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Research Article
Genetic Variability and Development of Cassava Based Products Using Morphometric and RAPD Markers

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Abstract

Background and Objective: Cassava (*Manihot esculenta* Crantz) and its product development are important to the diversification of the crop to enhance income, food sufficiency and security. Genetic variability among 12 Cassava (*Manihot esculenta* Crantz) varieties were assessed using morphometric and RAPD markers aimed toward product development from the varieties. **Materials and Methods:** Twelve morphometric characters and five random primers were employed in the genetic assessment analyses using descriptive statistics, Correlation Coefficient (CC) and Cluster Analysis (CA). **Results:** All morphometric characters were significantly different ($p \geq 0.01$) for the varieties. Harvest index (Hi) ranged from 0.41-0.46. The five random primers with an average of 55.2% polymorphism generated 139 polymorphic bands with primer P7 generating 68.05% of the cumulative variability observed. The RAPD analysis complemented the morphometric evaluation. The cluster analysis segregated the varieties into two major cluster groups with similar outcomes. **Conclusion:** The study provides improved understanding of the genetic basis of the varieties which can be exploited toward product development for commercial purpose and to ensure food security.

Key words: Cassava, *Manihot esculenta*, morphological, RAPD markers, product development

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important food crops in the sub-saharan Africa[1,2]. More than 500 million people in tropical areas depended on cassava for survival. Recent statistics on its global production was approximated[3] as 252 m t. In developing countries cassava is often cultivated by subsistence farmers, since the crop presents easy propagation systems, high drought tolerance and low demand for nutrients, producing reasonably well under critical conditions of climate and soil[4,5]. Cassava as a multipurpose food crop can be processed into chips, pellets, flour, alcohol or starch and used in a variety of industries[4,6,7]. The root is the most important product of cassava as food since it is a well-known material of high quality starch. In many African countries including Senegal, Ivory Coast and Sierra Leone, cassava leaves are an excellent source of proteins and vitamins[8,9]. In comparison with roots, the leaves are almost completely neglected in commercial terms, although they are available in abundance and there is considerable potential for its exploitation[10-12].

Major constraints to cassava cultivation and utilization includes low protein content of tubers, high content of the toxic glycosides; linamarin and lotaustralin in all tissues and poor shelf-life of tubers-microbial or secondary deterioration within 5-7 days after harvest[13-16].

Assessment of genetic diversity of important crops has been evaluated using morphological, biochemical and molecular markers. The genetic diversity useful for varied utilization especially for product development at local levels is integral to improved utilization and food security[17]. It is therefore, important that such efforts work directly with stakeholders particularly farmers and consumers as well to understand varied needs for cassava cultivation, processing and improvement of such cassava-based products such as garri (cassava flakes), cassava flour, chips and ethanol as well as development of new products[17].

Several varieties, particularly localized landraces have not been properly characterized to increase the utilization potentials and hence the need to characterize the accessions. In addition, there is the ever-increasing need to diversify the cassava utilization spectrum of the crop beyond food uses as recent studies have shown. Similarly, this study seeks to complement phenotypic characterization with molecular evaluation in the effort to investigate the genetic intra-specific variability important for utilization and genetic improvement. Therefore, the present study involved genetic characterization of some accessions of cassava using morphological and Random Amplified Polymorphic DNA (PCR-RAPD) as a pilot study toward cassava based product development.

MATERIALS AND METHODS

Areas of study: The study was carried out from April, 2016 to March, 2017 at the cassava experimental farm of Covenant University, Ado-Odo, Ota, Ogun state, Nigeria (latitude 6°37' N, longitude 3°42' E and altitude 41 m). The region is humid with average temperature of 28°C. The average rainfall is 1000 mm. The people of the area are mostly farmers, traders, artisans and government workers. Few agro-allied companies are located within the area which supports the agrarian nature of the area.

Stem acquisition and cultivation: A total of 20 varieties of cassava were collected: Ten from the Genetic Resources Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state and ten from farmers in Oyo, Osun, Ogun and Ekiti states, Nigeria. The passport data of samples collected was presented in Table 1. Twelve varieties were selected (Table 1), cultivated and used for this study. Experimental design and layout followed the procedures of Agre *et al.*[18]. Five cuttings (20-30 cm size) of each accession was planted horizontally by 100×100 cm per replicate (5×3×12) totaling 180 plant stands. No artificial/organic fertilizers were applied to the field/plant throughout the period of study.

Data collection: Standard descriptors for cassava were adopted and 12 morphometric characters selected for evaluation based on procedure of Fukuda *et al.*[19]. The characters were measured, weighed, counted and recorded in SI units. Measurements lasted for 9 months after planting and were recorded at 3 months interval. Harvest index (Hi) was evaluated according to Fukuda *et al.*[19].

DNA extraction, source of primers and quantification: DNA was extracted from young leaves from the apical region of each variety using CTAB method[20]. Ten primers with order number NG2017/4809 were synthesized and supplied by Inqaba biotechnical Industries (Pty) Ltd., South Africa. The primers were screened for polymorphisms out of which five were selected and used for this study based on clear bands and polymorphism. The concentration and purity of the extracted DNA was monitored using spectrophotometer

Table 1: Variety numbers and sources of planting materials used for the study

Varieties	Sources	Types	Local Name	Area	L/G	State
IBA010040	IITA	Breeding line	NA	IITA	Akinyele	Oyo
IBA950289	IITA	Breeding line	NA	IITA	Akinyele	Oyo
IBA980505	IITA	Breeding line	NA	IITA	Akinyele	Oyo
IBA011568	IITA	Breeding line	NA	IITA	Akinyele	Oyo
IBA011371	IITA	Breeding line	NA	IITA	Akinyele	Oyo
IBA980581	IITA	Breeding line	NA	IITA	Akinyele	Oyo
IBA30572	IITA	Breeding line	NA	IITA	Akinyele	Oyo
TMEB419	IITA	Breeding line	NA	IITA	Akinyele	Oyo
TMEB91934	IITA	Breeding line	NA	IITA	Akinyele	Oyo
1089A	IITA	Breeding line	NA	IITA	Akinyele	Oyo
OsNo001	Farmer	Landrace	Oko Iyawo	Ipetumodu	Ife North	Osun
OsNo002	Farmer	Landrace	NA	Akinola	Ife North	Osun
OyNo003	Farmer	Landrace	Arubielu	Ago-Are	Atisbo	Oyo
OyNo004	Farmer	Landrace	Safele/ege dudu	Iseyin Road	Ojongbodu	Oyo
OgNo005	Farmer	Landrace	Gbaguda	FUNAAB	Odeda	Ogun
OgNo006	Farmer	Landrace	Safele/ege dudu	Odeda	Odeda	Ogun
OgNo007	Farmer	Landrace	NA	Atan	Ado-Odo	Ogun
EkNo008	Farmer	Landrace	Unknown	Oye-Ekiti	Oye-Ekiti	Ekiti
EkNo009	Farmer	Landrace	Unknown	Oye-Ekiti	Oye-Ekiti	Ekiti
OyNo010	Farmer	Landrace	Oko Iyawo	Agurege	Atisbo	Oyo

Table 2: Descriptive statistics among the 12 varieties of cassava studied

Characters	Mean	SE	Range (cm)	Variance	Significance
LLL	18.16	0.70	13.88 (IBA011371)-22.68 (OsNo001)	5.89	p<0.01
WLL	4.47	0.12	3.80 (IBA30572)-5.3 (IBA980581)	0.18	p<0.01
RL	4.09	0.17	3.18 (IBA980581)-4.97 (OsNo001)	0.33	p<0.01
PL	27.44	1.11	22.42 (IBA011371)-35.5 (IBA950289)	14.89	p<0.01
PLH	262.93	7.24	218.00 (TMEB91934)-298 (TMEB419)	628.50	p<0.01
HFB	210.67	7.86	173.80 (TMEB91934)-261 (TMEB419)	740.69	p<0.01
NSR	9.09	0.82	6.00 (IBA980505)-15 (IBA010040)	8.08	p<0.01
NCR	5.25	0.55	1.00 (IBA010040)-8 (TMEB419,12)	3.66	p<0.01
NR	8.99	0.21	4.70 (IBA011371)-12.6 (OsNo002)	1.35	p<0.01
FR	10.27	1.20	8.70 (IBA980581)-14.2 (IBA950289)	2.67	p<0.01
Hi	0.45	0.12	0.41 (1089A)-0.46 (OsNo002)	0.23	NS

LLL: Length of leaf lobe, WLL: Width of leaf lobe, RL: Ratio of lobe, PL: Petiole length, PLH: Plant height at 9 months, HFB: Height at first branching, NSR: Number of storage root/plant, NCR: Number of commercial root/plant, NR: Number of root, FR: Fresh root, Hi: Harvest index

wavelength 260:280 nm using the NanoDrop ND-1000 Spectrophotometer. Extracted DNAs were stored at -40°C until used for RAPD-PCR.

RAPD-PCR characterization: The PCR amplification was carried out in a 25 µL reaction mixture containing 5 µL of DNA, 1.0 µL of 10 X standard *Taq* reaction buffer, 0.5 µL of 10 mM dNTPs, 1.0 µL of standard primer, 0.125 µL of *Taq* DNA polymerase and 15.875 µL of nuclease-free water. DNA amplification was performed in a thermocycler (C1000 Touch) programmed at initial denaturation temperature of 95°C for 30 sec, followed by 30 cycles of 30 sec at 95°C, 1 min at 45°C, 1 min at 68°C, a final stage of 5 min at 68°C and maintained at 4°C prior to analysis. The amplification products were electrophoresed on 1% agarose gels in 0.5XTBE buffer, stained with ethidium bromide and photographed under UV light.

Data analysis: Mean values of morphometric traits were estimated using Excel Microsoft (2013). The data were analyzed using descriptive statistics, one way ANOVA, coefficients of variation (CV %), Pearson Correlation Coefficient (PCC) and Cluster Analysis (CA). All statistical analyses were performed using SPSS and Paleontological Statistics Software package (version 3.15 for windows: Ohio, USA). Statistical significance was set at p<0.01. Cluster Analysis (CA) was performed based on Euclidean Distance using Unweighted Pair-Group Method of Arithmetic Averages (UPGMA). The total number of DNA bands and polymorphic bands were estimated and percentage polymorphisms recorded. Data matrix from RAPD profiles were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were subjected to genetic similarity matrix to generate cluster analysis.

RESULTS

Morphometric variation and harvest index (Hi) among the 12 varieties of cassava studied: The data in Table 2 showed the mean, SE, range, variance and significant differences of each quantitative trait of the cassava varieties evaluated.

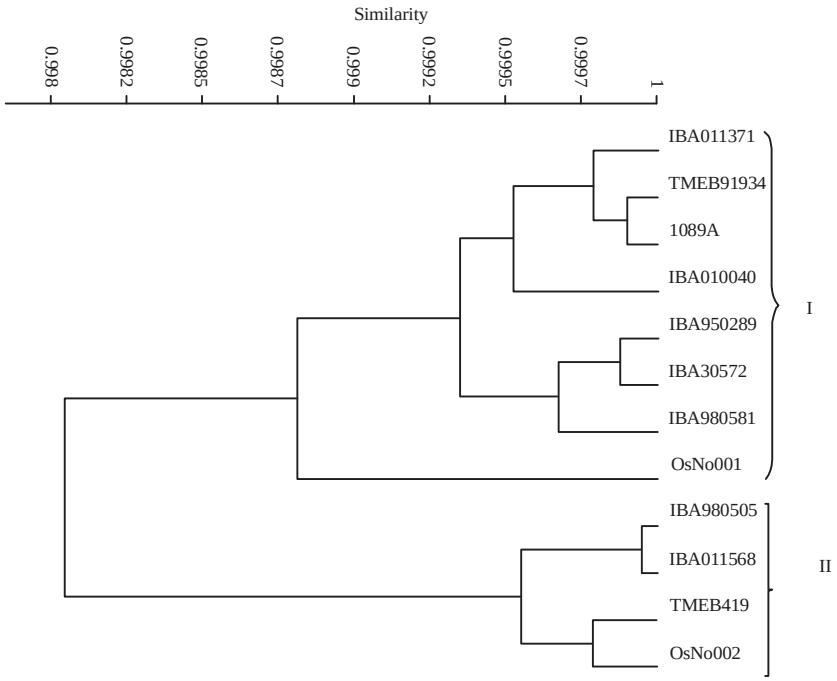

Fig. 1: Morphometric cluster analysis of the 12 varieties of cassava studied

Table 3: Correlation coefficients of 8 quantitative characters of the 12 cassava varieties studied

Characters	LLL	WLL	RL	PL	PLH	HFB	NSR	NCR
LLL	1							
WLL	0.25	1						
RL	0.77**	-0.42	1					
PL	0.42	0.10	0.37	1				
PLH	0.18	0.45*	-0.17	0.22	1			
HFB	0.16	0.41	-0.17	-0.01	0.94**	1		
NSR	0.21	0.13	0.11	0.08	-0.18	-0.12	1	
NCR	0.36	0.26	0.17	-0.36	0.01	0.22	-0.19	1

**Correlation is significant at 0.5 level (greater significance), *Correlation is significant at 0.45 level (moderate significance), LLL: Length of leaf lobe, WLL: Width of leaf lobe, RL: Ratio of lobe, PL: Petiole length, PLH: Plant height at 9 months, HFB: Height at first branching, NSR: Number of storage root/plant, NCR: Number of commercial root/plant

Length of leaf lobe ranged from 13.88 in IBA011371 to 22.68 in OsNo001, width of leaf lobe ranged from 3.8 (IBA30572) to 5.3 (IBA980581) while ratio of lobe ranged from 3.18 (IBA980581) to 4.97 (OsNo001). The number of root ranged from 4.7 in IBA011371 to 12.6 in OsNo002 while fresh root ranged from 8.7 in IBA980581 to 14.2 in IBA950289. There was no significant difference among the varieties with respect to harvest index.

Pearson correlation coefficients of the cassava accessions studied: The Table 3 showed correlation coefficients among eight quantitative characters of the cassava varieties studied. Ratio of leaf lobe (RL) positively and strongly ($p \leq 0.5$) correlated with length of leaf lobe (LLL) r = +0.77 while plant height at 9 months (PLH) was moderately correlated with width of leaf lobe (WLL) r = +0.45 and height at first branching (HFB) correlated with plant height at 9 months (PLH) r = +0.94.

Morphometric cluster analysis: At the similarity coefficient of 99.85%, the morphometric cluster analysis segregated the 12 varieties into two major cluster groups. Cluster group I consisted of 8 varieties (IBA011371, TMEB91934, 1089A, IBA010040, IBA950289, IBA30572, IBA980581 and OsNo001) while cluster group II comprised 4 varieties (IBA980505, IBA011568, TMEB419 and OsNo002). Figure 1 showed the cluster analysis and major cluster groups of the 12 varieties of cassava studied.

RAPD analysis: The sequences of each primer, the total number of bands per primer, polymorphic bands and

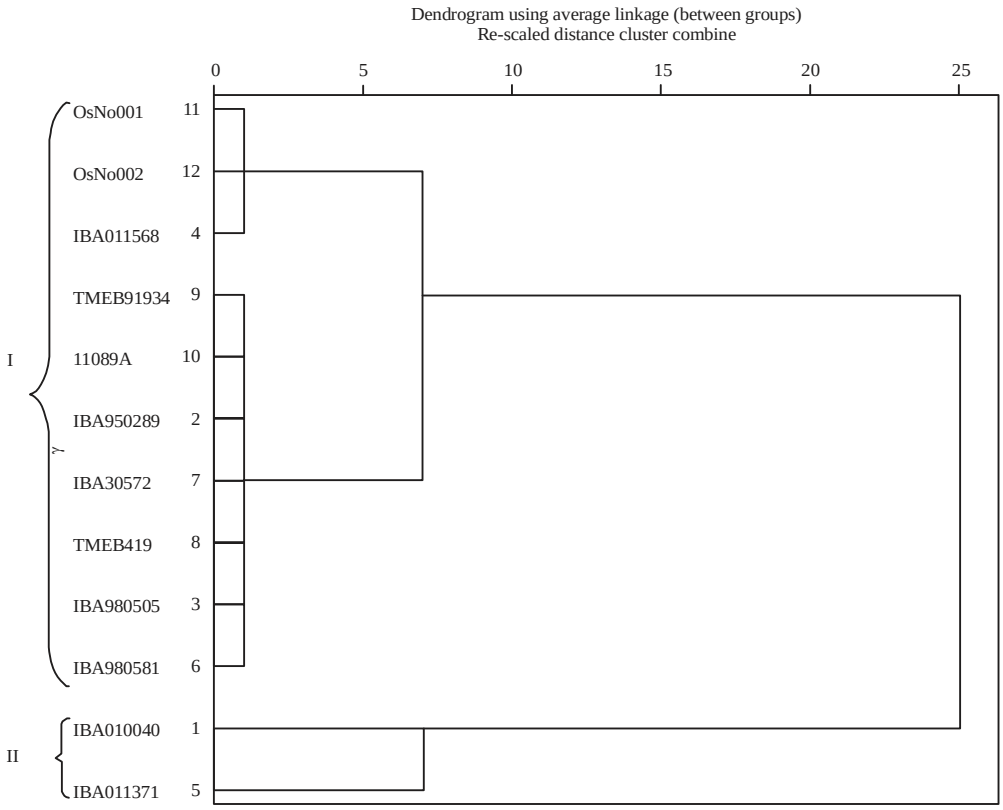

Fig. 2: RAPD cluster analysis of the 12 varieties of cassava studied

Table 4: Primers, sequences, number of bands, polymorphic bands and percentage polymorphic

Primers	Sequences	Number of bands	Polymorphic bands	Percentage
P1	CATTCGAGCC	60	23	38.3
P2	GTCTCCGCAA	24	12	50.0
P4	CCCGCTACAC	48	24	50.0
P5	AGCGAGCAAG	48	27	56.3
P7	CACAGGCGGA	72	49	68.05

Table 5: Summary of amplification patterns generated by the random primers used for this study

Parameters	Values
Total number of primers screened with all the 12 cassava accessions	10.0
Number of primers that produced polymorphic bands	5.0
Total number of bands amplified by the primers that generated polymorphic bands	252.0
Average number of bands per primer	50.4
Total number of polymorphic bands	139.0
Percentage of polymorphic bands	55.16%
Average number of polymorphic bands per primer	27.8

percentage polymorphisms were recorded in Table 4. Number of amplified fragments per primer ranged from 12 in primer (P2) to 49 in primer (P7). Primer P7 produced the highest number of polymorphic bands (49) with 68.05% polymorphisms while P2 produced the least (12) with 50% percentage polymorphisms (Table 4). The summary of amplification patterns generated by the five random primers is shown in Table 5.

RAPD cluster analysis: The IBM SPSS Statistics 20 segregated the 12 varieties of cassava studied into two major groups. Group I consisted two sub-groups, sub-group I made up of three varieties OsNO001, OsNO002, IBA011568 while sub-group II consisted of 7 varieties (TMEB91934, 1089A, IBA950289, IBA30572, TMEB419, IBA980505 and IBA980581). Cluster group two consisted of 2 varieties (IBA010040 and IBA011371) (Fig. 2).

DISCUSSION

Characterization and genetic variability studies enable effective selection of genotypes for hybridization for sound crop improvement and other utilization purposes[21,22].

The varieties studied showed good and promising agronomic traits useful for breeding and utilization purposes particularly towards product development. The number of storage root/plant was higher among the breeding lines compared to the two landraces variety. Varieties IBA010040 and TMEB419 showed higher number of storage root and commercial root per plant with higher harvest index values. These two varieties could be selected for breeding higher yield root yield, hence utilization as commercial root source for product development such as cassava chips, garri and ethanol. TMEB419 recorded higher number of commercial root/plant compared to OsNo001 while OsNo002 performed better with respect to number of yield related parameters. However, variety OsNo001 collected from farmers (Osun state) recorded higher leaf lobe length indicating higher photosynthetic rate which guarantees higher yield of cassava leaves and tubers for utilization purposes and hence product development. Growth dynamics, biomass, root yield and related traits have all been implicated in the sustainable utilization and production of amylose-free cassava starch and other derivable products[23,24]. Length of leaf lobe, width of leaf lobe, ratio of lobe, number of storage root/plant and number of commercial root/plant contributed significantly to the variations observed. Of all these traits however, both number of storage root per plant and number of commercial root per plant recorded significant values higher than 0.2 an indication that the breeding line TMEB91934 developed at IITA and in cultivation share considerable attributes with the two landraces variety collected from farmers (OsNo001 and OsNo002) as commercial tuber cultivars. These traits are heritable with significant correlation valuable in the selection of parent varieties for possible hybridization trials, breeding and product development which aligns with the study of Agre *et al.*[18], Sanoussi *et al.*[21] and Nadjiam *et al.*[24].

The level of polymorphism from this study is comparable to other RAPD analysis of cassava in Nigeria and elsewhere[25,26]. Variety OsNo001 that was isolated in the morphometric groupings was clustered in group I of RAPD with diverse varieties indicating higher genetic similarity. However, variety OsNo002 could possibly be the same variety with OsNo001 and could be cultivated in large scale for flour production similar to variety IBA011568 grouped together in cluster group I. The clustering of the two groups clearly suggested that individuals from each group could be explored for similar utilization purposes particularly cassava chips, flakes and flour production. The groupings also reflect the merit of RAPD over morphometric markers.

CONCLUSION

Selections based on the morphometric traits and cluster groupings identified in this study could contribute immensely to higher root productivity and for varied utilization as source of raw material for product development. The preliminary products generated from the 12 varieties are currently being evaluated for biochemical and quality assurance analyzes.

SIGNIFICANCE STATEMENT

The study discovered the genetic relationships and diverse utilization of the 12 varieties that can be beneficial toward product development for immediate and future commercial exploitation. The study will help the researcher to uncover the critical areas of cassava diversification that many researchers were not able to explore. Thus a new theory on genetic variability for varied product development of cassava using the studied varieties may be arrived at.

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