* accessions and three outgroup species: *D. bulbifera* (DBu19_64), *D. tokoro* (DTo19_54) and *D. japonica* (DJa200307). Genetic distances were estimated according to Euclidean coefficient similarity generated from 1052 AFLP markers using twelve primer combinations. All bootstrap values out of 100 replicates are shown at the corresponding forks.

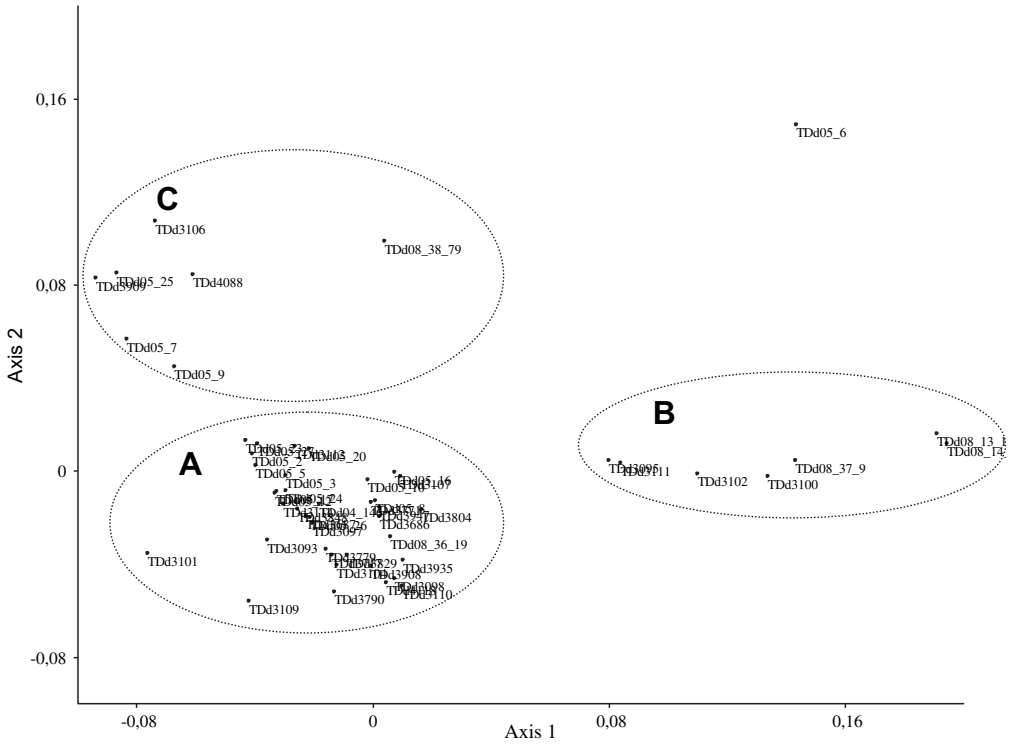

Fig. 2. Principal coordinate scatter plot of 53 *D. dumetorum* accessions revealing three distinct groups: A, B and C.

Table 5
Genetic distances among *D. dumetorum* accessions from three West African countries.

Countries	Benin	Nigeria	Togo
Benin	0.000	0.035	0.033
Nigeria	0.035	0.000	0.016
Togo	0.033	0.016	0.000
Mean distance to other countries	0.034	0.026	0.025

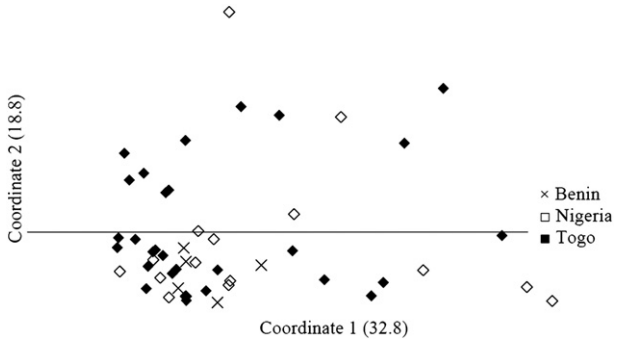

Fig. 3. Principal coordinate analysis based on selection of accessions from three countries.

variation and the second accounting for 12.3% of total variation are shown in Fig. 2. The main groups are A, B, C, with one outlier: TDd 05-6. Group A is composed of 38 accessions from all the six localities while group B is made up of seven accessions (three from Nigeria and four from Togo). The accessions in Group C are from Togo except TDd 08-38-79 which is from Nigeria (Fig. 2). Pairwise distances between countries differ greatly. The shortest distance was between Nigeria and Togo (0.016), whereas the longest distance was between Nigeria and Benin (0.035). Benin has the greatest mean distance to other countries (Table 5). This indicates that differentiation of accessions from Benin contributed most to the between-country

Table 6
Analysis of molecular variance format for the extraction of components of AFLP variation among countries and among individuals within countries.

Source of variation	Df	SSD[a]	MSD[b]	Variance component	% Total[c]	*P* value[d]
Among countries	2	362.1	181.1	3.6	3	<0.001
Individuals within countries	46	6091.1	132.4	132.4	97	
Benin	4	323.4	80.9			
Nigeria	14	1922.9	137.4			
Togo	28	3844.8	137.3			
Total	48	6453.2		136.1		

[a] Sum of squared deviations.
[b] Mean squared deviations.
[c] Percentage of total molecular variance.
[d] Probability level.

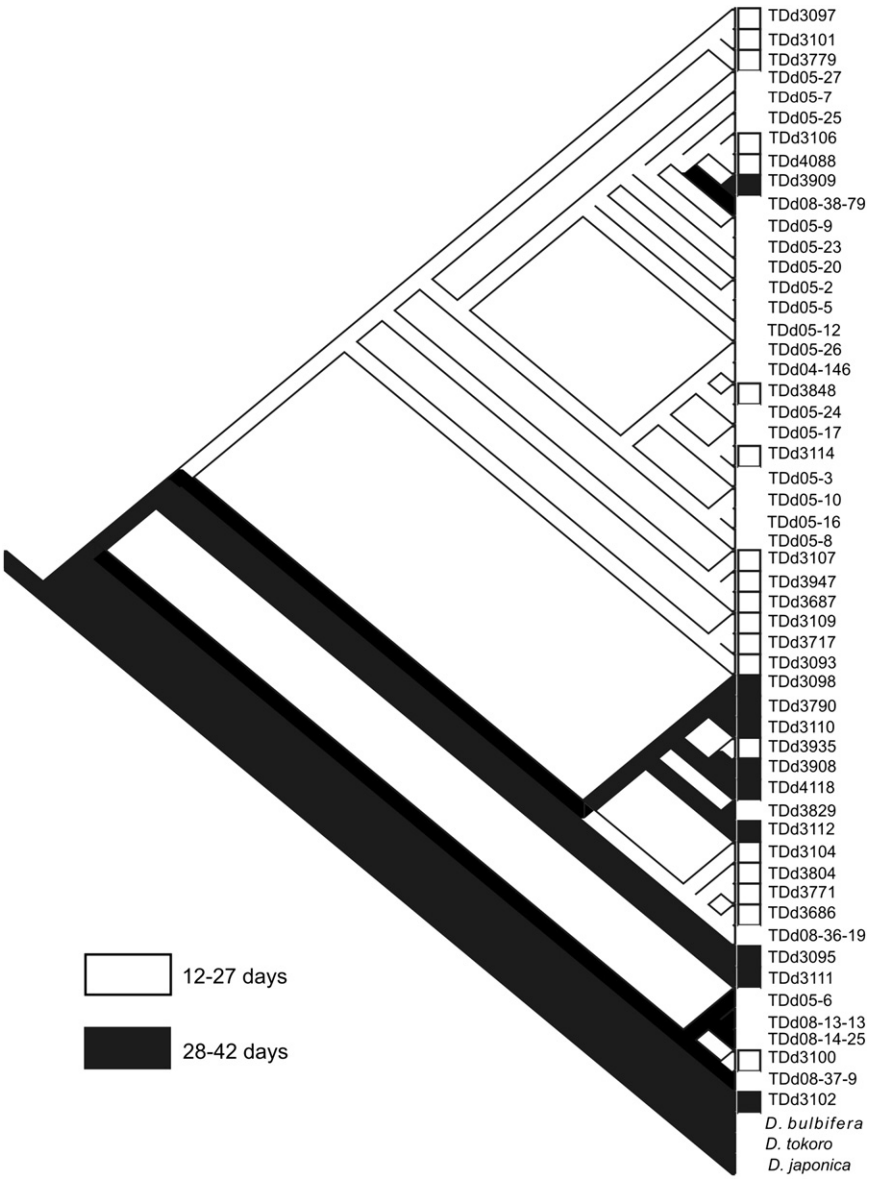

Fig. 4. Days to emergence optimized onto the AFLP tree. Missing data are indicated by the lack of box at the branch tip.

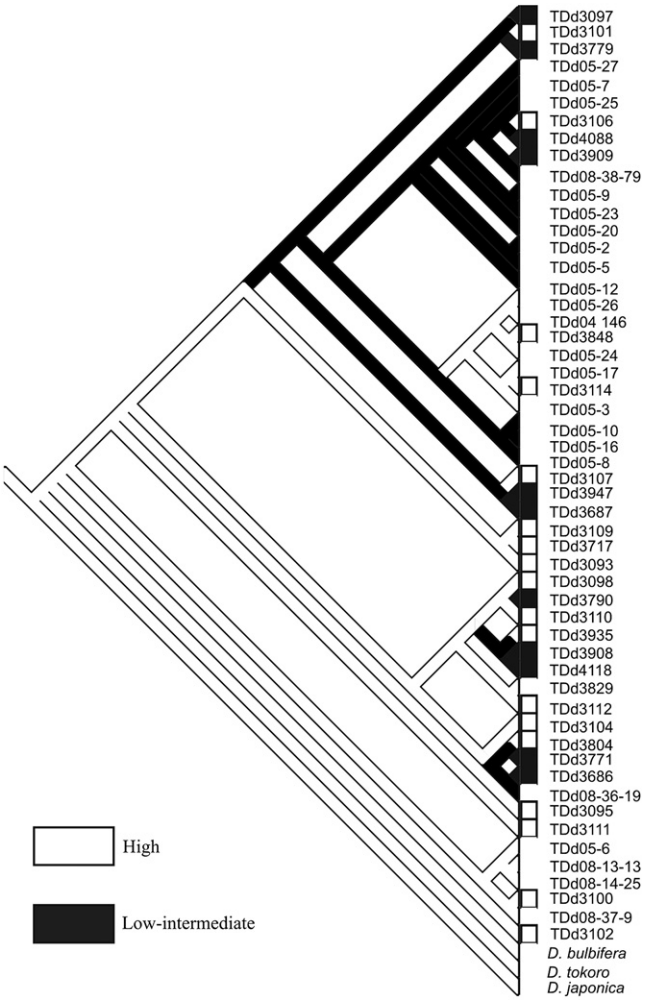

Fig. 5. Vigour of entire plant: high/low-intermediate optimized onto the AFLP tree. Missing data are indicated by the lack of box at the branch tip.

variation. The result of second PCoA (Fig. 3) based on grouping according to the three countries with at least five accessions each, in which 32.8% of total variation was expressed by axis one and 18.8% by axis two presented a similar topology. The populations (i.e. Benin, Nigeria and Togo) are intermingled (Fig. 3). The result of the analysis of molecular variance attributes only 3% of the total genetic variance to variation among populations while 97% was attributed to variation within populations (i.e. countries; Table 6). Of the accessions from the three countries, Benin has the lowest internal diversity (80.9) while Nigeria and Togo share almost the same degree of internal diversity of 137.4 and 137.3 respectively.

The Bayesian analysis of population structure detected three clusters ($P = 1$) similar to the groups revealed by the PCoA with a little difference. Clusters one and three of BAPs correspond to the PCoA clusters A and C consisting of 38 and seven *D. dumetorum* accessions respectively. BAPs cluster two consists of the same accessions found in the PCoA cluster B with the exception of TDd 05-6 that is present in BAPs cluster two, which is an outlier in the PCoA. The individuals from Benin, Congo, Gabon and Ghana were more or less scattered among others from Togo and Nigeria in the first cluster of 38 individuals. The second cluster contains five accessions from Togo and three from Nigeria whereas the third cluster is made up of five accessions from Togo with only two from Nigeria (Figure not shown). The optimization of 35 different morphological characters of leaf, tuber and corm of *D. dumetorum* accessions on the tree generated with the AFLP data revealed that there is little or no congruency between the AFLP data and the morphological characters. Four of the trees are presented in Figs. 4–7. Late emergence of leaves after planting (28–42 days, Fig. 4), high vigour of the entire plant (Fig. 5), coloured spot at the base of spine (Fig. 6) and tuber branching at the lower third part (Fig. 7) are shown to be ancestral characters that are still retained by some of the *D. dumetorum* accessions till the present time.

D. dumetorum has suffered neglect in the recent past with regard to research in the area of cultivar identification and genetic diversity whereas other species of *Dioscorea* have received much more adequate attention in studies ranging from domestication, isozyme pattern to genetic diversity using different molecular markers (Hamon and Touré, 1990; Dansi et al., 2000; Mignouna et al., 2002a, b; Mignouna and Dansi, 2003; Egesi et al., 2006; Silvia and Gustavo, 2006). A possible reason for

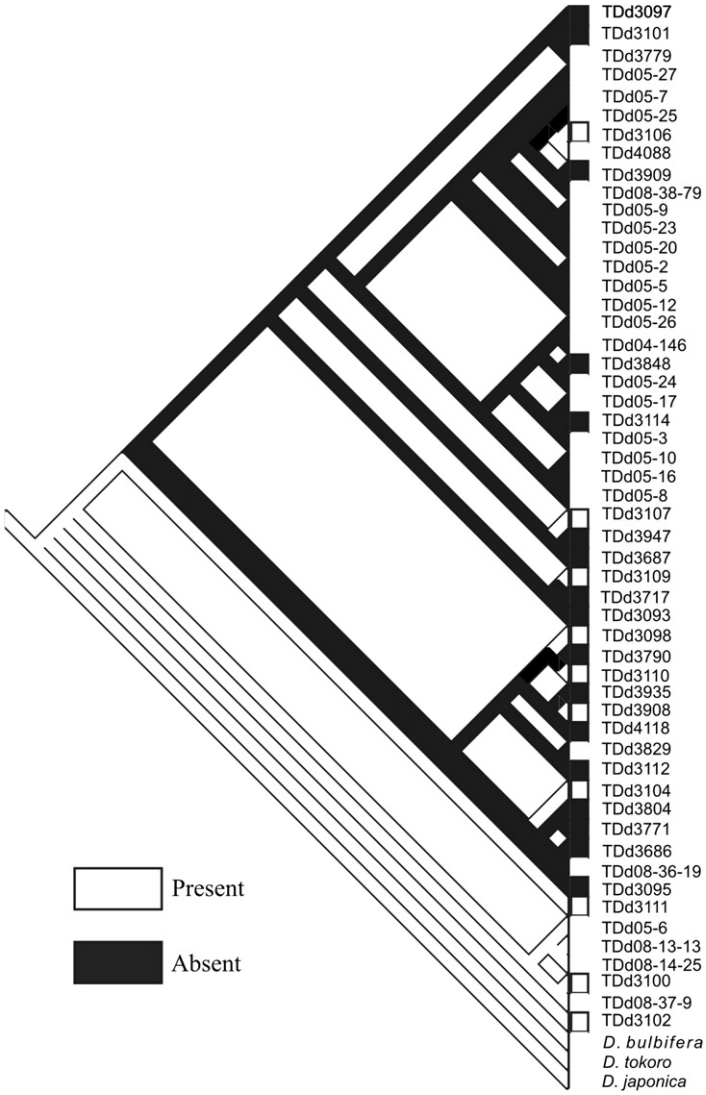

Fig. 6. Coloured spot at the base of spine optimized onto the AFLP tree. Missing data are indicated by lack of box at the branch tip.

this neglect might be the background bitter taste of the species that gave it the name 'bitter yam' or the tuber hardening after harvest (Afoakwa and Sefa-Dedeh, 2001). Most people, especially, the younger generation may not desire the bitter yam as a regular meal. This might be the reason why *D. dumetorum* is considered to be a plant that is consumed only during periods of serious food shortages. However, in spite of the lack of popularity of the bitter yam, it has been reported to possess more nutritive value than other well researched yam species such as *D. alata*, *D. cayenensis/D. rotundata* complex, *Dioscorea esculenta*, etc (Baquar and Oke, 1976; Mbome Lape and Treche, 1994). It has also been shown that the starch grains of *D. dumetorum* are smaller, more soluble and hence more digestible than those of other yam species (Szylit et al., 1977; Trèche and Guion, 1980; Amani et al., 2004; Sahore and Amani, 2007). *D. dumetorum* is also considered to be less demanding of manpower in its cultivation than *D. alata*, and *D. cayenensis*. In addition, the medicinal potency of *D. dumetorum* is another reason why this species deserves greater attention from biologists who work on plant genetics and crop evolution. Our data provide evidence to the unstructured genetic diversity among the 53 accessions of *D. dumetorum* from six West and Central African countries studied using the AFLP markers and the cpDNA. Sequence data from the *psb*A-*trn*H and *rpo*B-*trn*C spacers revealed no genetic variation in accessions that differ markedly in their AFLP pattern. For the *psb*A-*trn*H spacer, no polymorphisms were apparent among accessions from Benin, Nigeria or Togo. TDd 3829 (from Benin), TDd 3112 (from Nigeria), TDd 3097, TDd 05-20 (from Togo) produced DNA sequences of 335bp in length each with no evidence of a substitution or mutation in the length. Similar results were obtained from the *rpo*B-*trn*C spacer which produced DNA sequences of 459bp from the same accessions. In contrast, analyses of the AFLP data set of 1052 fragments illustrate a considerable level of variability in the genetic pool of the West and Central African *D. dumetorum* germplasm. The detection of 94.1% (average) polymorphism by the twelve AFLP primer combinations used in this study is an indication of the high quality marker profiling

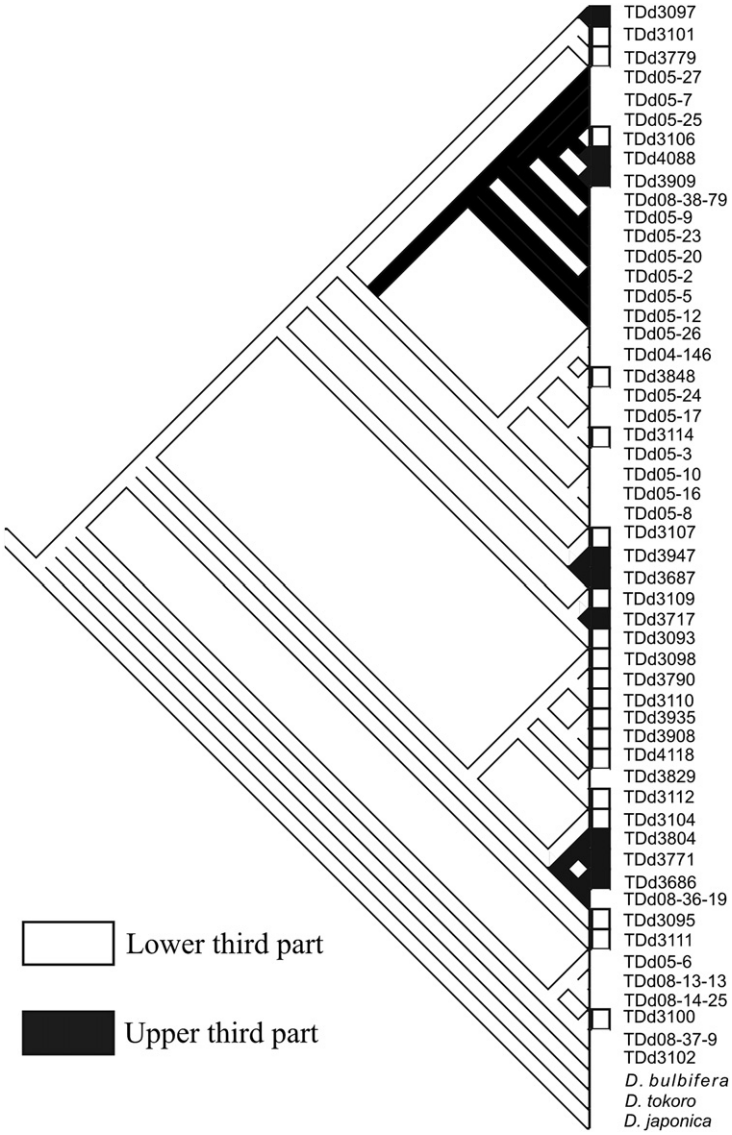

Fig. 7. Place where tuber branched optimized onto the AFLP tree. Missing data are indicated by lack of box at the branch tip.

obtained for the 53 accessions of *D. dumetorum* from West and Central Africa. This level of polymorphism is comparable to other AFLP diversity studies in several crop plants such as wheat (Barret and Kidwell, 1998), *Musa* sp. (Ude et al., 2002b), *Irvingia gabonensis* (Ude et al., 2006), *Sorghum* sp. (Wu et al., 2006), *Jatropha curcas* (Tatikonda et al., 2009) and in some species of *Dioscorea* (Malapa et al., 2005; Egesi et al., 2006; Li and Li, 2007; Tamiru et al., 2007). The generation of highest number of fragment, private fragments (fpr) as well as the highest percentage of polymorphic loci from the accessions from Togo compared to those from other countries is noteworthy. It is important to note that unique fragments detected from these accessions can be used to develop sequence tagged site markers that may be useful for the purpose of cultivar identification (Fernandez et al., 2002; Tatikonda et al., 2009). The Neighbour-joining (NJ) tree as expected, clearly separates *D. dumetorum* accessions from *D. bulbifera*, *D. tokoro* and *D. japonica*, which were used as outgroup species. The branch lengths demonstrate significant evolutionary divergence between the ingroup and outgroup belonging to different *Dioscorea* sections. However, *Dioscorea bubifera* belonging to the section *Opsophyton*, appeared to maintain the closest link to *D. dumetorum* accessions. This observation could be explained by the fact that *D. bulbifera* is present in both Asia and Africa, thus having possibly a common ancestry with *D. dumetorum* in Africa. A good observation could have been the relationship between *D. dumetorum* and *Dioscorea hispida* which are both of the section *Lasiophyton*, but this species was not analyzed. Relationships inferred from the AFLP analysis using both NJ, principal coordinate (PCo) analyses suggest several clusters, which do not show geographical correspondence. This observation is further strengthened by the results obtained from the analysis of molecular variance (AMOVA), in which the variance component among the countries was only 3% of the total variance, whereas much variability

was accounted for by the individual accessions within the countries (97%). The most likely explanation for this observation would be a high level of natural or human-mitigated migration, providing an opportunity for gene flow and interbreeding. Accessions from Nigeria clustered with ones from Togo in most cases while other accessions from Benin, Congo, Gabon and Ghana formed clusters that were interspersed between these two groups. This level of association within the gene pool of Nigeria and Togo *D. dumetorum* accessions which is also revealed by the two countries having internal diversities of 137.4 and 137.3, respectively, could be the result of interaction among farmers from the countries (e.g. Togolese migrant farmers in western Nigeria), encouraging a great exchange and introduction of the bitter yam germplasm through trade and agriculture. There is therefore an indication that the introduction of the *D. dumetorum* germplasm to other West and Central African countries has been much affected by the activities of farmers from Nigeria and Togo. The three genetic groups defined in our study consist of accessions from two or more localities of sampling. This finding, which is in line with that of Tamiru et al. (2007) on the genetic diversity in yam germplasm from Ethiopia, probably indicates that there are no effective geographical barriers in the West and Central African countries with regard to gene flow among the accessions of *D. dumetorum*. An additional suggestion would be that gene flow is frequent and recent. Dispersal of the trifoliate yam must have been greatly influenced by commercial activities among the six West and Central African countries. Most important, is the dispersal among local farmers that are engaged in the cultivation of this species of *Dioscorea*. West Africa is the centre of origin and diversity of *D. dumetorum* as well as other species which include *D. rotundata*/*D. cayenensis* complex, *D. bulbifera* (Asiedu et al., 1997). Some kind of semi-wild yam species domestication going on in West Africa has been said to support tremendous genetic variability in yam contrary to the situation in other crops where the deployment of improved varieties has led to loss of diversity and a narrowing of the genetic base (Mignouna and Dansi, 2003). Because fewer than five accessions each were analyzed from Congo, Gabon and Ghana, only a limited deduction can be made concerning the genetic variability within this sub-region. The only accession from Congo TDd 3717 formed a monophyletic group with TDd 109 from Ghana with a strong support (bootstrap value: 96%), indicating that this accession might have been introduced to Congo directly from Ghana. Accession TDd 3114 from Gabon, although present in group II consisting of mainly accessions from Togo, is only weakly related (bootstrap value: less than 50%) to the group hence appearing to be more or less isolated.

Deductions that could be made from the morphological character optimization on the tree generated with AFLP fragments are that: late emergence, high vigour of the entire plant, a coloured spot at the base of the spines, and tuber branching at the lower third part appeared to be ancestral characters in the common ancestors of all *D. dumetorum* taken in cultivation. These characters and others such as early onset of leafing, tuber skin colour and high branching have been part of desirable traits of choice by farmers for the cultivation of *D. dumetorum*. Most of the Nigerian accessions are characterized by low- intermediate vigour, whereas accessions from Togo have high vigour. This might have resulted in preference for accessions from this country for cultivation purposes. It is therefore not surprising, that the *D. dumetorum* genotype collection at the IITA contains predominantly accessions from Togo. Twining direction in certain accessions was found to be variable rather than strictly in the usual clockwise direction, i.e. climbing to the left. Accessions with the counter-clockwise twining habit are mostly from Benin. This may be part of the reason why accessions from Benin have the greatest mean distance to other countries in the PCo analysis, thus contributing most to the between-country variation. It might also have affected the choice of accessions from Benin for cultivation.

The detection of highest genetic diversity in accessions from Nigeria and Togo and their relationship to other accessions suggest that these countries are the centre of origin and diversity of *D. dumetorum*. This possibility is in agreement with the previous long speculations of West Africa as the origin and centre of diversity of *D. dumetorum* (Coursey, 1967; Okonkwo, 1985; Onwueme, 1978).

Our study shows the utility of AFLP markers to reveal polymorphisms in *D. dumetorum*. The relatively high level of genetic variation displayed by these accessions could be responsible for the advantage in adaptation to different local environmental conditions. The AFLP polymorphisms proved that the genetic diversity observed in the accessions of *D. dumetorum* seemed to be unstructured and suggested a rapid population expansion. The study has shown intraspecific variations in the accessions of *D. dumetorum* from six West and central African countries with a possible suggestion of an on-going gene flow among the accessions. The lack of correlation between genetic diversity observed among accessions and collection sites by the AFLP fingerprinting is attributable to biological dispersal and or to human activities leading to exchange of germplasm among farmers across the borders of the six countries.

Acknowledgements

The authors are grateful to M. Kever for practical support, E. Westberg, C. Uhink, G. Schorr, P. Teege and R. Scalone for some procedures in the data analyses. We would like to also thank A. Sartie, R. Adeleke and D. Dumet from the IITA for field data on the *D. dumetorum* accessions. The Alexander von Humboldt Foundation is gratefully acknowledged for the George Forster research fellowship granted to MAS to carry out this research at the Institut für Spezielle Botanik, Johannes Gutenberg-Universtät, Mainz.

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