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Identification of some Benin Republic's Guinea yam (*Dioscorea cayenensis/Dioscorea rotundata* complex) cultivars using Randomly Amplified Polymorphic DNA

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Abstract

DNA from twenty-three late maturing cultivars of Guinea yams (*D. cayenensis/D. rotundata* complex) from the Benin Republic that could not be separated using isozyme markers, were examined using randomly amplified polymorphic DNA (RAPD) markers with decamer primers of arbitrary sequence. All the twelve primers tested were informative and yielded 63 amplified DNA bands from which 47 (75%) were polymorphic. Although no single primer produced polymorphic bands in all cultivars, the great majority of the cultivars were separated with the combinations of polymorphic bands generated by various primers. Putative duplicates and cultivar misclassifications were identified. Many morphologically distinct cultivars were close. The dwarf cultivar Tam-Sam considered as derived from Tabane, appeared more distant from the latter than was believed. RAPD analysis was found as a practical tool for the identification of duplicates toward establishment of an accurate core collection of Guinea yams in Benin Republic and in the other countries of the African yam belt.

Introduction

In the context of the growing world population, cereals and pulses may not by themselves meet the food requirements in the future. In many of the developing tropical and subtropical countries, root and tuber crops have already been recognised as secondary staples to tide over food shortages.

Cultivated Guinea yam is one of the most important food crops, especially in the so-called 'yam belt' of West Africa. Because of its contribution to food security, yam has become a target for breeding new varieties with novel or improved characteristics. However, one of the prerequisites to this task is the characterisation of the existing traditional cultivars held by the farmers.

Morphological and isozyme markers have been recently used to characterise and to classify the different yam cultivars belonging to the species complex *Dioscorea cayenensis / D. rotundata* in Benin Republic (Dansi et al., 1998, 1999, 2000). Although interesting results were obtained by combining both methods, additional clarification and identification is required. In fact, no isozyme markers were found to separate the eleven cultivars (morphologically different) assigned to the cultivar group named TABANE (Dansi et al., 1998, 1999) as they are all electrophoretically identical (Dansi et al., 2000). Moreover, while farmers consider some of the eleven morphologically identical late maturing cultivars classified in the morphotype **Kinkerekou** (Dansi et al., 1999) as different (based on their cooking qualities and agronomics traits), no isozyme markers where found to separate within them.

It is known that the relatively narrow range of morphological traits and the limited number of polymorphic isozyme systems, are not adequate to discriminate all the cultivars of any given species, and that, identifying plant cultivars by molecular fingerprinting procedures is a practical necessity (Smith & Smith, 1992). Among the different types of molecular markers available, RAPD markers (Welsh & McClelland, 1990; Williams et al., 1990) are attractive because of their simplicity, versatility, modest cost, and ability to detect relatively small amounts of genetic variation (Ragot and Hoisington, 1993). RAPD markers have been already used for cultivar identification in a wide range of plant species including yams (Asemota et al., 1996; Sosinski & Douches 1996; Ramser et al., 1996, 1997; Ling et al., 1997; Al-Zahim et al., 1997).

Our primary objective was to use the PCR-based RAPD (Randomly Amplified Polymorphic DNA) analysis to distinguish some yam cultivars that could not be separated using isozyme markers. In addition, cluster analysis was used to examine the similarities among these cultivars, and to analyse the relatedness between the dwarf cultivar Tam-Sam and those belonging to the cultivar group TABANE.

Materials and methods

Plant material

The twenty-three yam cultivars analysed (Table 1) are part of Benin Republic's Guinea yam germplasm established in 1996 after a collecting survey throughout the country (Dansi et al., 1997) and maintained as a field collection at the International Institute of Tropical Agriculture (IITA, Cotonou). The twenty-three cultivars are all late maturing and belong to three of the cultivar groups (Table 1) defined within the Benin Republic Guinea yam germplasm (Dansi et al., 1999). For the purpose of the study, tubers of the 23 cultivars were planted in a screenhouse at IITA, Ibadan, Nigeria. (In the text, the cultivar groups' name will be written in capital letter while those of morphotype will be in bold character).

DNA isolation and RAPD analysis

Genomic DNA was isolated from leaf samples using the standard CTAB phenol/chloroform extraction procedures in a mini-prep format (Maniatis et al., 1982; Weising et al., 1995). DNA concentration was measured using an UV spectrophotometer (Beckmann DU-65) at 260 nm. DNA samples were diluted to 20 ng/μl for the RAPD analysis.

PCR reactions were performed in a volume of 25 μl containing 50 ng of DNA, 0.1 mM each of dATP, dTTP, dGTP and dCTP, 10 mM Tris-HCl (pH 8.3), 1.7 mM mgCl$_2$, 50 mM KCl, 0.1% Triton X-100, 0.4 μmol of single oligonucleotide decamer primer (Operon Technologies Inc, Alameda, CA. USA), and two units of Taq DNA polymerase (Promega). The mixtures were overlaid with one drop of mineral oil. Amplification was performed by first denaturing at 94 °C for 3 min followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C, and ending with 10 min at 72 °C. Amplifications were carried out in an automated thermal cycler (model 9600; Perkin-Elmer/Cetus). Amplified products were size-fractionated by electrophoresis in 1.5% agarose gels in 1X TAE buffer and bands were visualised by ethidium bromide staining and photographed under UV light. The primers used and listed in Table 2 are the best among those selected at IITA for the assessment of genetic diversity within West African Guinea yams.

Data analysis

Positions of unequivocally scorable RAPD bands were transformed into a binary matrix (1 for presence and 0 for the absence of a band at a particular position), and analysed phenetically. Pairwise distances between all samples were computed by NTSYS-pc 1.8 software package (Rohlf, 1993) using Jaccard's coefficient of similarity. Dendrograms were created by UPGMA cluster analysis (Sneath & Sokal, 1973; Swofford & Olsen, 1990).

Results and discussion

The RAPD analysis

The typical yields of DNA were 200 to 300 μg g^{-1} of leaf tissue. All extracted DNA was of high molecular weight with very little fragmentation, as indicated by gel electrophoresis. The twelve primers generated a total of 63 bands of which 47 were polymorphic. No single primer distinguished more than three cultivars but the majority of the cultivars were separated with the combination of polymorphic bands generated by all the primers. The low polymorphism revealed by each of the primers taken separately (Figure 1) is not surprising since cultivars analysed are closely related.

Table 1. List and agronomic characteristics of the yam cultivars analysed by RAPD. Information on the agronomic characteristics and the cooking qualities were recorded from the farmers (Dansi et al., 1997). Morphological traits are described in Dansi et al., 1998

A.N	Cultivar name	Cultivar group	Morphotype*	HV	NT	SA	RD	RI	DD	RW	TP	PL	WA
072	Assinabaro	KOKOROGBANOU	Kinkerekou	D	5	M	L	M	Lg	L	G	N	L
340	Awaya	KOKOROGBANOU	Kinkerekou	J	6	H	M	M	Lg	L	G	N	L
435	Chamba	KOKOROGBANOU	Kinkerekou	D	7	H	M	M	L	L	G	N	L
494	Gaki	KOKOROGBANOU	Kinkerekou	D	5	M	L	M	Lg	L	G	N	L
234	Hounbonon	TABANE	Hounbonon	J	5	H	M	H	M	H	G	N	L
357	Ihdonou	TABANE	Ihdonou	D	4	H	L	M	Lg	L	G	N	L
163	Kagourou1	TABANE	Kagourou1	D	3	H	L	H	S	L	G	N	L
334	Kagourou2	KOKOROGBANOU	Kinkerekou	D	7	H	L	H	S	L	G	N	L
480	Kandi	TABANE	Tabané	D	3	H	L	H	S	L	G	N	L
239	Kèkè	TABANE	Kagourou	D	3	H	L	H	S	L	G	N	L
075	Kinkerekou	KOKOROGBANOU	Kinkerekou	D	7	H	M	M	L	L	G	N	L
523	Kokoroagbessi	TABANE	Kagourou	D	4	H	L	M	Lg	L	G	N	L
091	Kokorogbara	KOKOROGBANOU	Kinkerekou	D	7	H	M	M	L	L	G	N	L
478	Komna	TABANE	Tabané	D	4	H	L	M	Lg	L	G	N	L
468	Kpadjoubakokpo	KOKOROGBANOU	Kinkerekou	J	6	H	M	L	S	L	G	N	L
481	Kpanatantangni	TABANE	Tabané	J	5	L	M	L	M	L	G	Y	H
329	Omoya	KOKOROGBANOU	Kinkerekou	J	6	H	M	M	Lg	L	G	N	L
327	Tabané	TABANE	Tabané	D	4	H	L	M	Lg	L	G	N	L
322	Tam-Sam	TAM-SAM	Tam-Sam	D	4	H	L	M	Lg	L	G	N	L
051	Tawounma	KOKOROGBANOU	Kinkerekou	D	5	M	L	M	Lg	L	M	N	M
192	Wohounko	KOKOROGBANOU	Kinkerekou	D	5	M	L	M	Lg	L	G	N	L
240	Yaka	TABANE	Yaka	D	3	H	L	H	S	L	G	N	L
333	Yakarango	TABANE	Yaka	J	5	H	M	H	M	H	G	N	L

*Morphotype is defined as a cultivar of a particular morphology. Cultivars belonging to the same morphotype are therefore morphologically identical (Dansi et al., 1999). Abbreviations: HP: Harvest period (D: December, J: January); NT: Average number of tubers produced per mound; SA: Storage aptitude; RD: Resistance to drought; TP: Texture of the pounded yam; PL: Presence of lumps in the pounded yam; RI: Resistance of the chips (dried yam) to insects; DRM: Duration of the dormancy; RW: Resistance to weeds; WA: Resistance to excess of water in the soil.; H: High; M: Medium; L: Low; G: Good; Y: Yes, N: No; Lg: Long.

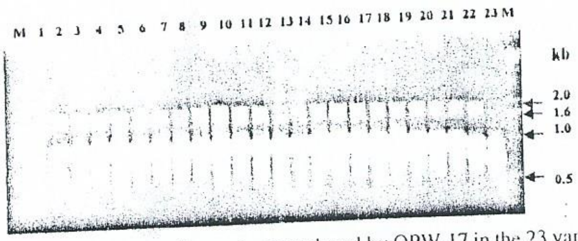

Figure 1. DNA banding pattern produced by OPW-17 in the 23 yam cultivars: (1) Tam-Sam; (2) Yaka; (3) Kandi; (4) Kinkerekou; (5) Awaya; (6) Kagourou-1; (7) Chamba; (8) Kagourou-2; (9) Keke; (10) Omonya; (11) Tabane; (12) Kpajoubakokpo; (13) Ihdonou; (14) Kokorobanou; (15) Wohounko; (16) Tawounma; (17) Kpanantantangni; (18) Komna; (19) Kokoroagbessi; (20) Assinabaro; (21) Gaki; (22)Yakarango; (23) Hounbonon. M = molecular size markers (1 kb ladder).

Diversity within the cultivar group TABANE

TABANE is one of the late maturing and old cultivar groups identified in Benin Republic's cultivated Guinea yam germplasm (Dansi et al., 1998, 1999). TABANE consists of eleven cultivars (assuming that each yam vernacular name corresponds to a unique cultivar) which are morphologically identical, for shoot characteristics. Based upon their tuber characteristics, the eleven cultivars were classified into five morphotypes (Table 3, Dansi et al., 1999). Taking into account their cooking qualities and their agronomic attributes, farmers considered some of the cultivar associations as incorrect, although recognising that individuals classified in the same morphotype are morphologically similar. Hence, for the farmers Komna and Kpanatantangni versus Yaka and Yakarango (Table 3) are different and should be classified separately. None of the seven isozyme systems used to identify and access genetic diversity among the Benin Republic Guinea yam cultivars was able to separate them (Dansi et al., 2000). Data recorded from the RAPD analysis clearly show that, apart from Kandi and Yaka which are identical, all the cultivars are different. In

Table 2. Sequences of the primers used for RAPD analysis

Primer	Sequence	Primer	Sequence
OPW-1	5′-CTCAGTGTCC-3′	OPW-14	5′-CTGCTGAGCA-3′
OPW-2	5′-ACCCCGCCAA-3′	OPW-15	5′-ACACCGGAAC-3′
OPW-5	5′-GGCGGATAAG-3′	OPW-16	5′-CAGCCTACCA-3′
OPW-6	5′-AGGCCCGATG-3′	OPW-17	5′-GTCCTGGGTT-3′
OPW-8	5′-GACTGCCTCT-3′	OPW-18	5′-TTCAGGGCAC-3′
OPW-12	5′-TGGGCAGAAG-3′	OPQ-4	5′-AGTGCGCTGA-3′

the dendrogram constructed by the UPGMA cluster analysis the eleven cultivars are partitioned into four clusters at the similarity level of 85% (Figure 1). A new classification is then obtained with the separation of some cultivars previously classified together, and the association of some cultivars that were belonging to different morphotypes (Table 3). Taking into account the information collected from the farmers on the cooking quality and the agronomic characteristics of these cultivars (Table 1), the present classification appeared as the one that better corresponds to the reality. In fact, contrary to the morphological classification, all the cultivars clustering together have the same agronomic and organoleptic characteristics. In a vegetatively propagated crop with frequent intraclonal variation of the tuber's traits such as yam, when classification is based only on the tuber traits (the eleven cultivars having identical shoot), errors cannot be avoided. It is therefore not surprising that putative cultivar misclassification is detected by RAPD markers.

Diversity within the morphotype Kinkerekou

The eleven cultivars assigned to the morphotype **Kinkerekou** have existed for a long period and are widely spread in the north of Benin (Dansi et al., 1999). In general, they are all high yielding cultivars, able to produce four to eight tubers per mound. They are easy to multiply and highly demanded in the production and in the trading of yam chips (dry tuber pieces). Although morphologically identical, many of the cultivars classified in the morphotype **Kinkerekou** seem to be different according to the farmers who distinguish them based on their agronomic traits and their cooking qualities. It is therefore evident that a classification based only on morphological traits is inadequate. Their separation using isozyme markers was not possible, as they have all shown the same patterns for all the seven enzyme systems used. In the dendrogram constructed based on the RAPD data (Figure 1), the separation of the 11 cultivars started only after the similarity level of 80% hence indicating that they are all very close. At the similarity level of 85%, the 11 cultivars were classified into tree different clusters: Thus, Chamba, Kagourou-2, Kinkerekou and Kokorogbara cluster together; Tawounma is associated to Wohounko, Assinabaro and Gaki while Awaya, Omonya and Kpajoubakokpo also cluster together. With regard to the close relationship between the individuals clustering together, each one of the three clusters can be considered as corresponding to a given cultivar and the individuals within the cluster as its different clones. Interestingly, it appeared as expected that some cultivars are genetically identical. Hence, Kinkerekou is a synonym of Kokorogbara, Assinabaro is another name of the clone Gaki and Awaya is thought to be a deformation (which is frequent in yam) of the name Omoya. The analysis of the agronomic data gathered on each of the 11 cultivars (Table 1) during the collecting survey (Dansi et al., 1997) corroborates the present classification based on the RAPD markers.

Relationship between Tam-Sam and Tabane

Tam-Sam is a special cultivar among the late maturing cultivars of Guinea yam of Benin Republic. Its presence in other countries of the African yam belt has never been reported. It is dwarf, unarmed (without thorns) with very small leaves of almost the size of the leaves of groundnut plant (Tam-Sam in *Bariba* language means "Yam groundnut"). The morphology of the shoot of Tam-Sam is unusual and could be mistaken as other plants than yam. Tam-Sam sometime develops leaves with non-chlorophyllous parts and more rarely some entirely non-chlorophyllous branches. It produced some tubers morphologically similar to those of Tabane, the cultivar which is most

Table 3. Morphological and RAPD marker-based classification of the cultivars belonging to the group TABANE

Classification based on morphological traits (Dansi et al., 1999)	New classification based on RAPDs
1 – **Hounbonon**	1 – **Keke**, Yaka/Kandi, Kagourou-1
2 – **Ihdonou**	2 – **Kpanantantangni**
3 – **Keke**, Kagourou , Kokoroagbessi	3 – **Tabane**, Komna, Kokoroagbessi, Ihdonou
4 – **Yaka**, Yakarango	4 – **Hounbonon**, Yakarango
5 – **Tabane**, Kandi, Komna, Kpanatantangni	

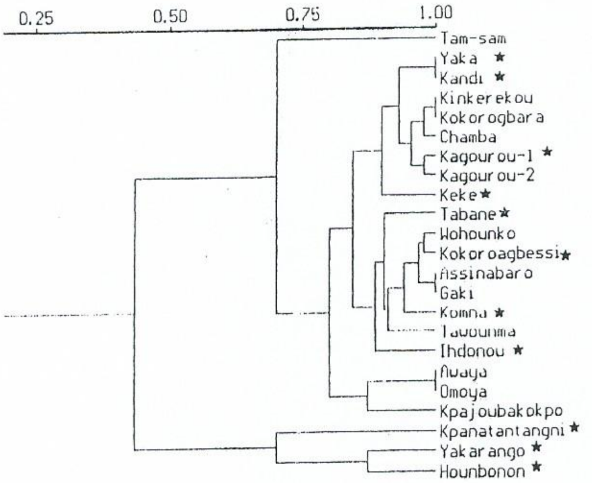

Figure 2. Dendrogram of the analysed twenty three Guinea yam cultivars constructed by UPGMA cluster analysis based on 47 RAPD markers. Individuals marked with star belong to group TABANE and the others (apart from Tam-Sam) belong to morphotype Kinkerekou.

close to it (Dansi et al., 1999). According to some farmers, Tam-Sam may have been spontaneously derived from Tabane during the ancient period. Considering that even single mutation can cause a significant morphological difference that might obscure an otherwise close relationship between cultivars, it has been hypothesised, based on morphological data, that Tam-Sam could have been derived from Tabane by a chloroplastic mutation (Dansi et al., 1999). If it is the case, then Tam- Sam is expected to be very close to Tabane. When included together with Tabane in a UPGMA cluster analysis, Tam-Sam appeared as a quite distinct cultivar, although related to Tabane (Figure 1). If the information collected from the farmers is correct, then the modification of Tabane's genome as the origin of Tam-Sam must be much deeper than assumed.

Relationship among all the analysed cultivars

No clear separation between the cultivars of the morphotype **Kinkerekou** and those belonging to the cultivar group TABANE was observed in the UPGMA cluster analysis (Figure 1) performed to examine the relationships among the 23 cultivars (all white and late maturing yams) involved in this study. Nevertheless, two clusters of TABANE (Kpanatantangni; Yakarango and Hounbonon) and one cluster of **Kinkerekou** (Awaya/Omonya and Kpajoubakokpo) remain separated. Surprisingly, the remaining two clusters of TABANE as well as the other two of **Kinkerekou** were merged into two new separate clusters, each exactly representing the combination of two clusters: one from TABANE and the other from **Kinkerekou**. The cultivars (all domesticated in their production zone) belonging to each of these novel clusters are more likely progeny of the same original parents with regard to their close genetic relationship. The dwarf Tam-Sam still appeared as a distinct cultivar. More interestingly, the two forms of Kagourou (Kagourou-1 and Kagourou-2) previously classified (based on their morphological traits) in two different cultivar groups (TABANE and KOKOROBANOU), cluster together and are almost identical. One therefore understands why, although morphologically different, farmers do consider them as two different forms of a single cultivar.

In total, the results obtained in this study show that farmers have a good knowledge of their yam cultivars and in terms of classification, identification and use, their knowledge would be valuable to geneticists and breeders. They also support the views of Asemota et al. (1996), Ramser et al. (1997) according to which, the technique may be practically applied for yam cultivar identification and can serve as an instrument to identify cultivar misclassification, help understand the relationships between cultivars and assist in the identification of putative duplicates towards the establishment of an accurate **core collection,** as shown by Novy et al. (1994); Virk et al. (1995).

In yam, cultivar identification via molecular marker-based analyses will be very useful because genetic variation is fixed within a line. Clonal propagation combined with polyploidy and a highly heterozygous genetic state (yam being allogamous) will result in a large number of phenotypic classes for fingerprinting. However, the low polymorphism individually revealed by the different primers lead suggest that in yam, when aiming to separate putative duplicates (e.g. cultivars already morphologically identical and with the same isozymic identity), AFLP and SSR which reveals very high levels of polymorphism may be better. Both methods are already perfected on Guinea yam at IITA for accurate classification of the traditional cultivars (Mignouna et al., 1998).

Conclusion

The results obtained in the present study have clearly shown the heterogeneity of the morphotype Kinkerekou and confirmed the existence of different cultivars misclassified within the cultivar group TABANE. Farmers are then right and in yam classification we recommend that their knowledge be capitalised by geneticists and breeders. Additional studies are still needed to clarify the origin of the dwarf Tam-Sam considered as derived from cultivar Tabane.

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