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Genetic Diversity and RAPD-Based DNA Fingerprinting of Some Members of the Cucurbitaceae in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author BEO designed the study. Author IOA wrote the protocol. Author EMIR wrote the first draft of the manuscript. Authors IOA and EMIR managed the analyses of the study. Author EMIR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The genetic variability and relatedness of 14 species from 12 genera of the Cucurbitaceae, an important horticultural family were examined using Random Amplified Polymorphic DNA (RAPD). The taxa (*Luffa aegyptiaca, Luffa acutangula, Cucumis melo, Cucumis sativus, Telfairia occidentalis, Trichosanthes cucumerina, Cucurbita moschata, Lagenaria breviflora, Cucumeropsis mannii, Zehneria scabra, Melothria scabra, Momordica charantia, Citrullus lanatus* and *Coccinia barteri*) collected randomly from the southern part of Nigeria were genotyped and their systematic interrelationships compared. DNA extracted from silica dried leaves of the species using DNeasy Plant Mini Kit (QIAGEN, Amsterdam, Netherlands) was amplified with fifteen Random Amplified Polymorphic DNA primers. The genetic similarities for the 14 species were estimated from banding profiles as a basis for dendrogram construction via the neighbour joining method conducted under

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parsimony criteria using DARWin 6 and NTSYS-PC. The RAPDs amplified 317 bands ranging from 120 to 2531bp producing monomorphic and polymorphic bands indicating considerable genetic differences among the studied cucurbit species. The genetic tree revealed circumscription of three major clusters. The first, a heterogeneous cluster comprising two groups with *Telfairia* occidentalis, *Trichosanthes cucumerina* and *Luffa* forming one group, and *Cucurbita moschata*, Lagenaria breviflora and *Cucumeropsis mannii* forming a second group. The second cluster consists of three groups; Group I comprised *Zehneria scabra* and *Melothria scabra* that clustered separately from *Momordica charantia*. Group II comprised closely related species *Cucumis sativus* and *Cucumis melo*. Group III comprised separately clustered *Citrullus lanatus*. The third cluster consisted of a group which comprised *Coccinia barteri*. The genetic diversity ranged between 40 to 99.9 %. The results were indicative that RAPD markers are useful in assessing genetic diversity of the Cucurbitaceae, providing an invaluable new tool for biological research and will be essential in expediting the process of breeding new and better adapted cucurbit species.

Keywords: Cucurbitaceae; genetic diversity; RAPD; genotyping.

1. INTRODUCTION

Cucurbitaceae is a family of economic important species, particularly those with edible fruits as they act as sources of vitamins, minerals and fiber [1,2]. They are mostly monoecious annuals that are trailing or prostrate, tendril producing, frost sensitive plants that thrive in warm seasons. They are associated with the origin of agriculture and human civilization and are amongst the first plants species to be domesticated in both the Old and New Worlds, and display a rich diversity of many traits [2]. The cucurbits are among the key horticultural crops grown in Nigeria as vegetables and fruits [3]; however, their production is faced with significant challenges [4] due to pathogen attack, poor knowledge of the genetics of the species in Africa, little or no national programmes and limitation to mandate.

Genetic diversity present in species represents a valuable genetic resource for breeding and genetic studies. Germplasm characterization is most valuable to scientific research and crop improvement, and genetic markers serve as the foundation for such characterization. Genetic marker data reveal the genetic profiles; help correctly identify germplasm, and their population structure for ex situ management [5]. Genetic markers may help optimize germplasm utilization strategies by identifying novel alleles of valuable traits, incorporate these latent traits into breeding populations and help to break existing productivity barriers in agriculture [2,6].

Misunderstanding systematic relationships had left some germplasm endangered in situ and without legal protection [7]. One of the most critical roles for genetic markers is the determination of systematic relationships [8]. Genomic and molecular diversity data available for cucurbits in Africa are scarce, with few researches conducted to illustrate the genetic variability and phylogeny of the Nigerian cucurbits and existing information is far from drawing a precise picture of the genetic relationships in this family. This hinders sustainable utilization and adequate conservation and management of the species. Hence, this study is aimed at investigating the diversity and phylogenetic relationships among the common species of the Cucurbitaceae in Nigeria using the Random Amplified Polymorphic DNA (RAPD) markers. Random Amplified Polymorphic DNA is a technique most commonly used in genetic studies for primary assay among the various molecular marker-assisted techniques available. It has been most popular fingerprinting technique because of its low cost, speed, requirement of minute amounts of plant material for analysis and being less restrictive as knowledge of pre-sequencing DNA data is not required. Though limited by low reproducibility RAPDs have provided very useful and informative data for inferring phylogenetic relationships within many families; and help in screening the different DNA sequences of two or more species and have been applied for less-known species [9]. RAPD markers have been used successful for characterizing and estimating of genetic relatedness and assessing genetic diversity among several crops [10,11,12]. RAPD markers have also been used in genome study, map construction and detection of polymorphism in varieties of different *Cucumis* species, and their advantages compared to RFLP markers have been reported [13,14].

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fourteen (14) species representing 12 genera of Cucurbitaceae in natural populations and under cultivation were randomly collected from different locations in the South-East and South-South of Nigeria and used for this study (Table 1).

2.2 DNA Extraction and Quantification

Total DNA from silica dried leaves was extracted using DNeasy Plant Mini Kit (QIAGEN, Amsterdam, Netherlands) following manufacturer's protocol. The quantity and quality of the extracted DNA were ascertained using a spectrophotometer (Nanodrop ND-2000) at the absorption wavelength of 260 and 280 nm; and the ratio of the corresponding absorbance values (A260/A280) was used to determine the protein and RNA contamination. In order to verify DNA integrity, 5µl each of DNA was subjected to gel electrophoresis on 0.8% agarose gel, stained with ethidium bromide and visualized under a UV transilluminator (BIORAD GelDoc XR Imaging system).

2.3 RAPD Analysis

A variant of the classical RAPD-PCR protocol [15] was performed in 25µl volume of PCR buffer and contained approximately 50ng genomic DNA, *Taq* standard buffer, 0.2µM dNTPs (containing dCTP, dGTP, dTTP, dATP), RAPD primer (SIGMA Life Science), *Taq* polymerase (Invitrogen Company name), 1.5 mM $MgCl_2$ and nuclease free water; using GeneAmp thermocycler (Applied Biosystems). The polymerase chain reaction conditions are as follows: an initial denaturation step of 94°C for 4 min., followed by 45 cycles of 94°C for 1 min., 37°C for 1 min, 72°C at 2 min. and a final extension step at 72°C for 5 min.

Amplified products were separated on 1.2% agarose gel (v/v) containing ethidium bromide in 1x TBE buffer at 70V; fragments were visualized and documented with a BIORAD GelDoc XR Imaging system. Sizes of amplicons were estimated by comparison with 50bp lamda (λ) DNA digested with *Eco*RI and *Hind*III (Thermo Fisher).

2.4 Genetic Diversity Analysis

Data matrices were obtained by scoring the RAPD profiles produced by individual primers. Presence or absence of fragments were recorded as 1 or 0 respectively, and treated as binary characters. Resulting matrices for all primers were used to calculate the genetic similarity and construct of similarity matrix within and among species based on Jaccard's coefficients [16]. Clustering and unrooted dendrograms displaying relationships among the 14 species were constructed based on the neighbour joining and parsimomy methods after a bootstrap for 1000 replicates using the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method with DarWin 6 [17] and NTSYS-PC 2.2 [18].

Table 1. Plant species used in this study

S/N	Name of species	Genera	Tribe	Location
1	*Cucumis sativus*	*Cucumis*	Cucumerinae	Emeyal, Bayelsa State
2	*Cucumis melo*	*Cucumis*	Cucumerinae	Opolo, Bayelsa State
3	*Citrullus lanatus*	*Citrullus*	Benincaseae	Edepie, Bayelsa State
4	*Cucurbita moschata*	*Cucurbita*	Cucurbitae	Aba, Abia State
5	*Telfairia occidentalis*	*Telfairia*	Jollifeae	Rumualogu, Rivers State
6	*Lufffa aegyptiaca*	*Luffa*	Benincaseae	Elele, Rivers State
7	*Luffa acutangula*	*Luffa*	Benincaseae	Oyigbo, Rivers State
8	*Momordica charantia*	*Momordica*	Jollifeae	Oyigbo, Rivers State
9	*Lagenaria breviflora*	*Lagenaria*	Benincaseae	Station Road, Rivers State
10	*Coccinia barteri*	*Coccinia*	Benincaseae	Elebele, Bayelsa State
11	*Melothria scabra*	*Melothria*	Cucumerinae	Otuoke, Bayelsa State
12	*Trichosanthes cucumerina*	*Trichosanthes*	Trichosanthae	Nembe, Bayelsa State
13	*Cucumeropsis mannii*	*Cucumeropsis*	Cucumerinae	Uyo, Akwa Ibom State
14	*Zehneria scabra*	*Zehneria*	Cucumerinae	Mbiama Rivers State

3. RESULTS AND DISCUSSION

Preliminary evaluation of 15 decamer RAPD primers on a pooled population of the sampled taxa resulted in the identification of 13 RAPD primers that produced high intensity bands with minimal smearing, and maximum polymorphic bands between the species; and thus were selected for the study (Table 2).

Table 2. Arbitrary primers used in the study

Primer name	Sequence (5' – 3')	Number of bands
OPA-01	CAGGCCCTTC	24
OPA-05	AGGGGTCTTG	32
OPA-09	GGGTAACGCC	20
OPA- 11	CAATCGCCGT	18
OPM-01	GTTGGTCGCT	24
OPM-07	CCGTGACTCA	23
OPM-10	TCTGGCGCAC	42
OPM-11	GTCCACTGTG	17
OPM-19	CCTTCAGGCA	19
OPM-20	AGGTCTTGGG	22
OPS-02	CCTCTGACTG	03
OPS-07	TCCGATGCTG	13
OPS-18	CTGGCGAACT	07
OPV-10	GGACCTGCTC	53
OPV-11	CTCGACAGAG	0

3.1 RAPD Fingerprints and Cluster Groupings

The RAPDs amplified 317 bands ranging from approximately 120 to 2531 bp, out of which four were monomorphic, and the remaining were polymorphic indicating considerable genetic differences among the studied species (Figs. 1 and 2). Of the 15 RAPD primers, primer OPA-09 (5'- ACTCCTGCGA-3') was consistently amplified as a single, intense fragment of approximately 750 bp for all the taxa. Primer OPS-02 produced monomorphic bands in all the specimens of *Trichosanthes cucumerina* and *Luffa aegyptiaca* but absent in other species, while OPS-18 consistently amplified as a single, intense fragment of approximately 700bp in *Zehneria scabra* and *Melothria scabra*.

The UPGMA (unweighted pair-group method with arithmetic average) based cluster analysis results revealed circumscription into three major clusters (Figs. 3 and 4). Cluster 1 is a heterogeneous cluster and is divided two major groups, Group 1 contains subgroup 1 with *Telfairia occidentalis* and *Trichosanthes cucumerina* having a sister origin and subgroup 2 consisting *Luffa aegyptiaca* and *Luffa acutangula*. Subgroup 2 is composed of *Lagenaria breviflora* and *Cucurbita moschata* with a sister origin and separates from *Cucumeropsis* mannii. Cluster 2 contains of two groups, Group 1 separates into two subgroups with subgroup 1 consisting and shows a sisterly origin of *Zehneria scabra*, *Melothria scabra* and *Momordica charantia*; subgroup 2 contains *Cucumis sativus* and *Cucumis melo*. Group 2 consist of *Citrullus lanatus*. Cluster 3 consists of *Coccinia barteri*. The Jaccard's similarity coefficient ranged between 0.4 and 1 (Table 3).

Table 3. Jaccard's Similarity Matrix generated from RAPD-PCR bands, Units 1-14 represent individual species - 1: *Melothria scabra;* 2: *Momordica charantia;* 3: *Luffa acutangula;* 4: *Lagenaria breviflora;* 5: *Trichosanthes cucumerina;* 6: *Cucumeropsis mannii;* 7: *Coccinia barteri;* 8: *Luffa aegyptiaca;* 9: *Cucumis melo;* 10: *Telfairia occidentalis;* 11: *Zehneria scabra;* 12: *Cucumis sativus;* 13: *Cucurbita moschata;* 14: *Citrullus lanatus*

Units	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.87												
3	1	1											
4	0.89	0.95	0.93										
5	0.91	0.96	0.89	0.83									
6	0.92	0.97	0.95	0.72	0.85								
7	0.98	1.04	1	0.96	0.98	0.99							
8	0.95	1	0.8	0.86	0.83	0.89	1						
9	0.87	0.92	1	0.89	0.9	0.91	0.98	0.94					
10	0.94	1	0.93	0.86	0.75	0.88	1	0.87	0.94				
11	•0.4	0.88	1	0.91	0.92	0.93	0.99	0.96	0.88	0.96			
12	0.88	0.94	1	0.91	0.92	0.93	0.99	0.96	0.73	0.95	0.89		
13	0.9	0.96	0.93	0.63	0.83	0.73	0.97	0.87	0.9	0.87	0.91	0.91	
14	0.93	0.98	1	0.92	0.94	0.95	1	0.97	0.92	0.97	0.94	0.94	0.93

●: Dissimilarity minimum value = 0.4; ♦: Dissimilarity maximum value = 1

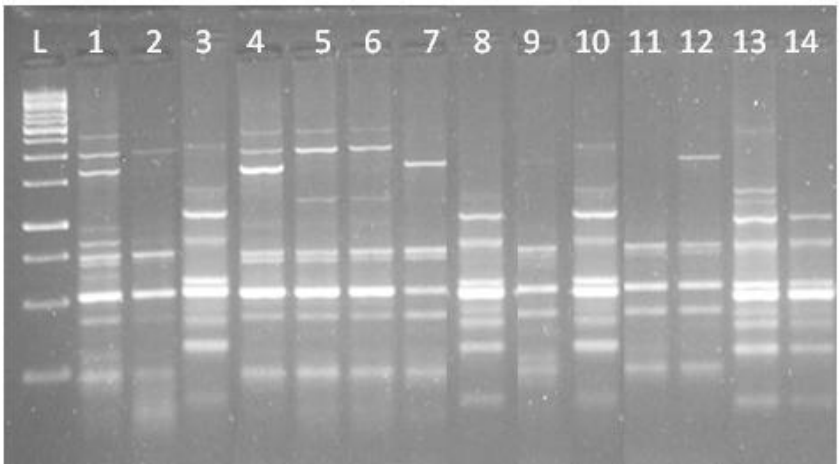

Fig. 1. Banding patterns obtained with primer OPM-07 for 14 species of Cucurbitaceae. 1: *Melothria scabra*; 2: *Momordica charantia*; 3: *Luffa acutangula*; 4: *Lagenaria breviflora*; 5: *Trichosanthes cucumerina*; 6: *Cucumeropsis mannii;* 7*: Coccinia barteri;* 8*: Luffa aegyptiaca;* 9*: Cucumis melo;* 10*: Telfairia occidentalis;* 11*: Zehneria scabra;* 12*: Cucumis sativus;* 13*: Cucurbita moschata;* 14*: Citrullus lanatus*, L: molecular weight (λ) (50 bp DNA ladder)

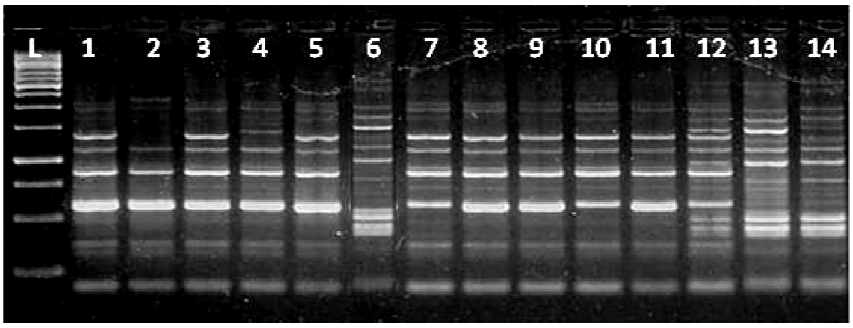

Fig. 2. Banding patterns obtained with primer OPA-05 for 14 species of Cucurbitaceae. 1: *Melothria scabra;* 2*: Momordica charantia;* 3*: Luffa acutangula;* 4*: Lagenaria breviflora;* 5*: Trichosanthes cucumerina;* 6: *Cucumeropsis mannii;* 7: *Coccinia barteri;* 8: *Luffa aegyptiaca; 9: Cucumis melo;* 10*: Telfairia occidentalis;* 11*: Zehneria scabra;* 12*: Cucumis sativus;* 13*: Cucurbita moschata;* 14*: Citrullus lanatus*, L: molecular weight (λ) (50 bp DNA ladder)

The introduction of molecular markers for the characterization of genetic materials presents a valuable tool for plant breeding and systematics. Adequate knowledge of existing genetic polymorphism is of fundamental interest for the efficient management of genetic resources; as crop genetic resources improvement is dependent on continuous infusions of wild relatives, traditional varieties and the use of modern breeding techniques [6,19]. Moreover, estimating genetic diversity can increase the efficiency of a breeding program, and has been considered as an important tool to quantify genetic variability in both self and cross-pollinated crops [20,21].

The RAPD markers produced amplified products and showed different polymorphisms in the 14 species studied. The capacity to produce RAPD fragments vary with primers and the species [22]. Of the 15 RAPD primers, primer OPA-09 (5'-ACTCCTGCGA-3') was consistently amplified as a single, intense fragment of approximately 750 bp for all the taxa. Primer OPS-02 (5'CCTCTGACTG-3') produced monomorphic bands in all the specimens of *Trichosanthes cucumerina* and *Luffa aegyptiaca* but absent in other species was identified and noted as putative markers specific for the aforementioned species, while OPS- 18 (5'-CTGGCGAACT-3') was noted as specific markers to only *Zehneria scabra* and *Melothria scabra* as it consistently

amplified as a single, intense fragment in the species. The most polymorphic arbitrary primer is OPV-10 (5'- GGACCTGCTC-3') producing 53 amplicons.

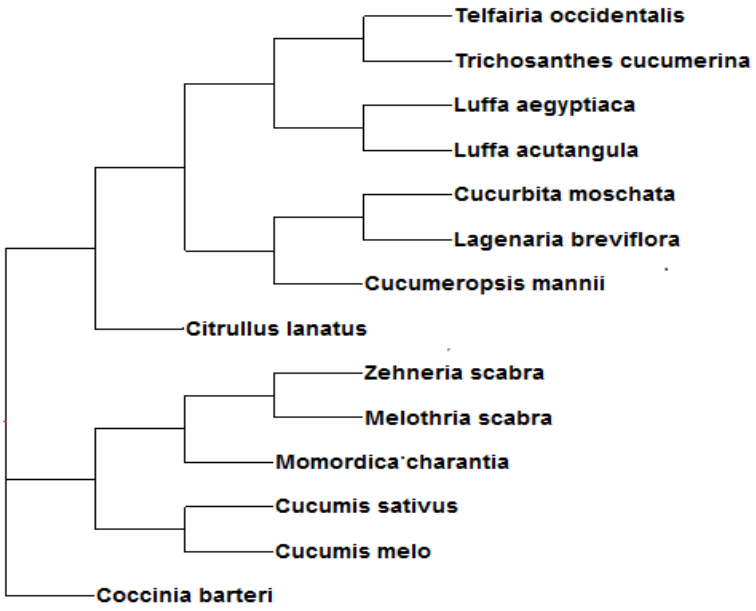

Fig. 3. Clustering relationships of 14 species of Cucurbitaceae based on RAPD (The dendogram was inferred using the parsimony method

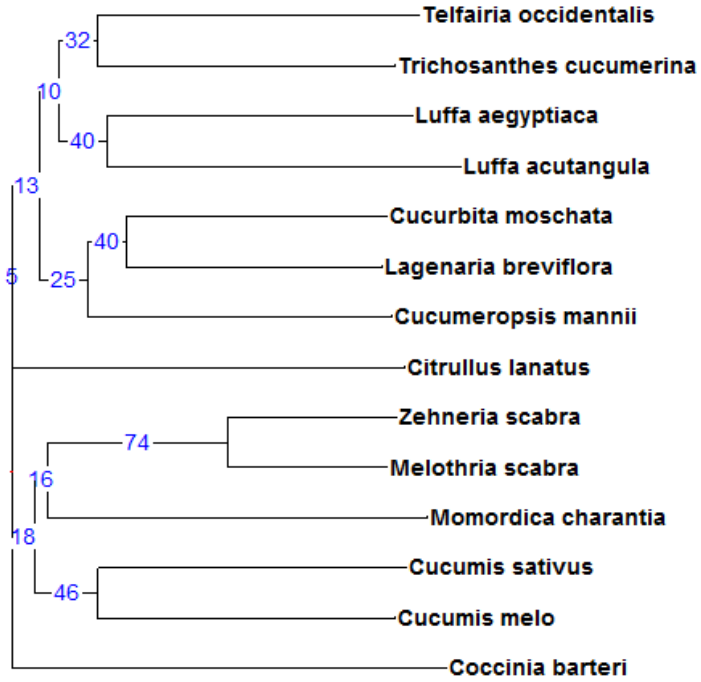

Fig. 4. Clustering relationships of 14 species of Cucurbitaceae based on RAPD (The dendogram was inferred using the neighbor-joining method

The UPGMA method provided insights into the degree of genetic similarity among the studied species and showed clusters formations, indicating the range of variation within and among the sampled genera. The dendogram produced from the cluster analysis corroborated and coincided with some earlier classification of the Cucurbitaceae [23,24]. *Luffa aegyptiaca* and *Luffa acutangula, Cucumis melo* and *Cucumis sativus* show close relationship respectively, and are clearly discriminated from the other cucurbit species.

The RAPD PCR using parsimony and neighbour joining methods produced dendograms with the same topology (Figs. 3 and 4). The use of both methods follows the assumption that when phylogenetic trees constructed from different methods agree, then the confidence in the tree and data used is increased [25].

The RAPD generated dendograms in this study showed *Coccinia barteri* as occupying the basal and an outgroup position suggesting that it is the first branching and possibly the oldest species of all the sampled taxa. This is supported by its morphological characteristics and previous report that it is parthenocarpic in nature and possessed anatomical features that are somewhat distinct from the other cucurbitaceae species [26].

Zenheria scabra, Melothria scabra and *Cucumis* are grouped together in a major cluster, also containing *Momordica charantia*. *Zehneria scabra* and *Melothria scabra* share the same ancestral gene pool, and morphologically both have most characteristics in common. This information is useful in identification and authentication of the species. Also revealed is homogeneity between *Lagenaria breviflora* and *Cucurbita moschata*.

Our results herein, showed a close relationship between the genera *Luffa*, Trichosanthes and *Telfairia*, supporting the works that suggest a parsimonious relationship between *Trichosanthes* and *Luffa* [27,28]. Furthermore, our results agree with previous findings based on biochemical [29] and chloroplast DNA sequence [28] data that different (or multiple) lines occur in the Benincaseae, indicating that the tribe Benincaseae is not monophyletic i.e. distinct lineages are present within the tribe, and that the subtribe Benincasinae is highly polyphyletic.

4. CONCLUSION

The results from this study show that RAPD markers are extensive polymorphic markers within the studied species. The knowledge of pattern of genetic diversity provided by the RAPD techniques will be useful in germplasm evaluation and breeding programs for the studied cucurbits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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