

Molecular Characterization of 36 Accessions of Two Genera of Cocoyam (*Colocasia* [Schott] and *Xanthosoma* [Schott], Araceae)

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Abstract	Article Information
<p>Most popular edible aroid commonly called cocoyam belong to the genera <i>Colocasia</i> (Schott) and <i>Xanthosoma</i> (Schott); are tuber crops introduced into Africa from Asia and tropical America respectively. These crops are grown in all parts of tropical and sub-tropical regions where they have high adaptability, acceptance and serve as auxillary crop plant for food and income security. The present study aims to collect cocoyam germplasm from local agriculture systems in Edo state, Nigeria and characterize them using molecular marker techniques. Random stratified sampling method was used to collect the plant genetic resources based on IBPGR and IPGRI descriptors. Collecting cocoyam germplasm is first reported in Nigeria in this study. Collected germplasm were planted out for trial in a home garden plot situated in the University of Benin, Nigeria. After 6 months, leaves were harvested for Random Amplified Polymorphic DNA (RAPD) analysis to characterize the accessions. RAPD-PCR was conducted at Biochemistry Division, Nigerian Institute of Medical Research (NIMR), Yaba-Lagos state. Differences in the pattern of bands produced after gel electrophoresis reveal the crops are diverse within the state. Dendrogram constructed from the data matrix suggests two trees with varied number of lines. These lines were inferred as different varieties of the species of the two genera. Some of the accessions precisely IA, HD, BB and JC were not clustered into any group. This suggests that these accessions are unique and distinct within and between the two genera of cocoyam. The banding pattern were similar within species and different between species, but was not able to distinguish all the varieties from one another. Cluster analysis gave 8-10 groups in two branches corresponding to specific associations with little filtration observed among these groups. The dendrogram implicates accession BB as been unique among all the thirty six accessions studied. Its intermediate response suggests either a branch off point or pure line. Jaccard's similarity clustered the accessions into six groups. The RAPD-PCR could serve as a basis for other characterization works on the 36 accessions of cocoyam for the basis of conservation and sustainable utilization</p>	<p>Article History:</p> <p>Received : 02-01-2015</p> <p>Revised : 13-03-2015</p> <p>Accepted : 18-03-2015</p> <hr/> <p>Keywords:</p> <p>Cocoyam</p> <p><i>Colocasia</i> and <i>Xanthosoma</i></p> <p>RAPD-PCR Characterization</p> <p>Molecular technique</p> <p>Arable crops</p> <p>Plant Conservation</p> <hr/> <p>*Corresponding Author:</p> <p>Ogwu, M.C</p> <p>E-mail:</p> <p>matthew.ogwu@uniben.edu</p>

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INTRODUCTION

Cocoyam is a non-specific terms, applied to various edible aroids mostly members of the monocotyledonous family Araceae and order Alismatales (Onwueme, 1978; Singh, 2004; APG, 2009; Mwenye, 2009). According to economic classification Cocoyams are root and tuber crops. Roots and tuber crops are among the oldest, most domesticated and important crops worldwide (Perez-Ponce, 2007; Lebot, 2009; Owusu-Darko *et al.*, 2014). *Colocasia* [Schott] and *Xanthosoma* [Schott] species (Cocoyam) are tuber crops consumed all over the tropics almost all year round. They are important tuberous vegetables cultivated for their edible tubers and tender leaves (Morton, 1972; Suja *et al.*, 2009). However, little is known about their extent of production, cultivation, types as well as the taxonomy of the plant (Talwana *et al.*, 2009, Baruwa and Oke, 2012).

There are two major varieties of *Colocasia*, namely *C. esculenta var esculenta*, with a large central corm, with suckers and stolons and *C. esculenta var antiquorum*, characterized with a small central corm and a large number of smaller cormels (Gomez-Beloz and Rivero, 2006; Seetohul *et al.*, 2008). Most edible species of *Xanthosoma* are either *X. sagittifolium* or *X. maffafa* (Okoye *et al.*, 2006). Their corms are often difficult to distinguish from those of *C. esculentus* after their leaves have been removed (Matthews, 2010). Many species and varieties exist in the genus *Xanthosoma*.

Colocasia is a major plant in South Eastern Nigeria (Opara *et al.*, 2013) whereas *Xanthosoma* is popular in South Southern and South Western regions of Nigeria. However, both genera are characteristically distinct and localized to different ecozones in Nigeria. Both genera are exotic to Africa. Introduction and spread occurred through

different routes. *Colocasia* has been cultivated in suitable parts of southern Africa for centuries and was probably introduced by the Portuguese traders. Its center of origin of *Colocasia* is thought to be South East Asia most probably in Eastern India and Bangladesh. *Xanthosoma* is thought to be from the new world most probably Bolivia and Nicaragua. *Xanthosoma* species are reported to have originated and first domesticated in tropical America, especially Central America and the Caribbean. It has since spread to South East Asia, the Pacific Islands and Africa. The spread of the crop to the South Pacific and Africa occurred in recent times (Onwueme, 1978; Janseens, 2001). It was spread by the Spanish and Portuguese, who also introduced it to Europe and Asia. It moved from the Caribbean, in the late 19th century to Africa, first to Sierra Leone and then to Ghana (FAO, 1990). *Colocasia* most likely spread through the African Continent, probably from Zanzibar in the east and the Mediterranean in the north (Eland, 2008) or through Egypt, the Nile basin (FAO, 1990; Mwenye, 2009).

In Nigeria especially southern political states, these crops thrive well in difficult terrains as weed as well as in cultivated land with poor record regarding yield and marketing systems. These crops contribute to food and income security in the Southern region of Nigeria. These notwithstanding, species of these genera are vulnerable to many factors. For example, *Colocasia esculentus* is seriously threatened to extinction in south eastern Nigeria as a result of its high susceptibility to bacterial leaf blight disease (Opara *et al.*, 2013). The focus of the world on a small number of crops for her needs is increasing the number of underutilized or neglected plants (Adelekan, 2012). Thus, there is an immediate need for collection, characterization and documentation in this study.

Beside morphological characterization, the use of biochemical and molecular characterization are becoming increasing important as they suggest more consistent and specific results. In spite of their relevance cocoyam is yet to receive deliberate research and development effort within tropical regions (Talwana *et al.*, 2009). According to Isikawa *et al.* (1989) they are applied in different systems to characterize plants and can be used as tags since they are located in loci (chromosomes) where they control specific traits within the organism after interaction with the environment. Molecular markers have several advantages over morphological markers for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait (Mwenye, 2009). Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs) and Microsatellites or simple sequence repeats (SSRs) are some of the marker techniques that have been developed and used for plants. The development of Polymerase Chain Reaction (PCR) led to the introduction of RAPD. DNA markers reveal polymorphism in a DNA sequence, or the presence or absence of a particular DNA sequence at a particular site in the genome (normally a restriction site or polymerase chain reaction (PCR) primer binding site). Plant genetic resources are an invaluable resource. The ability to identify genetic variation is indispensable to effective management and use of genetic resources (Rao, 2004). Beeching *et al.* (1993) stated that a prerequisite for any genetic improvement programme is knowledge of the extent of genetic variation present between genotypes

and the genetic distance between all closely related species with which hybrids could be produced. This can be achieved through the characterisation of the germplasm using either morphological, biochemical or genetic markers (Mwenye, 2009). The knowledge of genetic diversity result from analysis and characterization allows evaluation of genetic variability, which is a fundamental element in determining breeding strategies and genetic conservation plans (Gholiazadeh *et al.*, 2008, Mwenye, 2009).

This study is aimed at collecting cocoyam germplasm from southern parts of Nigeria (Edo state); to characterize using Random Amplified Polymorphic DNA analysis (RAPD); generate data that will be beneficial in breeding experiments and genetic conservation of the crop while contributing to knowledge on the taxonomy of these genera and understand observed agronomic polymorphism.

MATERIALS AND METHODS

Study Area: This lies between Longitude $06^{\circ} 04' E$ $06^{\circ} 43' E$ and Latitude $05^{\circ} 44' N$ and $07^{\circ} 34' N$. The climatic conditions are characterized by two distinct conditions of wet and dry seasons. It experiences high rainfall and humidity for most of the year with an annual average rainfall of 500 – 2,780 cm (Ikhile and Aifesehi, 2011). The vegetation is typical of tropical rainforest and slightly of a derived savannah (Osawaru and Ogwu, 2014).

Collection of Cocoyam Germplasm: Cocoyam germplasm missions were carried out in the study area in collaboration with the Plant Conservation Unit, Department of Plant Biology and Biotechnology, University of Benin, Benin City and the local indigenes between January and June 2013. Random stratified sampling was used to select farmers, home gardeners, and senior citizens and semi structured questionnaires were administered. Identification was based on IBPGR (1980) and IPGRI (1991) descriptor for *Colocasia* and *Xanthosoma* respectively. Fifty-four accessions were collected. For each accession, a detailed passport data was recorded. They were kept in the Department of Plant Biology and Biotechnology, for further evaluation and studies. The corms were put into miniset of about 100 g each waiting planting. Cut surfaces of the corm were allowed to dry by exposing it to sunlight for 2 days.

Planting Plot and Husbandry: Planting was done in a home garden plot in Benin City situated at Dentistry Quarters, University of Benin Teaching Hospital. Randomize block design (RBD) was used. The most important agronomic practices during cultivation was daily wetting with 1 liter of distilled water per stand as well as weeding, done once every three weeks.

Collection of Leaf Materials for RAPD-PCR Analysis: After 6 months, from each individual 2 - 3 fresh matured leaves were collected from 36 accessions based on the peculiarity attached by the donor and observations during trials, labelled and kept in polythene bags containing silica gel and stored in ambient temperature until DNA extraction. DNA extraction and RAPD-PCR was done at the Laboratory of Biochemistry Division, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos state, Nigeria. DNA extraction was done on all the accession but due to cost implication only thirty six randomly selected accessions were used for RAPD-PCR (Table 1).

Table 1: Passport information of the 36 accessions of cocoyam used for RAPD analysis

No	Accession No.	LGA	Exact Collection Point	Genus
1	ED/AC/AKD005	Akoko Edo	Igarra-Bokessimi	<i>Colocasia</i>
2	ED/AB/AKD003	Akoko Edo	Igarra-Bokessimi	<i>Colocasia</i>
3	ED/BA/EGR001	Egor	Upper Siluko	<i>Xanthosoma</i>
4	ED/BB/EGR003	Egor	Owode-Siluko	<i>Xanthosoma</i>
5	ED/CB/ECT003	Esan Central	Irrua	<i>Xanthosoma</i>
6	ED/DC/ENE005	Esan North East	Uromi Ogbidi	<i>Colocasia</i>
7	ED/DB/ENE003	Esan North East	Uromi-Agbor rd	<i>Colocasia</i>
8	ED/EA/ESE001	Esan South East	Ubiaja-Ariah	<i>Xanthosoma</i>
9	ED/EC/ESE005	Esan South East	Ubiaja-Ukhualen	<i>Colocasia</i>
10	ED/FA/EWE001	Esan West	Iruekpen	<i>Colocasia</i>
11	ED/FC/EWE005	Esan West	Opoji	<i>Colocasia</i>
12	ED/GA/ETC001	Etsako Central	Fugar-Ogbona	<i>Xanthosoma</i>
13	ED/GC/ETC005	Etsako Central	Avianwu	<i>Xanthosoma</i>
14	ED/HA/ETE001	Etsako East	Agenebode	<i>Colocasia</i>
15	ED/HC/ETE005	Etsako East	Igiode	<i>Xanthosoma</i>
16	ED/IA/ETW001	Etsako West	Avielle Ubiane	<i>Xanthosoma</i>
17	ED/IC/ETW005	Etsako West	Aviele Ubiane	<i>Xanthosoma</i>
18	ED/JC/IGB005	Igueben	Idumogo	<i>Colocasia</i>
19	ED/IB/IGB003	Igueben	Eguare	<i>Colocasia</i>
20	ED/KA/IKB001	Ikpoba okha	Ikpoba hill	<i>Colocasia</i>
21	ED/KB/IKB003	Ikpoba okha	Idogbo	<i>Colocasia</i>
22	ED/LB/ORD003	Oredo	3 rd Ogbewise	<i>Colocasia</i>
23	ED/LC/ORD005	Oredo	Okpenede	<i>Colocasia</i>
24	ED/MA/ORH001	Orhionmwon	Abudu	<i>Xanthosoma</i>
25	ED/MB/ORH003	Orhionmwon	Uvbe	<i>Xanthosoma</i>
26	ED/NC/ONE001	Ovia North East	Ekhiador	<i>Xanthosoma</i>
27	ED/NA/ONE001	Ovia North East	Ofunwengbe	<i>Xanthosoma</i>
28	ED/OB/OSW003	Ovia South West	Ugbogui	<i>Colocasia</i>
29	ED/OC/OSW005	Ovia South West	Obaretin	<i>Colocasia</i>
30	ED/OA/OSW001	Ovis South West	Ikoha	<i>Xanthosoma</i>
31	ED/PA/OWE001	Owan East	Afuze	<i>Xanthosoma</i>
32	ED/PB/OWE003	Owan East	Afuze	<i>Xanthosoma</i>
33	ED/QB/OWW001	Owan West	Sabongida Ora	<i>Xanthosoma</i>
34	ED/QC/OWW005	Owan West	Ovbiokhumrin	<i>Xanthosoma</i>
35	ED/RA/UHW001	Uhunmwode	Eyaen village	<i>Xanthosoma</i>
36	ED/RC/UHW005	Uhunmwode	Ehor	<i>Xanthosoma</i>

DNA Extraction and RAPD-PCR Analysis: DNA was extracted using IDT Protocol adopted from Keb-Llanes *et al.* (2002). The concentration and purity of DNA was estimated spectrophotometrically at 260 nm, and 260/280 nm ratio was determined using a DU 530 Beckman spectrophotometer. Purity was also checked on a 0.8 % agarose gel electrophoresis, using 0.5X TBE buffer. All the stock DNA samples were maintained at -20°C. DNA samples were diluted to working concentration of 50ng/μl for use in RAPD analysis. The DNA aliquots were maintained at 4°C. Agarose gel QC was carried out to assess the extracted DNA.

RAPD-PCR reactions were carried out using the Ready-To-Go RAPD Analysis beads, which are room temperature-stable were optimized for RAPD reactions. Each bead contains thermo stable polymerases (AmpliTaq™ DNA polymerase and Stoffel fragment), dNTPs (0.4mM each dNTP in a 25 μl reaction volume), BSA (2.5μg) and buffer (3mM Magnesium Chloride, 30mM Potassium Chloride), and 10mM Tris (pH 8.3) in a 25μl reaction volume. To this 25pmol of primer and 50ng of genomic DNA were added.

Amplification was carried out on a Techne Master Thermocycler, Ver B. 1.7 PCR machine. The machine was programmed to 1 cycle of 5 minutes of initial denaturation at 94 °C. This was followed by 40 cycles of 1 minute at 94 °C for denaturation, 1 minute at 36 °C for annealing, and 2 minutes at 72 °C for extension. The final extension was carried out at 72 °C for 7 minutes. The primer set used for the present analysis is OPA-9 a 10-mer RAPD primers. Attempts were made to utilize OPA-2

AND OPA-13 but both produced no electrophoretic bands. OPA-9 was screened and used in RAPD-PCR of extracted DNA from Cocoyam. The sequence of the primer is given thus 5'- GGG TAA CGC C -3'.

After the PCR, 6μl of the RAPD products were resolved on a 1.6 % agarose gel using 0.5X TBE buffer. To carry out the agarose gel electrophoresis, molten agarose was used and Ethidium bromide and mixed well. This was poured into the electrophoresis cast and allowed to solidify at room temperature. When the agarose was solidified, the comb was removed and electrophoresis apparatus was filled with 0.5X TBE. The samples were mixed with 2μl of the loading buffer and loaded in the wells. A 1000bp ladder was also loaded in one of the wells. This was then electrophoresed at a constant voltage of 70V. The gel product was placed on a UV transilluminator and photographed under using a 15 mega pixel Sony digital camera.

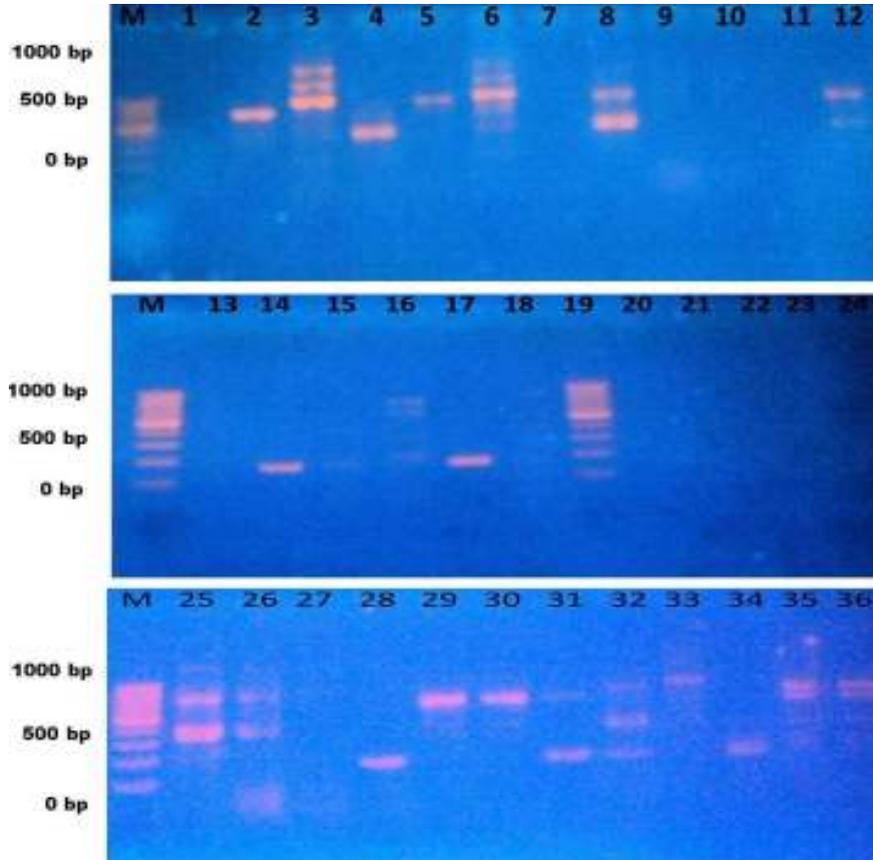
Data Analysis: Dominant marker exhibits a present/absent behaviour on the electrophoresis gel. A matrix was generated assuming each RAPD band as a unit character, and presence of a band was represented as 1 and absence as 0. This matrix was used for generating the Jaccard's similarity coefficient for RAPD bands using Multivariate Analysis System of Multivariate Statistical Package (MSVP). Dendrograms wasalso constructed to depict (1) each marker band represented the phenotype at a single biallelic, distinct locus and, (2) that populations are in Hardy-Weinberg equilibrium.

RESULTS

Results are presented in Plate 1 and Figures 1 and 2. The electrophoresis product of RAPD analysis is presented in Plate 1. The electrophoresis product showed the differential response of the different cocoyam accessions. No bands were obtained for some accessions at all the bp while others responded differently at each bp.

Clustering of cocoyam accessions based on Jaccard's similarity coefficient by MVSP software is presented in Figure 1. The two branches obtained separated the accessions into *Colocasia* and *Xanthosoma*.

Analysis of the binary matrix obtained from scoring electrophoretic product of the RAPD analysis based on their polymorphism is presented in Figure 2.



M = Marker, bp = Base pair

For the purpose of expressing RAPD-PCR result the accession number were used thus: 1= AC, 2= AB, 3= BA, 4= BB, 5= CB, 6= DC, 7= DB, 8 = EA, 9= EC, 10= FA, 11= FC, 12= GA, 13= GC, 14= HA, 15= HC, 16=IA, 17= IC, 18=JC, 19= JB, 20= KA, 21=KB, 22= LB, 23= LC, 24= MA, 25= MB, 26= NC, 27= NA, 28= OB, 29= OC, 30= OA, 31= PA, 32= PB, 33= QB, 34= QC, 35= RA, 36= RC

Plate 1: RAPD-PCR electrophoretic result of 36 accessions of Cocoyam

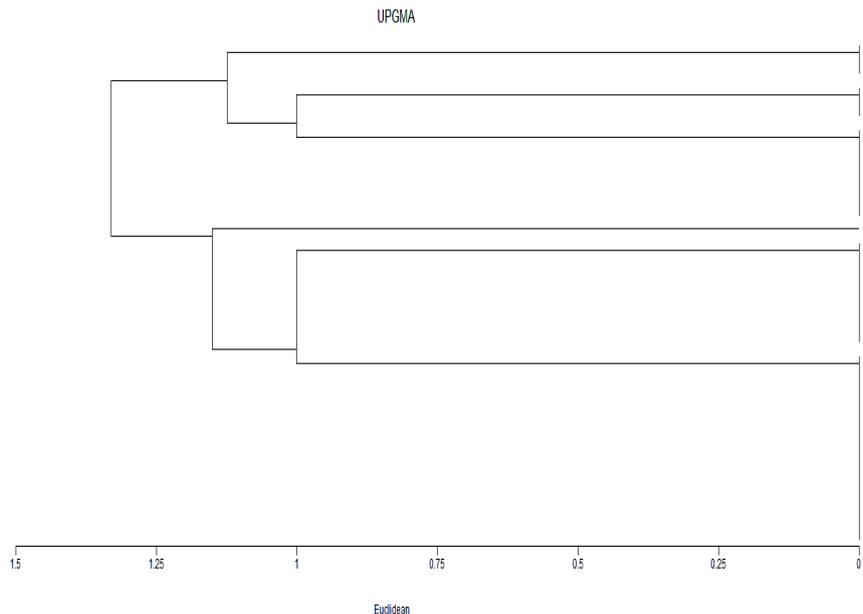


Figure 1: Dendrogram depicting the Jaccard's similarity coefficient of the 36 cocoyam accessions

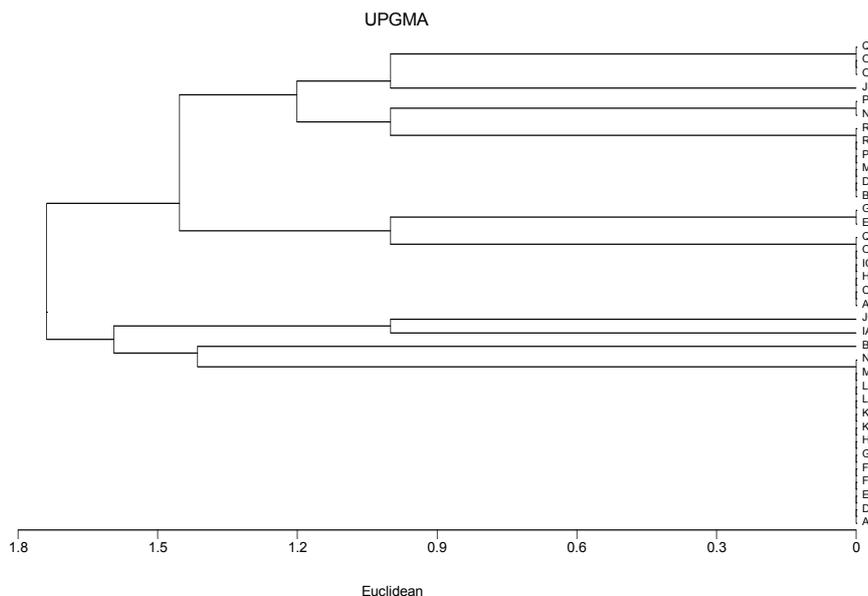


Figure 2: Dendrogram depicting the polymorphic characteristics of the 36 cocoyam accessions

DISCUSSION

Cocoyam is an important tuber crop in tropical and sub-tropical regions. These crops when properly harnessed can contribute to alleviate the suffering of good number of households (Baruwa and Oke, 2012). They have been ignored in spite of their adaptability, acceptance and commercial food value and have received little attention by researchers (National Academy of Science, 1975; Ekwe *et al.*, 2009, Mwenye, 2009; Global forum for underutilized species, 2009). All these can be implicated for the underutilized status of cocoyam. There is misunderstanding as to the use of vernacular names for this crop without distinction among the genera (Janseens, 2001, Mwenye, 2009). Names and naming for these crops need to be standardized to ease identification. The study attempt to provide data that could be used to elucidate key issues and improve knowledge on these crops. Knowledge of genetic diversity and relationships among cocoyam germplasm may play significant role in breeding programmes to biotic and abiotic requirements as well as conservation goals.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology as such information is integral for rational use of plant genetic resources (Aladele, 2009). Such information will be relevant in elucidating genetic relationship within and among species (Chakravarthi and Naravaneni, 2006). Markers and marker techniques are now essential to conserve and use plant genetic resources.

Despite these production figures little data is available on these crops. Different national and international organizations, local communities, and even researchers from different disciplines identify and evaluate biodiversity differently (Caillon and Degeorges, 2007). These can include most especially agro morphological, biochemical or anatomical and molecular considerations. In this study, attempts were made to understand the diversity within the cocoyam genera collected from different locations within Edo State, Nigerian. The primer OPA-9 gave 108 scorable bands (Plate 1). The morphological variants were distinguished by the primer used. This is similar to what Perez-Ponce (2007) reported for RAPD analysis of 44 accessions of cocoyam. A total of 44 amplicons were

generated in the study. These amplicons ranged between 0 bp and 1000 Bb. The amplification obtained showed that the 36 cocoyam accessions responded to the primer different. This was not in total agreement with the observations of Thaker *et al.* (2011), that one band absent in *Colocasia* and present in *Xanthosoma* shows some variants are present which cannot be easily placed in either genera. The presence of one band and/or the absence suggests two significant findings: (i) A distinct variation in DNA pattern was observed between two morphological variants of two cocoyam genera and (ii) Furthermore, DNA amplification patterns using RAPD primers showed polymorphism within DNA from morphological variants (Thaker *et al.*, 2011). DNA polymorphisms are indeed very useful for genetic mapping and can be defined as any difference in the nucleotide sequence between individuals including can be single base pair changes, deletions, insertions, or even changes in the number of copies of a given DNA sequence. SNPs (single nucleotide polymorphisms) (Nature Education, 2013).

RAPD analysis is generally considered a very good starting point for characterization studies of species (Williams *et al.*, 1990). This has also been proven true by the present investigation. This distinctions observed by their molecular characterization will be handy during morphological and biochemical characterization. More so, the results show that modified method is optimal for DNA isolation from morphological variants of taxa as in Cocoyam. Method of extracting pure DNA is important and is not the same for all plant species. The DNA extracted method adopted in this study can also be suggested as suitable for molecular analysis of the two genera.

The quality of extracted DNA was analyzed by means of agarose gel electrophoresis (0.8%), followed by ethidium bromide staining (0.5 mg/ml). The purity of the DNA was estimated by spectrophotometry by calculating the A260/280 and A260/230 ratios, and the yield was estimated by measuring absorbance at 260 nm. RAPD profile suggests DNA polymorphism among both variants, which proves that there was either environmental/chemical influence or certain variation that lead to

mutation in plant genome. For future studies, the primers selection can be extended in order to effectively compare their effects. Production of cocoyam in many tropical and subtropical countries is mainly for family subsistence through income and food security. In the study by Okoye *et al.* (2006) to determine allocative efficiency of cocoyam in part of Nigeria their result suggest that age, education, farm size, farm experience, fertilizer use and credit access have direct contribution less so for extension visit, family size and membership of cooperative societies.

RAPD results suggest two distinct gene pools as revealed from gel electrophoresis. This was also observed and exploited during the selected process for the markers and primers to be used. As markers and primers pooled for used were first tested to determine their efficiency in distinguishing them. Genetic mapping involves measuring the amount of recombination that occurs between two markers during meiosis. If there is a low amount of recombination between markers, then they are linked (i.e., located very close to each other on the same chromosome). Originally, the markers used by geneticists for genetic mapping experiments included alleles of genes that produced different physical phenotypes (traits) (Nature Education, 2013). DNA polymorphisms can be used in much the same way as these physical phenotypes. Different forms of DNA polymorphisms can be tracked using a variety of techniques. Some of these techniques include Southern blots, PCR (polymerase chain reaction), and hybridization techniques using DNA microarray chips. These techniques allow us to determine if a specific DNA polymorphism is linked to a specific phenotype. We can then conclude that the gene responsible for a given phenotype maps to a chromosomal location very close to the DNA polymorphism (Nature Education, 2013).

DNA polymorphisms are such a powerful tool for mapping human diseases that several projects are underway to identify DNA polymorphisms in humans and to make this data publicly available to scientists worldwide. Two groups that are involved in these massive efforts include the SNP consortium (TSC) and the DNA Polymorphism Discovery Resource. These genetic variations between individuals (particularly in non-coding parts of the genome) are exploited in DNA fingerprinting, which is used in forensic science. Also, these genetic variations underlie differences in our susceptibility to disease. Respond to pathogens, chemicals, drugs, vaccines, and other agents. The principle will be applicable in breeding programs.

The banding patterns were similar within species and different between species, but not able to distinguish all the varieties from one another. Cluster analysis on pooled data from the primers gave 8-10 groups corresponding to morphological species. In the dendrogram of Jaccard's similarity coefficients, six groups were identified and two trees formed individual branches of the dendrogram (Figure 4). The first cluster included four genotypes from region with 3 branches. The first and second clusters were made up of 3 accessions each; including PB, RA and QC (in the first cluster) and RC, NC and IA (in the second cluster). The third cluster in the first tree contains seven accessions including MB, NC, QB, MA, HC, EA and BA. The second tree with also three branches/clusters, the first cluster is the smallest with only accession BB

while the second cluster has eight accessions (OC, PA, IC, CC, GC, FA, DC and AC). The last cluster is the largest with fourteen accessions including OC, LB, LC, KB, KA, JC, JC, HA, CC, DC, GA, FC, EC and AC. The dendrogram implicates accession BB as been unic among all the thirty six accessions studied. It may be inferred that it belongs to a separate variety from either of the two genera of cocoyam. All the other accessions studied had similar genotypes as reflected by the dendrogram. More so, this also suggests that the pairs grouped together have the same genotype but collected at different locations and time.

Markers are assumed to amplify only dominant allele per locus hence amplification products as shown in figure 1 is assumed to refer to the allele at that locus. A locus can be considered polymorphic by the presence or absence of bands on particular accession that polymorphic when absence in some and monomorphic if presence in all the accessions (Al Nashash *et al.*, 2007). On this basis the dendrogram on figure 4 was constructed to depict accessions with polymorphic and monomorphic characteristics. A total of 10 groups were formed from the dendrogram. The first branch of the tree has six clusters thus: PB, RA and QC are group together, accession JC was isolated in its own group, RC and NC are grouped together, MB, NC, QB, MA, EA and BA; GC and FA while OC, PA, IC, CC, DC and AC were also place together in a group. The second branch of the tree has four groups mostly made up of monomorphic groups with only one accession including IA, HC and BB while the polymorphic group in this branch including 13 accessions (OC, LB, LC, KB, KA, JC, HA, OC, DC, GA, FC, EC and AC). The grouping of cocoyam accessions with similar banding pattern is in accordance with the results of Shnell *et al.* (1999). These suggest genetic variation exists among and within the accessions used for the study. Some accessions were unic and require further studies using molecular, biochemical and morphological tools. All the accessions were clearly split into two groups which may imply different genera.

CONCLUSION

This RAPD-PCR study implicates high level of polymorphism in their genetic makeup. There is need for further molecular characterization studies for these genera to assist in more systematic elucidation. More so, there is need to use different markers for RAPD-PCR analysis of the cocoyam accessions. Due to cost and infrastructure challenges could not be applied in the present study. Diversity of the crops in Edo state is enormous. Most of their varieties are represented. Cost was a limiting factor in this aspect of the study. Others include land and research facilities. With the assistance of effective policy and Government Department, these and other limiting factors can be put to check. Mobilization of small scale cocoyam farmers will contribute to ensure proper management and utilization of yields.

Conflict of Interest

Authors declared no conflict of interest

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