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Genetic Analysis of Selected Cassava (*Manihot esculenta*) Genetic Pool in Africa Assessed with Simple Sequence Repeats

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Abstract: Genetic markers are widely used tools in genotype and species identification. The main objective of this study was to analyze the genetic diversity and relationships among cassava genotypes identified in the African gene pool. Genomic DNA from Africa several cassava genotypes were analyzed using simple sequence repeats (SSR) markers. A total of seventeen landraces around Africa, three elite lines from International Institute of Tropical Agriculture (IITA) and four inter-specific hybrid lines were analyzed. Ninety six SSR primers produced 31% polymorphic and 5% monomorphic markers, totaling 82. All the 82 markers were used to construct the dendrogram based on UPGMA. The cassava genotypes analyzed were genetically closely related, with a coefficient of genetic similarity ranging from 0.43 to 0.86, exhibiting similar SSR amplification profiles. This may imply low genetic variability among the genotypes studied.

Key words: Cassava · Genotypes · Manihot esculenta · SSR markers · Polymorphic

INTRODUCTION

Manihot esculenta Crantz (cassava) was initially introduced to Africa 400 years ago, where its cultivation for food spread throughout 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, although its distribution was less extensive. Cassava, which is generally propagated vegetatively, is one of the major sources of food in Africa [1]. Cassava has moved from being a subsistence crop to a full commercial crop for its income generating capacity. The crop is now widely cultivated for its vast industrial potentials. Cassava has the advantage of being well adapted to a wide range of environmental stresses. It grows very well in less fertile soil in contrast to many other crops that are highly vulnerable to environmental stresses during critical stages of plant development [2], but shows different growth behaviors in different locations as a result of variation in climatic and soil conditions [3]. One of the best methods to increase cassava production to serve as the main food security and cash crop in Africa and developing countries is by the development of better varieties that are resistant to diseases, pests and drought [2].

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 [4, 5]. Studies have been conducted earlier to assess the variability based on biometrical characters as well as RAPD (randomly amplified polymorphic DNA) markers [6, 7]. There is a wide range of molecular techniques available to assess genetic variability of a species. Due to their co-dominant inheritance, robustness and amenability to high throughput, SSRs or microsatellites have become a tool of choice for investigating important crop germplasm [8]. SSR markers have been confirmed to be the most informative and appropriate for cassava [9]. Perera *et al*. [10] also supported SSR markers as the most informative for plants.

Useful attributes of all SSR markers are codominance (many alleles are found among closely related individuals), technical simplicity, sensitivity, analytical simplicity (data are unambiguously scored and highly

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reproducible) and are high abundance (markers are uniformly dispersed throughout genome as frequently as every 10 kb and therefore are ideal tools for many genetic applications. Microsatellites are ubiquitously present in eukaryotic genomes and are highly polymorphic [11]. At present more than 500 SSR markers are available in cassava which will provide genetic tags for various phenotypes in cassava. The objective of the present study was to analyze the genetic diversity and relationships among cassava genotypes identified in the Africa gene pool.

MATERIALS AND METHODS

Plant Materials: The study was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan (7°26'N, 3°54'E) a transition zone between humid and sub-humid tropics. A total of 24 genotypes, including both landraces and elite genotypes, were provided by IITA for this study. The names and source of these genotypes are described in Table 1. About 200 to 250 mg fresh and young leaves sample were collected, frozen in liquid nitrogen and stored at -80°C until use for DNA extraction. In total 24 genotypes were analyzed.

Table 1: List of the 24 African cassava genotypes used in this study and their geographical or genetic origin

Genotypes	Type	Origin
TME 28	Landrace	Nigeria
TMS 30572	Elite line	IITA, Nigeria
TME 419	Landrace	Togo
TME 117	Landrace	Nigeria
TME 3	Landrace	Nigeria
TME 9	Landrace	Nigeria
TME 7	Landrace	Nigeria
TME 1	Landrace	Nigeria
TMS 4(2) 1425	Elite line	IITA, Nigeria
TMS 30555	Elite line	IITA, Nigeria
TMS 388	Landrace	Uganda
TME 1786	Landrace	Kenya
TME 530	Landrace	Malawi
TME 6	Landrace	Nigeria
TME 225	Landrace	Benin
TME 230	Landrace	Togo
TME 232	Landrace	Togo
TME 279	Landrace	Nigeria
TME 638	Landrace	Ghana
TME 568	Landrace	Angola
02/0577	Castor-cassava hybrid	-
00/0214	Interspecific hybrid	-
02/0593	Castor-cassava hybrid	-
00/0210	Interspecific hybrid	-

Dna Extraction: Total cellular DNA was isolated from frozen young leaf samples using the method described by Dellaporta and Hicks [12], with some modifications. The concentration of each sample was determined using the DNA quantification kit from Bio-Rad and the purity was determined using a spectrophotometer (Variant Cary 100 UV-VIS spectrophotometer). The quality of the DNA was checked on 1% agarose gel electrophoresis in 1 x TAE buffer by using 0.5µg/ml Ethidium Bromide staining technique for 5minutes and then distained in water, so as to visualize the DNA bands in the gel.

Amplification of Ssr Markers: Eighty six SSR primers were used for preliminary amplification of isolated DNA. The amplification reactions were conducted following the protocol proposed by Williams *et al.* [13] with a final volume of 10 µl containing: 1.0 µl of DNA (10ng/µl); 1.0 µl 10x PCR buffer containing 15 mM $MgCL_2$ (Promega), 0.8 µl of dNTPs (2.5 mM each), 0.2 µl of TaqDNA polymerase (Promega; 5 u/µl), 2.0 µl of the forward and reverse primer (1 µM each) and 5.0 µl of distilled water. The amplifications of the primers were carried out in a BIORAD-MyCycler-thermocycler with the following amplification conditions: a denaturing step at 95°C for 3min.; followed by 40 cycles, each one consisting of denaturation at 94 °C for 1min.; primer pairing with the DNA strand (35°C for 1 min.) and fragment extension step of 7 minutes at 72°C for 2 min and after 40 cycles, a last extension step at 72°C. The PCR products were then run on a 3.5% superfine resolution (SFR) agarose gel in 1 x TBE bufferat 80V for 2h. Primer pairs that failed to amplify any product or that have a faint product under these conditions were tested under the same three annealing temperatures with three different $MgCl_2$ concentrations: 1, 2 and 4 mM. The gels were stained with ethidium bromide and photo-documented under UV light.

Data Analysis: Each band was scored as present (1) or absent (0) and the generated data was assembled into a matrix. The data matrix was then analyzed using Numerical Taxonomy System of Statistic v.2.0j [14]. Similarity matrix between pairs of the cassava genotypes were calculated for the combination of data from 86 primer pairs by selecting Similarity for Quantitative Analysis (SIMQUAL) using the method of Jaccard [15] Similarity Coefficient. The similarity data matrix was then used for cluster analysis, which was based on the unweighted pair-group method with arithmetic mean (UPGMA) to determine genetic relationships among the germplasm studies. The clustering obtained was used to generate dendrograms.

RESULTS AND DISCUSSION

Out of 86 primers tested, 51 produced non-polymorphic fragments, 1 gave non-scorable band and 3 had no amplification. The remaining 31 primers amplified from 1 to 24 polymorphic fragments which ranged from 50 to 500 bp. Polymorphic and monomorphic SSR loci were detected (Fig 1). In some cases, it could not be unambiguously determined whether a particular SSR locus was monomorphic or polymorphic. However, as shown in Fig.1, lanes with no bands were observed, indicating either absence of alleles (null alleles) or alleles below the detection limit. Due to the limited resolution of the SFR gels to about 5 bp, the number of alleles determined in this assay, represent a minimum estimate. The actual number of alleles per locus as well as the number of alleles per genotypes may be higher.

For some SSR loci, the bands observed on the SFR gels were larger than the expected size, based on the predicted location of the primers on the sequence. Since the primers for PCR were based on cDNA sequences while the PCR reaction for amplification of the SSR was carried out on genomic DNA, these larger bands most likely indicate the presence of intron sequences. The high degree of SSR polymorphism that was observed in cassava in this study is comparable to the results of other crop species [17, 18]. About 31% of the of SSR markers were polymorphic in all cassava genotypes under study. SSR markers have been used to study genetic diversity in a large number of plant species, including wheat, sunflower and many other crops. In this study, they also showed clear distinctions in the cassava genotypes.

In addition to measuring genetic diversity, verification in mutual relatedness of these cassava genotypes from different regions in Africa was assessed. The dendrogram constructed on NTSYS using similarity index based on UPGMA showed genetic similarity among cassava genotypes, with the coefficient of genetic similarity ranging from 0.43 to 0.86. At 0.70 similarity coefficient, the 24 cassava genotypes clustered into ten main groups (Table 2). There was a strong genetic relationship between the Nigeria landraces and strong similarities between TME 1786 from Kenya and TME 530 from Malawi and between TME 225, TME 638 and TME 568 (Fig.2). This indicates the strength of SSR markers in detecting relationships and diversity among germplasm.

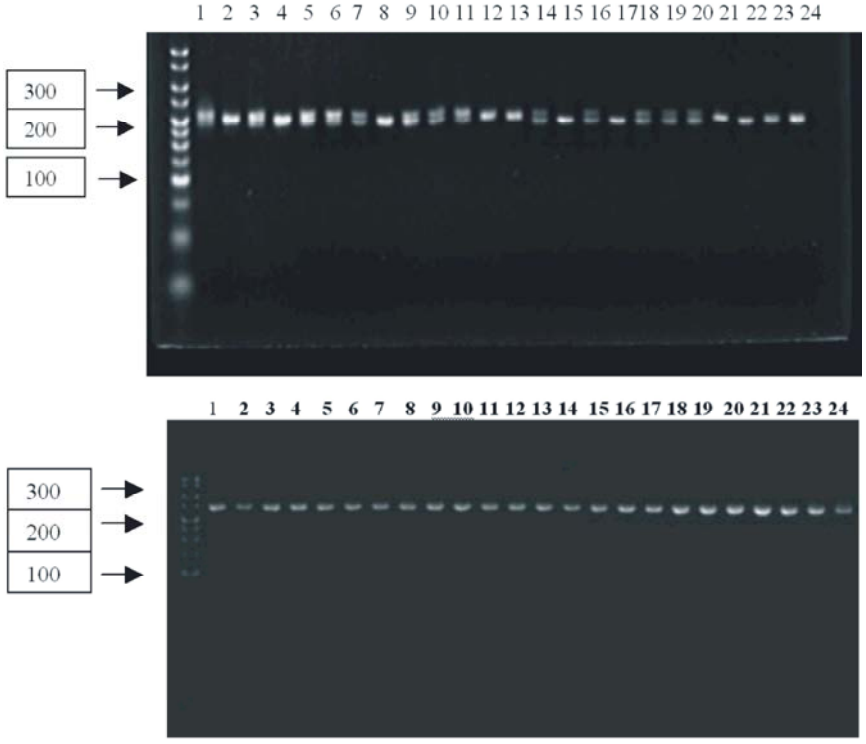

Fig. 1: Allelic variation of selected SSR loci of cassava genotypes
a: Example of allelic variation of cassava genotypes by primer pair # 153
b: Example of a monomorphic DNA locus by primer # 145

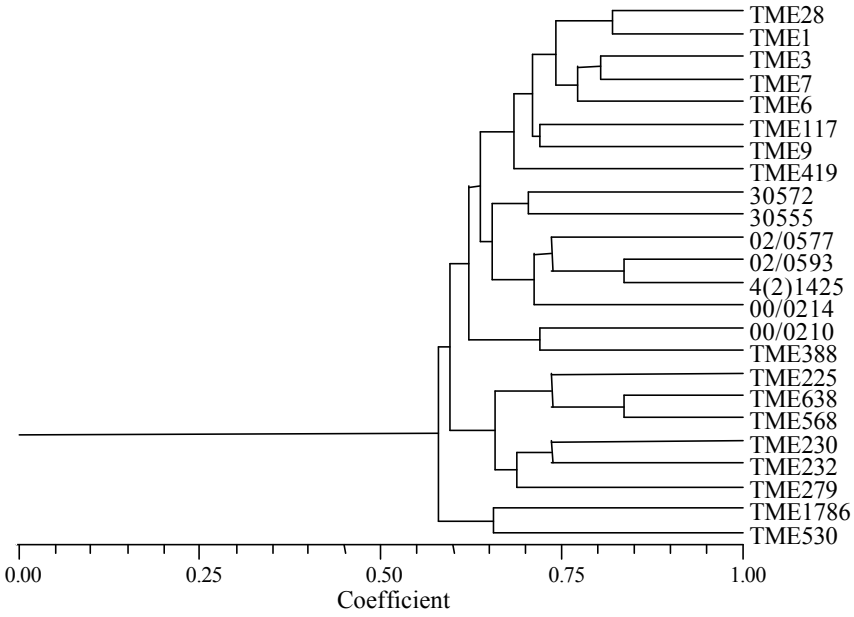

Fig. 2: Dendrogram of cassava genotypes obtained with NTSYS similarity coefficient, representing genetic relationships among 24 Africa cassava (*Manihot esculenta)* genotypes.

Table 2: Dendrogram cluster groupings of the cassava genotypes

Cluster groups	Accessions
Group I	TME 530
Group II	TME 1786
Group III	TME 279
Group IV	TME 232 and TME 230
GroupV (two subgroups 'a' and 'b')	Subgroup 'a' - TME
	638 andTME 568
	Subgroup 'b' - TME 225
Group VI	TME 388 and 00/0210
Group VII (three subgroups	Subgroup 'a' - 00/0214
'a', 'b' and 'c')	Subgroup 'b'- 4(2) 1425
	and 02/0593
	Subgroup 'c' 02/0577
Group VIII	30555 and 30572
Group IX	TME 419
Group X (four subgroups	Subgroup 'a' - TME 9 and
'a', 'b', 'c' and 'd')	TME 117
	Subgroup 'b' - TME 6
	Subgroup 'c' -TME 7 and TME 3
	Subgroup 'd' - TME1 and TME 28

This finding may indicate that SSR markers can successfully determine genetic relationships among genotypes and identify genotypes.

The similarity value among 24 genotypes from diverse geographic regions ranged from 0.43 to 0.86. This may imply low genetic variability among the 24 cassava genotypes studied. This narrow variability is a drawback from the point of view of breeders, because they need high genetic variability to improve agronomic traits and the genotypes are selected only based on very few agronomic traits such as maturation time, height and yield. Increasing genetic variability is crucial to breeding programs.

For this aim, the closely related species could be the useful source of variation for plant breeders because within the genus interspecific crosses are usually feasible and efficient in terms of selection pressure [19]. In conclusion, using genotypes from different eco-geographical groups in breeding program will allow widening of genetic base in cassava.

The SSR markers efficiently discriminated all genotypes in this study. Hence, they may be readily used in understanding relationship level, establishing germplasm collections and integrating markers into genetic linkage maps establishing germplasm core collections.

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