Molecular Evaluation of *Garcinia kola* Heckel Accessions Using RAPD Markers

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Abstract

The genetic relationships among twenty-five accessions of *Garcinia kola* using six Random Amplified Polymorphic (RAPD) primers were evaluated in this study. The highest volume of total genomic DNA (2218/μl) was recorded in ON4 from Ikare, while the highest DNA concentration of 1.93 gl was found in OS3 from Ilesa. The highest Polymorphic Information Content (PIC) and gene diversity of 93.77% and 0.94 respectively were revealed by primer OPO2 compared to other primers. The dendogram generated from Unweighted Pair Group with Mean Average (UPGMA) clustering delineated two groups, A and B, consisting of 21 and 4 accessions respectively. This study clearly showed the level of molecular diversity in the accessions and the information provided could be utilized for genetic improvement and conservation of *Garcinia kola*.

Keywords

Molecular Diversity, Genetic Relationships, RAPD, Genetic Improvement and Conservation

1. Introduction

Garcinia kola Heckel (family, Guttiferae) is an indigenous medicinal fruit tree commonly found in moist forest zones of western and central African countries such as Nigeria, Cameroon, Sierra Leone, Gabon and Congo Brazzaville [1] [2]. It is a spreading forest tree that reaches up to 90 feet height and over 5 feet girth; commercially important and highly exploited Non-timber Forest Product (NTFP) with various uses, most especially as chewing stick and for its therapeutic functions such as fever, cough, heart burn, liver disorder and mouth infections remedies [3]. As a result of its over-exploitation, *Garcinia kola* is currently

threatened and listed among species of concern for conservations in the Sub-Saharan Forest Genetic Resources (SAFOGEN) programme [4] [5]. The medicinal importance also confirmed its anti-bacteria [6], anti-hepatoxic [7], antioxidant [8], anti-inflammatory [9] and hypoglycaemic [10] properties.

Diversity study based on genetic relationships requires appropriate strategies in germplasm management and plant breeding programmes. This determines the selection and categorization of germplasms into heterotic groups for hybrid breeding, so as to develop new and improved cultivars with desirable traits [11] [12]. The development of molecular markers is one of the most important aspects in the field of molecular genetics [13]. The use of molecular markers in identification of cultivars compared to morphological markers is affected by environmental factors, stage of development and other conditions [14]. Molecular markers detect polymorphisms efficiently due to their high resolutions and reliabilities in the identification of plant species [15] [16]. Various types of molecular markers used to study genetic diversity in many plant species included, Random Amplified Polymorphic DNA (RAPD), DNA Amplification fingerprinting (DAF), Inter-Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSRs), Express Sequence Tags (ESTs), Simple Nucleotide Polymorphism (SNPs) and Sequence Tagged Sites (STSs) [17] [18]. Inter-Simple Sequence Repeats (ISSR) and Random Amplified Polymorphic DNA (RAPD) are the two PCR based techniques that have been used to study genetic variations among the *Garcinia* species [19]. However, RAPD had received lots of attention from population geneticists due to its simplicity and rapidity in revealing DNA-level variation. It had been successfully used to investigate genetic variation in *Garcinia cambogia*, *G. cowa*, *G. hombroniana*, *G. indica*, *G. mangostana* and *G. xanthochymu*s [19] [20] [21]. However, there is limited information on molecular studies of *Garcinia kola*in Nigeria. Therefore, the aim of the study was to establish a genetic relationship among accessions of *Garcinia kola* using RAPD primers.

2. Materials and Methods

2.1. Study Location and Sources of Materials

The study was carried out at the Bioscience centre of the International Institute of Tropical Agriculture (IITA), Ibadan. Twenty-five accessions (Table 1) of *Garcinia kola* seeds were sourced from farmers' farms within Seven States in Nigeria, Abia, Anambra, Ekiti, Ondo, Ogun, Osun and Oyo. They were raised into seedlings in the nursery farm of Department of Botany, University of Ibadan.

2.2. DNA Extraction

Five fresh leaf samples were collected from each accession early in the morning. A total of 125 samples were lyophilized at −80˚C. DNA extraction was carried out using modified procedure of [22] reported by [23]. Each sample (200 mg)

Table 1. Nanodrop and DNA concentration of *Garcinia kola* accessions.

S/N	Accession	Total Genomic DNA extracted (µl)	DNA Concentration (260/280 gl)
1	AB1	453.0	1.87
2	AB2	695.4	1.83
3	AN1	752.5	1.47
4	EK1	554.7	1.74
5	OG1	521.0	1.44
6	OG2	662.6	1.60
7	OG3	786.9	1.64
8	OG4	373.8	1.83
9	ON1	338.6	1.79
10	ON2	630.5	1.79
11	ON3	285.6	1.84
12	ON4	2218.0	1.86
13	OS1	557.5	1.87
14	OS2	809.2	1.90
15	OS3	853.5	1.93
16	OS4	778.5	1.90
17	OS5	657.6	1.82
18	OS6	427.7	1.76
19	OS7	663.0	1.75
20	OS8	757.8	1.76
21	OY1	686.7	1.57
22	OY2	609.0	1.85
23	OY3	776.5	1.79
24	OY4	654.8	1.42
25	OY5	921.5	1.90

was powdered in liquid nitrogen and transferred into 1.5 ml eppendorf tube and 700 ul of 2% pre-heated CTAB extraction buffer (20 mM EDTA, 0.1 M Tris-HCl, pH 8.0, 1.4 M NaCl, and 0.4% β-mercaptoethanol) was added to the solution which was incubated at 65˚C for 45 min, gently by mixing and inverting every 15 mins. 500 µL of choloroform-isoamyalcohol (24:1) was added to the tubes gently mixed for 1 min and centrifuged for 10 min at 1200 rpm and 600 µL of the supernatant was transferred to a fresh tube. This procedure was repeated twice.

2.3. DNA Purification

500 µL of the supernatant was transferred to a fresh tube containing 700 µL of cold Isopropanol (−20˚C). The samples were gently mixed by inversion and centrifuged at 12,000 rpm for 10 mins after which the DNA adhered to the bottom of the tube. The liquid solution was then released and the DNA pellet was

washed with 700 μL of 70% ethanol to eliminate salt residues that adhered to the DNA and dried for approximately 12 hours ensuring that the tubes were inverted over a filter paper at room temperature. The pellet was re-suspended in 100 μL TE buffer and 5 μL RNA in each tube. The solution was incubated at 37°C for 1 hour and stored at −20°C.

2.4. DNA Quantification and Gel Electrophoresis

The purity was checked by electrophoresis and spectrophotometric analyses. 1.5 μl of DNA samples was used to obtain 1.8 - 2.0 ratio at 260/280 absorbance level and the concentration from which the working dilutions were prepared for Polymerase Chain Reaction (PCR). On 1.5% agarose gel for electrophoresis, 2 μl of the stock DNA samples were loaded and visualized under a UV light Transilluminator (Model-2, Upland, CA, USA) to check the quality of the extracted DNA molecules.

2.5. RAPD-PCR Amplification

Six RAPD primers in Table 2 used for the PCR reactions were conducted at a final volume of 10.2 mL, containing 0.2 mL Taq polymerase (5 units·mL^{-1}), 1.0 mL 10× Amplification Buffer (Mg$^+$ Free), 1.0 mL MgCl$_2$ (50 mM), 0.5 mL Forward Primer (5 pmoles·mL^{-1}), 0.5 mL Reverse Primer (5 pmoles·mL^{-1}), 1.0 mL dNTP's (2.5 mM each), 3 mL de Milli-Q H$_2$O and 3 mL DNA (5 ng·mL^{-1}). The PCR reaction conditions were initial denaturation at 94°C for 5 mins, followed by 40 cycles of denaturation at 94°C for 30Sec, annealing at 37°C for 30secs and elongation at 72°C for 1 min. This is followed by a final elongation step at 72°C for 7 minutes which holds temperature at 10°C forever. These amplified products or fragments (of up to 3.0 kb) were separated on agarose gels (1.5% - 2.0%) and visualised under UV light and after staining with 1.5% ethidium bromide.

2.6. RAPD Data Analysis

The molecular data were analysed using the procedure adopted by [24]. The amplified fragments were scored as 1 for presence and 0 for absence. The level of polymorphisms was estimated from binomial data through dividing the polymer bands by the total number of scored bands. NTSYS-pc version 2.02e was used to determine the allelic frequency, allele no, gene diversity and Polymorphic Information Content (PIC) while Power marker version 3.25 software was used to compute pairwise Jaccard's similarity coefficients. The similarity matrix was also used in cluster analysis using an Unweighted Pair-Group Method and Arithmetic mean (UPGMA) to generate a dendogram.
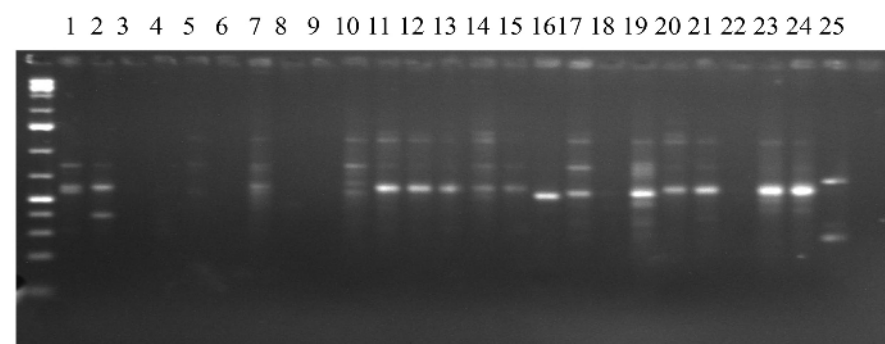
3. Results

3.1. Nanodrop and DNA Concentration of *Garcinia kola* Accessions (Plates 1-6)

The Nano drop and DNA concentration of the *Garcinia kola* accessions (Table 1) were found to be at 260/280 gl. The quality of genomic DNA concentration

Table 2. Allele frequency, gene diversity and polymorphic information contents (PIC) of *Garcinia kola* accessions using RAPD primers.

Primers	Sequence (5'-3')	Major Allele Frequency	Sample size	Allele number	Gene diversity	PIC (%)
OPO2	TGA TCC CTGG	0.12	25	20	0.94	93.77
OPB10	CTG CTG GGAC	0.20	25	14	0.90	89.49
OPB12	CCT TGA CGCA	0.32	25	12	0.84	82.37
OPH05	AGT CGT CCCC	0.24	25	14	0.87	86.26
OPH06	ACG CAT CGCA	0.12	25	12	0.91	89.77
OPH09	TGT AGC TGGG	0.12	25	18	0.93	93.05
Mean ± S.D		0.19 ± 0.03	25 ± 0.00	15 ± 1.09	0.90 ± 0.01	89.12 ± 0.01
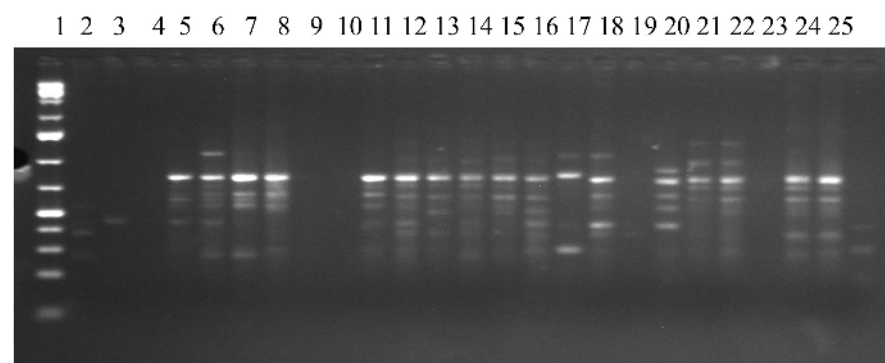
Plate 1. Gel showing 25 *Garcinia kola* accessions using OPO2. 1—AB1, 2—AB2, 3—AN1, 4—EK1, 5—OG1, 6—OG2, 7—OG3, 8—OG4, 9—ON1, 10—ON2, 11—ON3, 12—ON4, 13—OS1, 14—OS2, 15—OS3, 16—OS4, 17—OS5, 18—OS6, 19—OS7, 20—OS8, 21—OY1, 22—OY2, 23—OY3, 24—OY4, 25—OY5.

Plate 2. Gel showing 25 *Garcinia kola* accessions using OPB10. 1—AB1, 2—AB2, 3—AN1, 4—EK1, 5—OG1, 6—OG2, 7—OG3, 8—OG4, 9—ON1, 10—ON2, 11—ON3, 12—ON4, 13—OS1, 14—OS2, 15—OS3, 16—OS4, 17—OS5, 18—OS6, 19—OS7, 20—OS8, 21—OY1, 22—OY2, 23—OY3, 24—OY4, 25—OY5.

was generally good. The result revealed that OS3 had the highest concentration at 1.93/ul from the total of 853.50/ul of genomic DNA extracted. However, the highest total genomic DNA of 2218.0/ul was extracted from ON4 and the corresponding DNA concentration was 1.86/ul. The lowest total genomic DNA of 285.6/ul was extracted from ON3 and the DNA concentration was 1.84/ul.

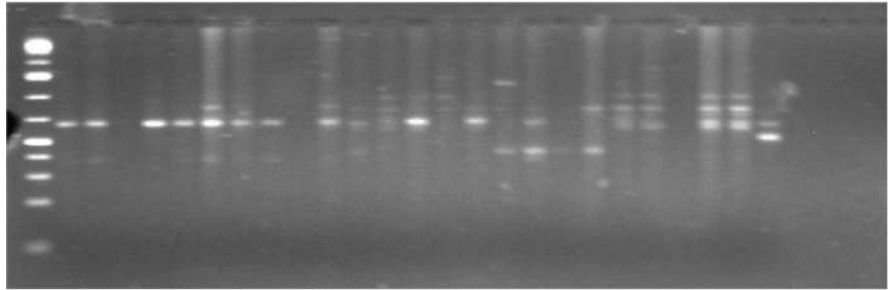1 2 3   4 5 6 7 8 9 10 1112 13 1415 1617 1819 2021 22 232425

Plate 3. Gel showing 25 *Garcinia kola* accessions using OPB12. 1—AB1, 2—AB2, 3—AN1, 4—EK1, 5—OG1, 6—OG2, 7—OG3, 8—OG4, 9—ON1, 10—ON2, 11—ON3, 12—ON4, 13—OS1, 14—OS2, 15—OS3, 16—OS4, 17—OS5, 18—OS6, 19—OS7, 20—OS8, 21—OY1, 22—OY2, 23—OY3, 24—OY4, 25—OY5.

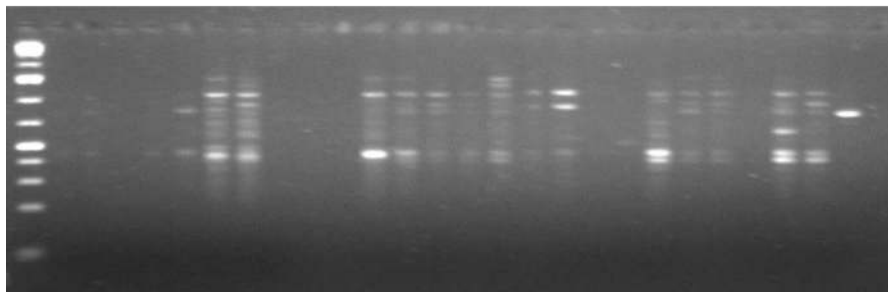1 2 3 4 5 6 7 8 9 10 11 1213 14 15 16 17 18 19 2021 22 23 24 25

Plate 4. Gel showing 25 *Garcinia kola* accessions using OPH05. 1—AB1, 2—AB2, 3—AN1, 4—EK1, 5—OG1, 6—OG2, 7—OG3, 8—OG4, 9—ON1, 10—ON2, 11—ON3, 12—ON4, 13—OS1, 14—OS2, 15—OS3, 16—OS4, 17—OS5, 18—OS6, 19—OS7, 20—OS8, 21—OY1, 22—OY2, 23—OY3, 24—OY4, 25—OY5.

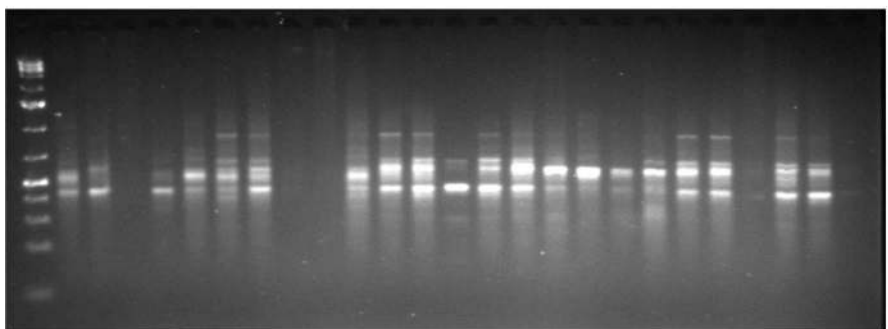1 2 3   4 5 6 7 8 9   10 11 12 13 14 1516 17 18 1920 21 22 23 24 25

Plate 5. Gel showing 25 *Garcinia kola* accessions using OPH06. 1—AB1, 2—AB2, 3—AN1, 4—EK1, 5—OG1, 6—OG2, 7—OG3, 8—OG4, 9—ON1, 10—ON2, 11—ON3, 12—ON4, 13—OS1, 14—OS2, 15—OS3, 16—OS4, 17—OS5, 18—OS6, 19—OS7, 20—OS8, 21—OY1, 22—OY2, 23—OY3, 24—OY4, 25—OY5.

3.2. Frequency and Diversity of Alleles and Polymorphic Information Content (PIC) of *Garcinia kola* Accessions

The six RAPD primers were polymorphic across the accessions and a total of 90 alleles were identified as shown in Table 2. The percentage of gene diversity

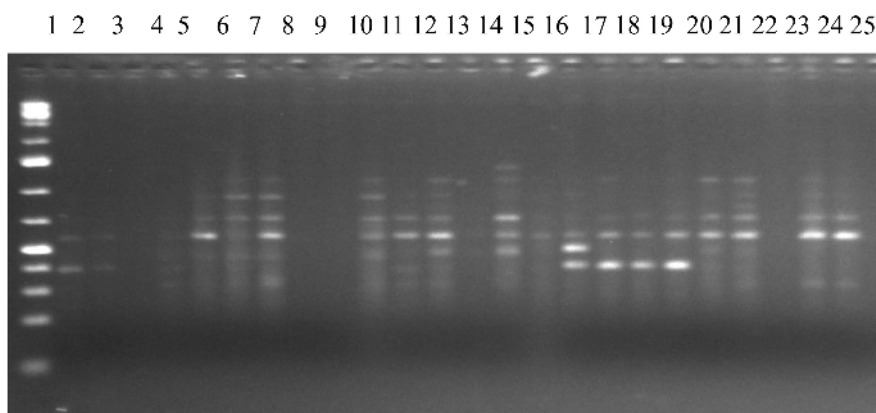1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

Plate 6. Gel showing 25 *Garcinia kola* accessions using OPH09. 1—AB1, 2—AB2, 3—AN1, 4—EK1, 5—OG1, 6—OG2, 7—OG3, 8—OG4, 9—ON1, 10—ON2, 11—ON3, 12—ON4, 13—OS1, 14—OS2, 15—OS3, 16—OS4, 17—OS5, 18—OS6, 19—OS7, 20—OS8, 21—OY1, 22—OY2, 23—OY3, 24—OY4, 25—OY5.

recorded was 89.92% while the percentage Polymorphic Information Content (PIC) observed in the population was 89.12%. The major allele frequency range from 0.12 (revealed by OP02, OPH06 and OPH09) to 0.32 (by OPB12) and the mean value was 0.19. The number of alleles found range from 12 to 20 and the mean value was 15. The highest number of alleles (20) was revealed by OPO2 while the least (12) was revealed by OPB12 and OPH06. The level of gene diversity ranged from 0.84 (OPB12) to 0.94 (OP02) and the mean value was 0.90. The highest PIC (93.77%) and gene diversity (0.94) were recorded in OPO2 primer.

3.3. Dendogram Showing Genetic Relationship of *Garcinia kola* Accessions

The dendogram for the RAPD profiling of the twenty five accessions of *Garcinia kola* within Nigeria (Figure 1) produced two clusters, A and B. Cluster A consists of twenty-one accessions (OG4, OY2 OY3, OS3, OG1, OS2, OY5, OS6, OG2, AN1, OS4, OS8, OS7, EK1, AB2, OY4, OS1, ON4, OY1 and ON3) while Cluster B consists of only four accessions (ON1, OS2, OG3 and ON2). Cluster A had two sub-clusters C and D. Sub-cluster C consists of OG4 and OY2, while sub-cluster D comprises of OY3, OS3, OG1, OS2, OY5, OS6, OG2, AN1, OS4, OS8, OS7, EK1, AB2, OY4, OS1, ON4, OY1 and ON3. Each cluster consists of *Garcinia kola* accessions that are more genetically similar to each other compared to other accessions. The non-clustering accessions branching from cluster B (ON1), sub-cluster D (OY5 and OS2), and sub-cluster E (OS5) were genetically distinct from other accessions in their immediate clusters or sub-clusters.

4. Discussion

Generally, *Garcinia* species lack adequate genomic information to design specific markers [19] [25]. Hence, RAPD is very suitable for determining genetic diversity and relationships in *Garcinia kola*. The six RAPD primers used in this study
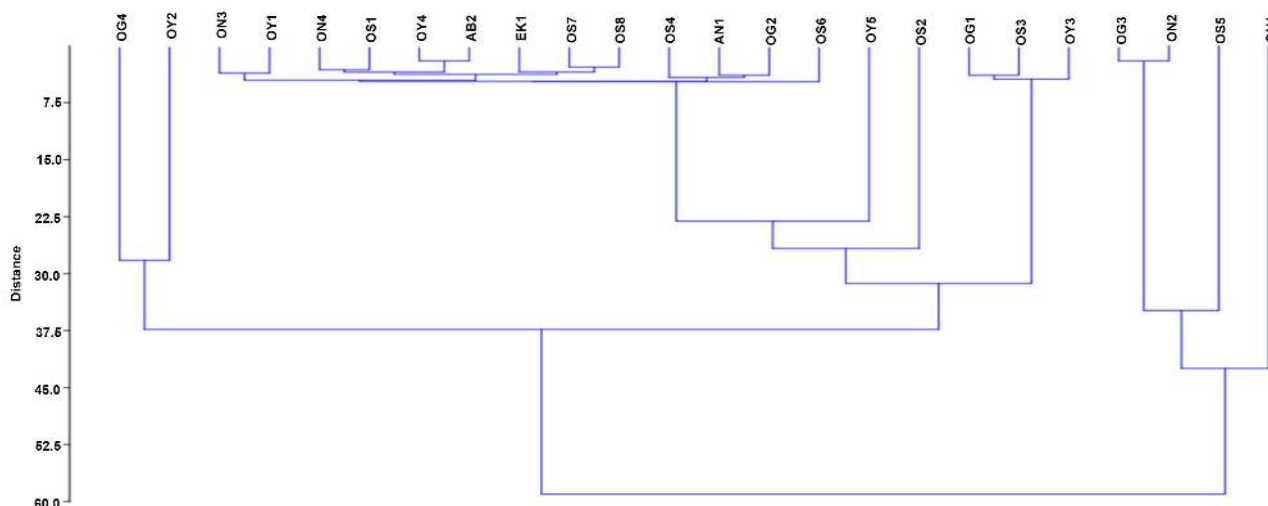
Figure 1. Dendogram showing genetic relationship among twenty five accessions of *Garcinia kola*.

were highly polymorphic across all the accessions, in agreement with the findings made by [26] and [19] who reported that, RAPD markers can be used effectively to determine variations based on geographical or morphological features within the same species. The polymorphisms revealed by the six decamer primers were high and very close and this further indicates that, they are all good and reliable for genetic diversity assessment in *G. kola.* There is a high degree of diversity among the accessions studied. The differences in bands revealed by gel plates of the accessions contributed to variability in the allele frequencies, number of alleles, gene diversity and Polymorphic Information Contents (PIC). The highest value found in OP02 suggested its consideration mostly for genetic studies. The dendogram generated from the cluster analysis revealed an unpredictable clustering pattern of accessions from the same and or different provenances. The genetic diversity revealed by RAPD primers considered in this study agrees with the observation made by [27]. The diversity of *Garcinia* species reported by [28] [29] and [30] using Inter Simple Sequence Repeat (ISSR), Isozyme and Randomly Amplified DNA Fingerprinting (RAF) markers respectively differed due to variation in efficiency of the markers [31]. Also confirmed the phylogenetic relationship of *Garcinia* species using Standard ITS primers. The relationship among the accessions of *Garcinia kola* from the cluster based on their genetic similarities and differences is in accordance with the observation made by [17] [26] and [32]. This shows that are variations which could be due to genetic and environmental factors. Therefore, this information will assist future *Garcinia kola* breeders in improving the available genetic base through hybridization techniques by selecting appropriate parents via trait performance and cluster pattern.

5. Conclusion and Recommendation

The study indicates that all the six RAPD primers are effective in studying ge-

netic diversity in *Garcinia kola* and they could be recommended for future molecular research on *Garcinia kola* and other related tree species. Presently, information on genetic diversity of *Garcinia kola* in Nigeria is very scanty; hence this study could serve as a source of valuable information for tree geneticists and breeders to help in improvement and conservation of this important tree crop.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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