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GENETIC POLYMORPHISM OF CASSAVA WITHIN THE REPUBLIC OF BENIN DETECTED WITH RAPD MARKERS

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ABSTRACT

A knowledge of the extent of genetic variation in a crop is a prerequisite for its improvement. In cassava (*Manihot esculenta* Crantz) such knowledge is lacking especially at the DNA level. The genetic variation among 28 cassava varieties collected from different locations in the Republic of Benin was investigated using random amplified polymorphic DNA (RAPD). A set of 10 random sequence 10-mer primers out of 100 that were screened detected polymorphisms and generated 78 amplified DNA bands. Based on an unweighted pair group method with arithmetic averages (UPGMA) cluster analysis, the accessions were categorised into 6 groups. The results, summarised in a dendrogram, show the genetic diversity among this cassava germplasm.

***Key Words*: Cultivar identification, genetic diversity, *Manihot esculenta*, RAPDs**

RÉSUMÉ

La connaissance de l'importance de variation génétique chez une culture est nécessaire pour son amélioration. Chez le manioc (*Manihot esculenta* Crantz), une telle connaissance manque surtout au niveau du DNA. La variation génétique parmi les 28 variétés du manioc recueillies de différentes localités du Benin était étudiée en utilisant un polymorphe amplifié sélectif du DNA (RAPD). Un ensemble de 10 amorces de 10-mer d'une sélection séquentielle sur 100 qui avaient été depistés ont detecté le polymorphisme et ont généré 78 bandes de DNA amplifié. Sur la base de l'analyse de faisceau de la méthode d'un groupe de paire non-pondéré avec les moyennes arithmétiques (UPGMA), les variétés étaient divisées en six groupes. Les résultats, résumés en dendogramme, montrent la variation génétique chez les différents types du plasme germinatif du manioc.

***Mots Clés*: Identification variétale, variation génétique, *Manihot esculenta*, RAPDs**

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major carbohydrate food for millions of people worldwide, and one of the most important crops in the tropics. In tropical Africa, cassava is cultivated mainly for its storage root which is the single-most important source of calories in the diet (Cock, 1982; Hahn, 1989). Efforts are being made to develop cassava varieties with high and stable yield across different agro-ecologies. Success in these efforts depends on the availability

of an extensive level of genetic variability. Some amount of variation necessary for a genetic improvement programme of cassava cultivars already exist within the several germplasm collections from different parts of Africa (Beeching *et al.*, 1993). However, there is no adequate information on the extent of genetic variation available in this crop.

Genetic variation studies using isozyme analysis has been attempted in cassava (Hussain *et al.*, 1987; Ramirez *et al.*, 1987; Lefevre and Charrier, 1993), but the results showed low level of polymorphism. Besides, isozyme patterns were found to be influenced by stage of plant development as well as the part of the plant from which sample was taken. There are other molecular techniques that can detect variability within and among several crop species. Two of these widely used techniques are restriction fragment length polymorphisms (RFLPs) (Helentjaris *et al.*, 1986) and specific amplification of polymorphic DNA fragments by PCR (Weining and Langridge, 1991). Using specifically amplified cassava cDNA as probes, Beeching *et al.* (1993) detected RFLP in *Manihot* germplasm, assembled from different parts of the world. Their study revealed significant levels of polymorphism both within and between the species. Bertram and Schaal (1993) have also used RFLP to study the phylogeny of *Manihot* and the evolution of cassava. This technique, unlike isozyme analysis, detects more polymorphisms and has the advantage of being consistent between tissues. Nevertheless, the intense labour and time inputs (Kazan *et al.*, 1993) and the requirements of useful probes most times render RFLP analysis cumbersome.

The polymerase chain reaction (PCR) allows for the detection of random amplified polymorphic DNAs (RAPDs) using arbitrarily designed short primers to amplify DNA (Welsh and McCleland, 1990, 1991; Williams *et al.*, 1990). RAPD requires no previous sequence information or probe identity for the fingerprinting of cultivars. Moreover, the DNA fragment electrophoretic patterns generated are able to discriminate between interspecific and intraspecific variation in several plant species (Caetano-Anolles *et al.*, 1991; Hu and Quiros, 1991; Hadrys *et al.*, 1992). Marmey *et al.* (1994) have successfully detected RAPDs using 8 primers, among 3 species of *Manihot* and 19 cultivars of *M. esculenta*. However, these cultivars and species were assembled from many countries in Africa, India and South America.

Our aim was to determine whether RAPDs are useful for cultivar discrimination and identification in cassava populations collected from a small geographical region. In this study, PCR-RAPD was evaluated for its ability to detect polymorphisms within 28 germplasm accessions collected from the Republic of Benin in West Africa.

MATERIALS AND METHODS

Plant material. The cassava clones used in this study were collected from farmers' fields in at least 11 locations in the Republic of Benin (Fig. 1, Table 1). Most of the accessions came from the southern part of the country (between latitudes 6°N and 9°N) where cassava is mostly cultivated. The plants were grown from stem cuttings and maintained in the screenhouse of the Germplasm Resources Unit at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. DNA was extracted from plants grown in pots placed in the screenhouse.

DNA isolation. The procedure used for total genomic DNA extraction was that of Saghai-Maroof *et al.* (1984) with some modifications. Young leaf tissue from single plants of each accession was ground to a fine powder in liquid nitrogen. Approximately 100 mg of the powdered tissue was suspended in 600 µl of 2 x CTAB buffer (50 mM Tris, pH 8.0; 0.7 M NaCl; 10 mM EDTA; 1% hexadecyltrimethylammonium bromide; 0.1% 2-mercaptoethanol) and incubated at 55°C for 30 min. DNA was purified by two extractions with chloroform:isoamyl alcohol (24:1) and precipitated with ice-cold absolute ethanol. After washing with 70% ethanol, the DNA was dried and resuspended in 100 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA concentration was measured using a DU-65 UV spectrophotometer (Beckman Instruments Inc., Fullerton CA, USA) at 260 nm. To check for degradation of the DNA, the samples were loaded

onto a 1.0% agarose gel in 0.5 x TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) and electrophoresed.

PCR and electrophoresis. DNA primers tested in this study were purchased from Operon Technologies (Alameda, CA ,USA) and each was 10 nucleotides long. Two concentrations of each template DNA (24 ng and 96 ng per reaction) were used to test reproducibility and eliminate sporadic amplification products from the analysis. Amplifications were performed in 12 µl reaction mixture consisting of genomic DNA, 1 x reaction buffer (Promega Corporation, Madison WI, USA), 100 mM each of dATP, dCTP, dGTP and dTTP, 0.2 µM primer, 2.5 µM $MgCl_2$ and 1 U of *Taq* polymerase (Boehringer Mannheim GmbH, Germany). A single primer was used in each reaction. The reaction mixture was overlaid with 50 µl of mineral oil to prevent evaporation. Amplification was performed in a thermowell microtiter plate using a PTC-100^a programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA). The cycling programme involved an initial denaturation at 94°C for 3 minutes and followed by 40 cycles of 94°C for 1 minute, 35°C for 1 minute and 72°C for 2 minutes; and a final extension at 72°C for 10 minutes. Amplification products were maintained at 4°C until electrophoresed.

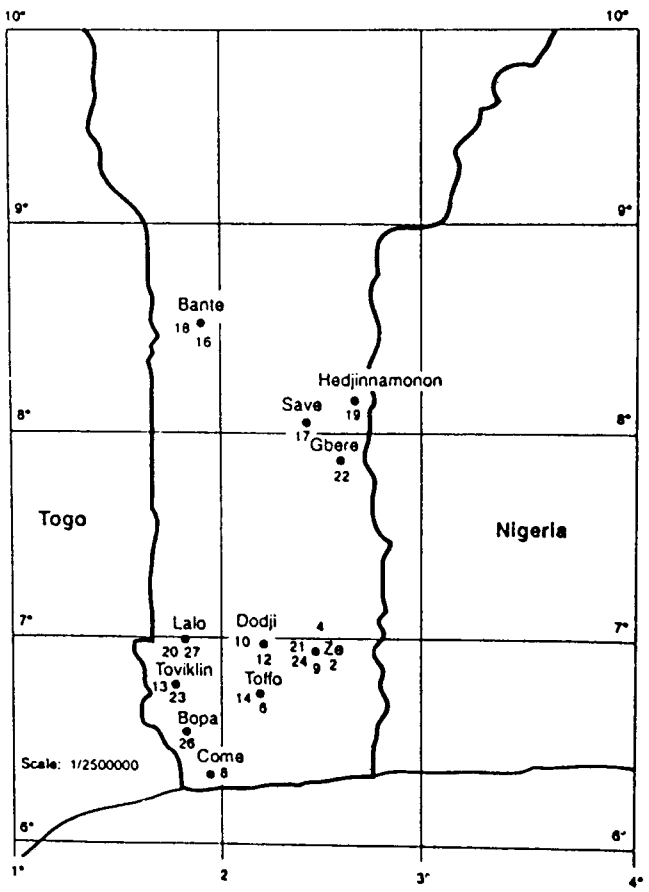


Figure 1. Sketch map of Benin Republic showing areas of cassava collection. The code numbers near each village name on the map correspond to the Labaratory numbers for the cassava accessions in Table 1.

The reaction products were resolved by electrophoresis in a 1.4 % agarose gel in 0.5 x TBE buffer at 150 V for 2.5 hours. A 1-kb ladder (Life Technologies, Gaithersburg, MD, USA) was included as size marker. Gels were visualised by staining with ethidium bromide solution (0.5 $\mu g\ ml^{-1}$) and banding patterns were photographed over UV light using a red filter.

Data analysis. RAPD markers that were consistently reproduced in at least two replicate PCR reactions were further analysed. Fragment sizes of RAPD were estimated from the gel photograph by comparison with the 1-kb ladder marker. From the whole set of reproducible RAPD bands generated in the 28 cassava clones by the different primers, bands were scored as present (1) or absent (0). A data matrix with fragments in columns and accessions in lines was assembled from these scores. Only the polymorphic markers were included in the data set.

Arbitrarily primed PCR characters were analysed using Genstat 5 Release 3.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K.). A similarity/distance matrix was developed using Jaccard (1901) similarity coefficient (Sneath and Sokal, 1973), which does not consider the joint absence of a marker as an indication of similarity. The similarity index of Jaccard between plants i and j is given by:

$$Sij = a/(a+b+c)$$

where a is the number of characters present in both i and j, b is the number of characters present in i but not in j, c the number of characters present in j but not in i. A distance matrix index was calculated from the dissimilarity as follows; (*Dij*

TABLE 1. List of 28 cassava accessions collected from different locations in Benin Republic for evaluation by PCR-RAPD analysis

Lab. No.	IITA accession No.	Collection code	Cultivar name	Village
1	TMe 2020	_	B'Hombete	_
2	TMe 2059	RB92/0199	Kalaba	Ze
3	TMe 2001	344	_	_
4	TMe 1911	RM92/01180	Dossimoire	Ze
5	TMe 2034	_	Meta A	_
6	TMe 1951	RM92/0173	_	Toffo
7	TMe 1879	1494	_	_
8	TMe 2052	RM92/0064	Youovi	Come
9	TMe 2060	RB92/0153	Fléké	Ze
10	TMe 1938	RB92/0126	Miklomidè	Dodji
11	TMe 2038	310	_	_
12	TMe 1885	RB92/0139	Feguse	Azanlinghame, Dodji
13	TMe 1906	RB92/0115	Todoukon	Toviklin
14	TMe 1900	RB92/0175	Hombete	Ahigondo, Toffo
15	TMe 1876	1284	_	_
16	TMe 1921	RB92/0004	Onichoutin	Bante centre
17	TMe 1918	RB92/0043	Wolodo	Save
18	TMe 1972	RB92/0012	Gbaruraye	Gotcha, Bante
19	TMe 1922	RB92/0059	Lohopké	Hedjinnamonon
20	TMe 2056	RB92/0111	Mamoe	Ahodjen, Lalo
21	TMe 1962	RB92/0162	Glokjia	Ze
22	TMe 1923	RB92/0051	Olobekpe-saint	Gbere
23	TMe 2066	RB92/0118	Firite-nonte	Toviklin
24	TMe 1937	RB92/0192	Samynom	Ze
25	TMe 1862	1147	_	_
26	TMe 1956	RB92/0084	Siton	Hangnanme
27	TMe 2055	RB92/0104	Kataoli	Kpoha Dogbo
28	TMe 1868	1169	_	_

= 1 - *Sij*). Clustering was done using the unweighted pair group method with arithmetic averages (UPGMA) algorithm (Sneath and Sokal, 1973).

RESULTS

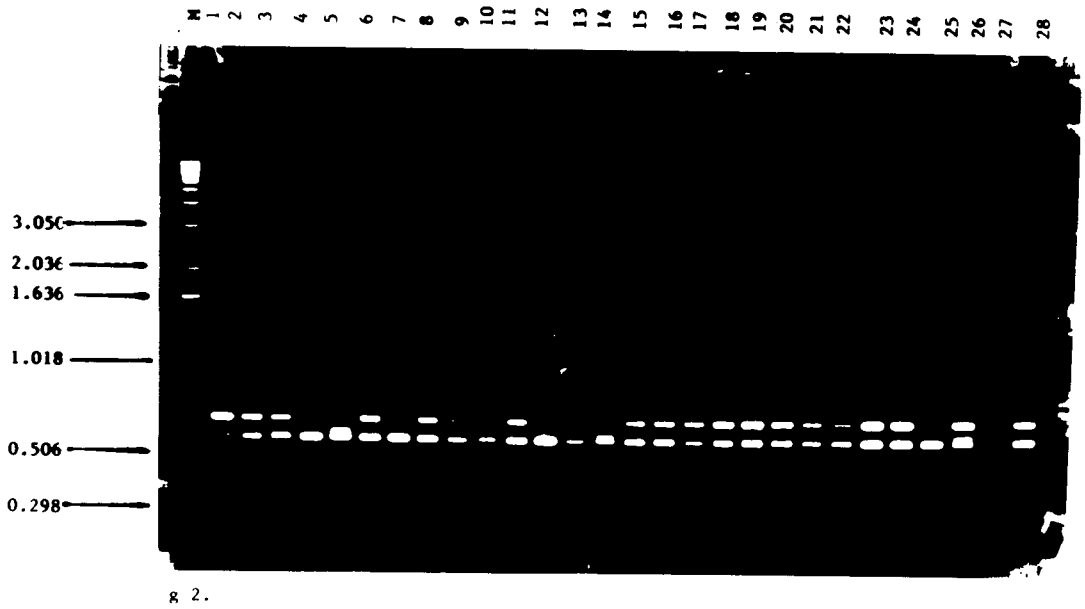
One hundred random oligonucleotide primers (Operon Technologies sets A, B, D, E and O) were evaluated for their ability to prime PCR amplification of cassava genomic DNA. In a preliminary survey, genomic DNA of two of the accessions, TMe 1900 and TMe 1921 collected from Ahingodo, Toffo and Bante, respectively (see Fig. 1), were used as templates. Eighty one of the primers did not amplify the two DNA templates or resulted in only limited amplifications that were visualised on gels as faint bands or smears. The remaining 19 primers were able to amplify the genomic DNA, giving reproducible RAPD amplification patterns with individual fragments that stained intensely. Ten of these primers (Table 2) were chosen for the whole experiment and used to amplify the DNA of the various accessions. Examples of typical PCR amplification products are shown in Figure 2.

The 10 informative oligonucleotide primers detected polymorphisms among the 28 cassava accessions. The primers revealed a total of 78 clear and easily scorable bands. The number of bands per primer ranged from 6 to 12. The size of DNA bands that were produced in the PCR reactions ranged from 300 to 2100 bp, but most of the bands were between 500 and 1000 bp. None of the primers was specific for any of the accessions. However, primers OPE-03 and OPE-04 produced a 480 bp and 1200 bp bands, respectively, in TMe 1862 and TMe 2052 only. Also a 920 bp band was generated in TMe 1876 and TMe 2052 only by primer OPF-01, while a 900 bp band generated by OPO-20 was specific only to TMe 1918 and TMe 1921. Examples of polymorphic bands used for discrimination among the accessions are shown in Figure 2. Only twenty five (32%) of the 78 bands were polymorphic among the cassava accessions. Data from the scores were subjected to UPGMA analysis. Jaccard similarity coefficient ranged from 100% for the most closely related accessions to approximately 40% for those most distantly related, with a mean of 69%.

With our PCR conditions, TMe 1876 showed the highest polymorphic bands while TME 1906 showed the least. None of the primers could provide enough markers to discriminate between all the cassava populations. Identical RAPD patterns (100% Jaccard similarity coefficient) using the ten primers were observed for four pairs of the accessions; TMe 1900 and TMe 2055, TMe 1918 and TMe 1921, TMe 1972 and TMe 2056, and TMe 2059 and TMe 2066. Also, TMe 1937 and TMe 1951 as well as TMe 1906 and TMe 1911 has 95% and 94% similarity coefficients, respectively. The similarities between accession TMe 2052 and accessions TMe 1906, TMe 1900 and TMe 2001 were 40%, 42% and 42%, respectively. Based on the average cluster analysis, the accessions formed 6 groups at the 70% Jaccard similarity coefficient level (Fig. 3).

TABLE 2. Oligonucleotide primers that showed genetic discrimination among the cassava accessions using the PCR-RAPD analysis and number of polymorphic bands among total fragments generated

Operon code	Nucleotide sequence (5' to 3')	No. of fragments amplified	No. of polymorphic bands
OPA-12	TCGGCGATAG	12	2
OPD-08	GTGTGCCCCA	8	1
OPD-18	GAGAGCCAAC	9	3
OPE-03	CCAGATGCAC	6	4
OPE-04	GTGACATGCC	7	2
OPF-01	ACGGATCCTG	8	3
OPF-10	GGAAGCTTGG	6	1
OPF-13	GGCTGCAGAA	9	3
OPO-10	TCAGAGCGCC	7	2
OPO-20	ACACACGCTG	6	4
		78	25



g 2.

Figure 2. RAPD electrophoretic profiles of 28 accessions of *Manihot esculenta* genomic DNA amplified with primer OPE-03 (Table 2). Lane M: 1-kb DNA size marker; 1: TMe 2020; 2: TMe 2059, 3: TMe 2001; 4: TMe 1911; 5: TMe 2034; 6; TMe 1951; 7: TMe 1879; 8: TMe 2052; 9: TMe 2060; 10: TMe 1938; 11: TMe 2038; 12: TMe 1885; 13: TMe 1906; 14: TMe 1900; 15: TMe 1876; 16: TMe 1921; 17: TMe 1918; 18: TMe 1972; 19: TMe 1922; 20: TMe 2056; 21: TMe 1962; 22: TMe 1923; 23: TMe 2066; 24: TMe 1937; 25: TMe 1862; 26: TMe 1956; 27: TMe 2055; 28: TMe 1868.

50%

Figure 3. Dendrogra
accessions collected
analysed by an unwe

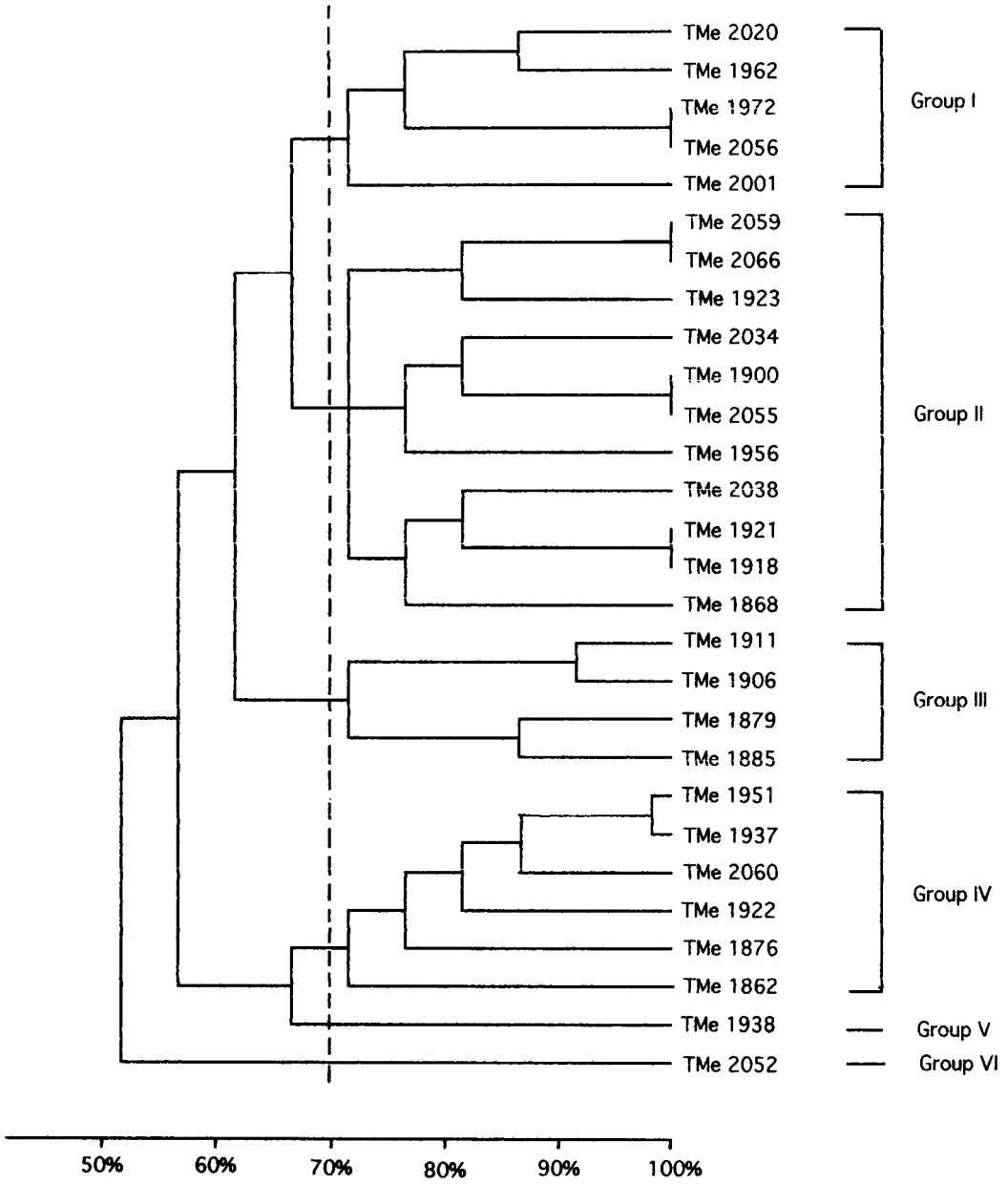


Figure 3. Dendrogram of percent similarities (axis below dendrogram) among the genomes of the 28 cassava accessions collected in the Republic of Benin in pairwise comparisons based on the Jaccard similarity index and analysed by an unweighted pair group method with arithmetic averages (UPGMA).

DISCUSSION

PCR-RAPD technique provided means for genetic analysis of cassava cultivars collected from farmers' fields in a small geographical region (Republic of Benin, 116,347 km^2). This methodology was developed to identify *Manihot* species and 19 cassava cultivars assembled from many countries in Africa, India and South America (Marmey *et al.*, 1994). The use of RAPD analysis also revealed extensive polymorphism in other species (Carlson *et al.*, 1991; Hu & Quiros, 1991; Welsh *et al.*, 1991; Reiter *et al.*, 1992; Wolff & Rijn, 1993), showing that RAPD technique is able to detect diversity at the intraspecific level.

Using the RAPD patterns generated by the 10 random primers with G+C content ranging from 60 to 70%, it was possible to detect genomic polymorphisms in *Manihot esculenta*. Only a combination of RAPD patterns allowed the unambiguous characterisation of the cassava accessions. Although the cluster analysis assigned the accessions into 6 groups, none of these, except TMe 2052, were classified according to geographical origin. TMe 2052, the only accession collected from Come (Fig. 1) differed considerably from all the others, having only 40 and 42% similarities with TMe 1900 and TMe 2001, respectively. Similarly, the dendrogram generated by Marmey *et al.* (1994) using 8 primers and 19 cassava accessions did not provide a strong group structure based on geographical origin. Nevertheless, their study indicated genetic diversity within the population studied with only 2 accession pairs having zero genetic distances.

Moreover, the accession pairs having 100% Jaccard similarity coefficients [TMe 2059 (Ze) and TMe 2066 (Toviklin); TMe 1918 (Save) and TMe 1921 (Bante); TMe 1900 (Toffo) and TMe 2055 (Lalo); and TMe 1972 (Bante) and TMe 2056 (Lalo)] were also found to have originated from different locations as indicated after the accession numbers. Although they were collected from relatively distant regions (Fig. 1), our RAPD analysis indicated that these accessions were closely related based on their similarity coefficients. This situation is not unusual (Beeching *et al.*, 1993) particularly for an introduced crop such as cassava, which is predominantly vegetatively propagated. The basic similarities also observed among the cassava accessions used in this study was also not unexpected due to the small geographical region from which the plants were collected.

Cultivar identification is an important issue for breeders and germplasm managers. The application of RAPDs seems very valuable in this regard. Using RAPD to determine the introgression of desirable traits such as disease resistance should therefore allow breeders to select related or unrelated parental germplasm material to maximize variability in their breeding programs as has been pointed out by similar studies in cassava (Chàvez, 1990; Beeching *et al.*, 1993; Marmey *et al.*, 1994). There are over 2000 accessions of cassava germplasm currently maintained at IITA. In collaboration with national scientists and agricultural authorities in Africa, the Institute intends to continue to assemble the germplasm collection in Africa. The methodolgy of PCR-RAPD established in this study with additional assessment of other primers and techniques will be intensified for the investigation of diversity and detection of duplicate germplasm samples. This will enhance the efficiency of germplasm management and the use of cassava germplasm. A more extensive sampling of populations from different parts of tropical Africa would definitely reveal a greater genetic variability.

In summary, this study reveals the genetic diversity of cassava accessions collected fron the southern part of the Republic of Benin. We suggest that RAPD, or other appropriate genetic markers, be used whenever possible in evaluating collections of cassava cultivars.

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