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Evaluation of cassava (*Manihot esculenta* Crantz) germplasm collections using RAPD markers

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Summary

Randomly amplified polymorphic DNA (RAPD) markers combined with the Simple Matching index for calculating the distance matrix were used to determine the genetic diversity of African cassava clones. A preliminary study of three *Manihot* species using 20 primers clearly showed that RAPD markers were relevant for analyzing their genetic diversity. DNAs from 19 cultivars of *Manihot esculenta* Crantz (cassava) were amplified using 8 primers. Cultivars were well discriminated and coherently distributed, comparing to previous results from isozymes and RFLP studies. Genetic diversity analysis, characterization of collections and study of introgression are the domains that RAPD markers can contribute to the improvement of cassava.

Abbreviations: PCR – Polymerase Chain Reaction, RAPD – Randomly Amplified Polymorphic DNA, RFLP – Restriction Fragment Length Polymorphism

Introduction

The genus *Manihot* originates from Latin America where 98 species are found (Rogers & Appan, 1973). *Manihot esculenta* Crantz (cassava) was initially introduced to Africa 400 years ago, where its cultivation for food spread through the tropical and subtropical regions. The second *Manihot* species present in Africa, *M. glaziovii* Mueller Von Argau, was introduced 200 years ago as a source of rubber, its distribution, however, was less extensive (Jones, 1959). Cassava, which is generally propagated vegetatively, is one of the major sources of food in Africa (Cock, 1982). The roots, which are an excellent source of carbohydrates, have a very low protein content. In addition the roots have a high content of cyanogenic glucosides (De Bruijn, 1971) which often necessitates extensive processing before the cassava is edible. These areas, together with disease susceptibility (Silvestre & Arraudeau, 1983) and the problem of post-harvest deterioration (Plumbley & Rickard, 1991), are recognised as targets for cassava breeding.

Genetic improvement of cassava is to a certain extent limited by a poor knowledge of genetic diversity within the species. Isoenzymes have been used as a method to estimate genetic diversity within cassava, but low polymorphism was detected and the technique was not reproducible (Hussain et al., 1987; Ramirez et al., 1987; Lefevre, 1989; Lefevre & Charrier, 1993). However restriction fragment length polymorphisms (RFLPs) have been recently used for this purpose with some success (Beeching et al., 1993). In addition to their use in assessing genetic diversity, these approaches are useful in the detection of accession duplications within germplasm collections.

Analysis of random amplified polymorphic DNA (RAPD) (Williams et al., 1990) has been successfully used to assess genetic diversity within several

plant groups including conifers, *Brassica* and wheat (Carlson et al., 1991; Hu & Quiros, 1991; Vierling & Nguyen, 1992), and for other purposes (for a review Hadrys et al., 1992). Recently this analysis has been applied to tropical crop plants for which genomic or cDNA libraries do not exist or are not readily available for generating RFLP data (Lashermes et al., 1993; Wilde et al., 1992). In this paper we report a study of three *Manihot* species clearly showing the applicability of RAPD markers as genetic markers and the use of RAPDs for the evaluation of genetic diversity of cassava. A comparison with previous results issued from different analyses (isozymes, RFLPs) is discussed.

Material and methods

Plant material

Manihot accessions (Table 1) were selected from the collection kept *in vitro* in our laboratory (Brizard et al., 1992). Ten accessions representing the species present in Africa (two *M. glaziovii*, four *M. esculenta* and two interspecific controlled hybrids) and one species from South America (two *M. caerulescens* Pohl) were chosen in order to see if RAPD markers were applicable for studying the genetic diversity. For detecting variation within *M. esculenta*, 19 clones were used. DNA was extracted according to the method of Dellaporta et al. (1983).

PCR amplification

Decamer oligonucleotide primers (kit OPK) were purchased from Operon (USA). Amplification reactions were done in volumes of 25 μl containing 10 mM Tris-CHl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 0.1 mM of each dATP, dCTP, dGTP and dTTP (Boehringer), 100 ng of genomic DNA, 0.4 μM of primer and 1 unit of Taq polymerase (Perkin Elmer Cetus). PCR reactions were performed using the Trio-ThermoBlock apparatus (Biometra/Germany). Samples were subjected to one step of four minutes at 94° C, 45 repeats of the following cycle; one minute at 94° C, one minute at 35° C, two minutes at 72° C, and then a final step of six minutes at 72° C. Amplification products were analysed by electrophoresis in 2.0% agarose gels in TBE buffer at 2.5 volt/cm for four hours and detected by staining with ethidium bromide.

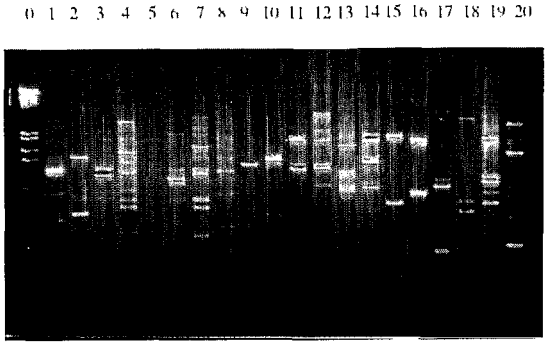

Fig. 1. Example of amplification products from *M. esculenta* variety Kasimbidgi Red using the 20 primers. Lane 0: Lambda DNA digested by Eco RI + Hind III. Lane 1: OPK1; Lane 2: OPK2; Lane 3: OPK3; Lane 4: OPK4; Lane 5: OPK5; Lane 6: OPK6; Lane 7: OPK7; Lane 8: OPK8; Lane 9: OPK9; Lane 10: OPK10; 11: OPK11; 12: OPK12; 13: OPK13; 14: OPK14; 15: OPK15; 16: OPK16; 17: OPK17; 18: OPK18; 19: OPK19; 20: OPK20.

Data analysis

For each primer, band levels were recorded and presence or absence of an amplified product was respectively coded one and zero for all accessions. From these data, a matrix of distances based on the coefficient of Simple Matching (Sokal & Michener, 1958) was calculated. The Simple Matching index of similarity was:

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

where

a = number of amplified products in both i and j,
b = number of amplified products in i but not in j,
c = number of amplified products absent in i but present in j,
d = number of amplified products absent in i and j but scored for other genotypes.

For the creation of the distance matrix, coefficients were calculated using the following formula:

$$Dij = 1 - Sij = (b + c)/(a + b + c + d)$$

A hierarchical ascendant clustering analysis (Benzecri, 1973) was performed using the computer program Taxo (Serres & Rioux, 1986).

Results

Twenty different primers of arbitrary sequence were used for the study; the amplified products could be

Table 1. List of accessions surveyed for RAPD markers

RAPD analysis	Identificator collection	Species	Origin
Study between species			
caeA	MC1	*M. caerulescens*	South America
caeB	MC3	*M. caerulescens*	South America
glaC	127-I	*M. glaziovii*	Côte d'Ivoire
glaD	glaziovii	*M. glaziovii*	Côte d'Ivoire
escE	7902	*M. esculenta*	Côte d'Ivoire
escF	Kasimbidgi Red	*M. esculenta*	Kenya
escG	30337	*M. esculenta*	Nigeria
escH	57	*M. esculenta*	Madagascar
hybI	GE 3-3	*M. gla* × *M. esc*	Côte d'Ivoire
hybJ	GE 31-2	*M. gla* × *M. esc*	Côte d'Ivoire
Intraspecific study			
escE	7902	*M. esculenta*	Côte d'Ivoire
escF	Kasimbidji Red	*M. esculenta*	Kenya
escG	30337	*M. esculenta*	Nigeria
escH	57	*M. esculenta*	Madagascar
escK	4762	*M. esculenta*	South America
escL	H43	*M. esculenta*	Madagascar
escM	Chokorote	*M. esculenta*	Kenya
escO	5318/34	*M. esculenta*	Kenya
escP	Mwakasanga	*M. esculenta*	Kenya
escQ	4748	*M. esculenta*	South America
escR	Kasimbidji	*M. esculenta*	Kenya
escS	Garimoshi	*M. esculenta*	India
escT	Nusu Rupia	*M. esculenta*	India
escU	Viro 9	*M. esculenta*	Côte d'Ivoire
esvV	30555	*M. esculenta*	Nigeria
escW	A13	*M. esculenta*	Côte d'Ivoire
escX	30786	*M. esculenta*	Nigeria
escY	Bonoua Rouge 1	*M. esculenta*	Côte d'Ivoire
escZ	TA49	*M. esculenta*	Côte d'Ivoire

detected for most primers (Fig. 1). Within an individual plant the 20 primers each detected different RAPD profiles containing DNA bands between 0.3–3.2 kilobases (kb).

Variability between species

Of the 20 primers tested, two (OPK5 and OPK18) gave pattern with no or very faint bands (and not repeatable) for the ten accessions, and three (OPK1, OPK6 and OPK10) revealed a pattern too difficult to interpretate. The number of band levels for the 15 other primers ranged from five to 24 per primer (Table 2). The average number of bands detected per primer for all the plants was 14, while the average number of bands detected in each species was close to five, indicating a high degree of polymorphism. This polymorphism was apparent with all primers and was not only between species but also within species (Fig. 2).

The dendrogram constructed by hierarchical cluster analysis, based on the distance matrix (data not shown), and shown in Fig. 3, distributes the genotypes in different clusters. The two *M. caerulescens* clones are clustered and well separated from the others. With-

Table 2. Sequence of the primers used and number of fragments detected for each of them for the interspecific study

Primer N°	Sequence	All plants.	Within the 4 *M. esculenta* accessions	
		Number of fragments detected	Number of fragments detected	Number of polymorphic fragments detected
OPK1	CATTCGAGCC	*		
OPK2	GTCTCCGCAA	13	7	2
OPK3	CCAGCTTAGG	11	8	6
OPK4	CCGCCCAAAC	17	11	8
OPK5	TCTGTCGAGG	**		
OPK6	CACCTTTCCC	*		
OPK7	AGCGAGCAAG	24	11	7
OPK8	GAACACTGGG	23	15	14
OPK9	CCCTACCGAC	7	2	0
OPK10	GTGCAACGTG	*		
OPK11	AATGCCCCAG	16	12	10
OPK12	TGGCCCTCAC	23	13	8
OPK13	GGTTGTACCC	8	3	2
OPK14	CCCGCTACAC	10	6	3
OPK15	CTCCTGCCAA	11	5	3
OPK16	GAGCGTCGAA	5	2	0
OPK17	CCCAGCTGTG	12	7	3
OPK18	CCTAGTCGAG	**		
OPK19	CACAGGCGGA	10	7	4
OPK20	GTGTCGCGAG	13	10	7

* : primer that gave a pattern too difficult to interpretate.

** : primer that did not amplify DNA for all the plants.

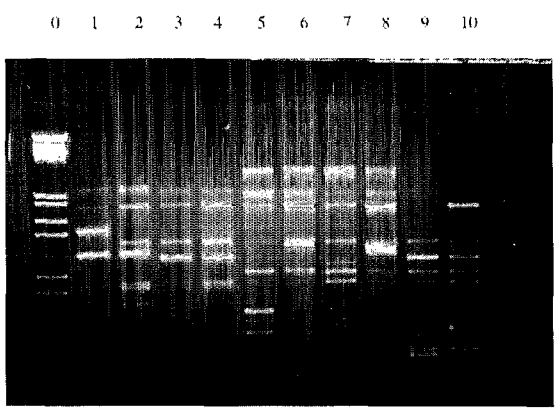

Fig. 2. Example of amplification products from 10 *Manihot* accessions using the primer OPK12. Lane 0: Lambda DNA digested by Eco RI + Hind III. Lane 1: caeA; Lane 2: caeB; Lane 3: glaC; Lane 4: glaD; Lane 5: escE; Lane 6: escF; Lane 7: escG; Lane 8: escH; Lane 9. hybI; Lane 10: hybJ.

in the latter, the two species are well separated with the hybrids positioned between the two parental species. The two *M. glaziovii* clones are close; the variation within the four *M. esculenta* clones is no less than within the other species.

Variability within M. esculenta

The 19 *M. esculenta* accessions DNA were amplified using eight primers that had previously shown an informative polymorphism. The primers used were primers OPK2, OPK3, OPK4, OPK8, OPK12, OPK14, OPK17, OPK19. 85 amplified products levels were recorded. The RAPD profiles (Fig. 4) were scored and a distance matrix calculated based on the Simple Matching index (data not shown). Values from the distance matrix are homogeneous. This is apparent on the

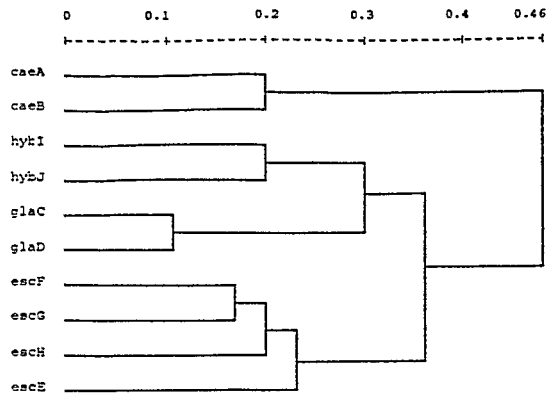

Fig. 3. Dendrogram showing genetic relationships between *Manihot* accessions constructed by hierarchical clustering analysis using RAPD markers.

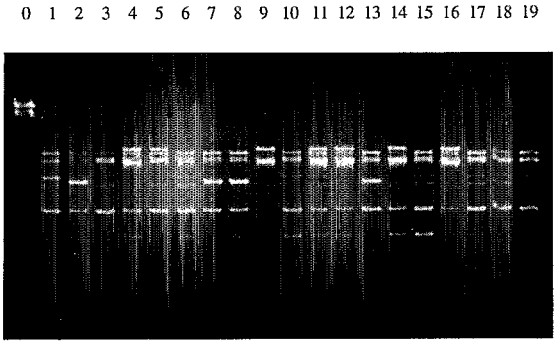

Fig. 4. Example of amplification products from 19 *M. esculenta* accessions using the primer OPK14. Lane 0: Lambda DNA digested by Eco RI + Hind III. Lane 1: escE 11: escR; Lane 2: escF 12: escS; Lane 3: escG 13: escT; Lane 4: escH 14: escU; Lane 5: escK 15: escV; Lane 6: escL 16: escW; Lane 7: escM 17: escX; Lane 8: escO 18: escY; Lane 9: escP 19: escZ; Lane 10: escQ.

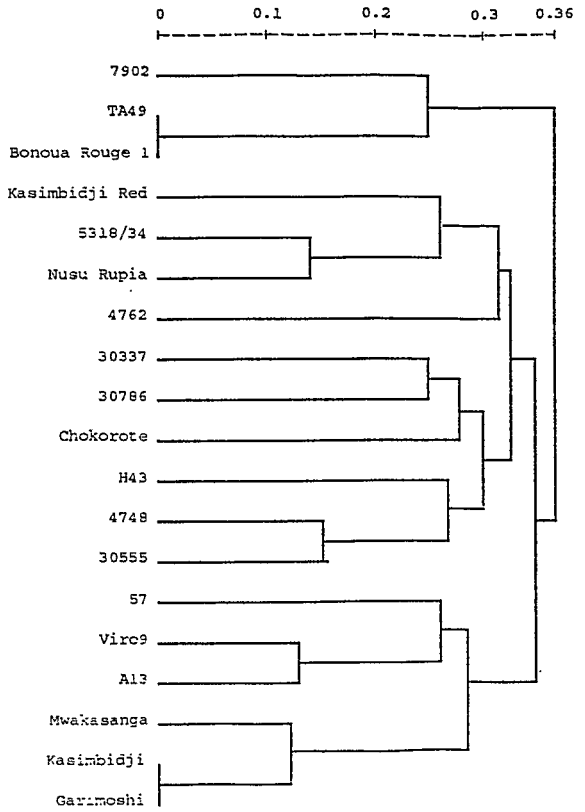

Fig. 5. Dendrogram showing genetic relationships between *Manihot esculenta* accessions constructed by hierarchical clustering analysis using RAPD markers.

dendrogram which provides little evidence of strong group structure (Fig. 5).

Discussion

Genetic diversity

The Simple Matching index is not a straightforward pair-wise comparison between two genotypes. It also takes into account the whole of information given by all the genotypes, e.g. the absence of an amplified product (a band) in two genotypes which is present in others is given equal weighting as the shared presence of a band in calculating similarity. This is in contrast to the Jaccard Index which scores shared presences while ignoring shared absence (Jaccard, 1901). With RAPD markers, fragments are randomly amplified and no relationship can be made between the absence of a fragment and the presence of a new one. Therefore, the index of Simple Matching is appropriate for analysing RAPD markers as it includes joint absences of a fragment in the numerator and denominator.

The distribution of species observed on the dendrogram (Fig. 3) is coherent and clearly shows that the RAPD technique and the analytical methods used are powerful tools for the studying the genetic diversity of *Manihot* species.

Concerning the intraspecific analysis, the results show at this level of investigation an interesting genetic diversity within African cultivars of cassava, which is perhaps surprising bearing in mind the introduction of a limited number of genotypes from Latin America (Jones, 1959; Silvestre & Arraudeau, 1983; Lefevre

& Charrier, 1993). Although the dendrogram is quite homogeneous, three groups can be considered: one consisting of cultivars 7902, Bonoua Rouge 1, and TA49, the second consisting of cultivars 57, Viro 9, A13, Mwakasanga, Kasimbidji and Garimoshi, and the third one made up of the remainders. This distribution correlates the RFLP analyses done on the same cultivars (Beeching et al., 1993) where the first two groups are detectable in the dendrogram issued from RFLP datas. The cluster containing cultivars 57, Viro 9, A13, Mwakasanga, Kasimbidji and Garimoshi is highly conserved. This study demonstrates a genetic diversity within the African *M. esculenta* accessions that could be used for a programme of genetic improvement of cultivars.

Collection characterization

The RAPD technique could also contribute to a better characterization of collections and so to elimination of duplicates. RAPD markers tend to be more discriminating than isozymes. Cultivars Viro9 and A13 (both from Côte d'Ivoire) were identical, as well Mwakasanga and Kasimbidgi (both from Kenya) using isozymic electrophoresis techniques (Lefevre, 1989). They are different with RAPD markers, with a distance of 0.13 for Viro9 and A13 and 0.12 for Mwakasanga and Kasimbidji. They were also different using RFLP markers (Beeching et al., 1993). Cultivars Kasimbidji (from Kenya) and Garimoshi (from India) are identical whatever the techniques used (isozymes, RFLP, RAPD). The two varieties could be identical as cassava was taken from Brazil to India via East Africa in the eighteenth century (Cock, 1985). Labelling errors within the collection is probable with the cultivars Bonoua Rouge 1 and TA49. Lefevre had found them different using isozymes (Lefevre did the isozymes experiments in Côte d'Ivoire using the *in vivo* collection – personnal communication); the two cultivars are now found similar with RAPD and RFLP markers. This is probably due to an error while maintaining the collection *in vitro* (the two cultivars have consecutive identification numbers): the two clones are identical. RAPD technique is efficient for fingerprinting: it is more discriminating than isozymes and can detect duplicates within collections.

Markers of introgression

RAPD analyses can be used to evaluate gene flow between species (Arnold et al., 1991; Quiros et al., 1991). *M. glaziovii* species has recently become more important because of the increasing pressure of diseases on cassava in Africa: spontaneous forms of *M. glaziovii* contain various types of resistance to African cassava mosaic virus (Hahn et al., 1980). Hybrids between the two species can be constructed and have been shown to occur spontaneously (Lefevre, 1989). RAPD markers could be used to follow introgression of desirable traits, such as disease resistance into cassava.

RAPD technique is a powerful tool which can contribute to the improvement of cassava through an evaluation of African cassava collections. RAPD analyses can be used for an assessment of genetic diversity, fingerprinting of clones and the detection of gene flow between species. Furthermore, this technique is less restricting than the RFLPs (no hybridization and no use of radioisotopes), and therefore is more convenient for use in research centres in developing countries.

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