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Molecular Relationship among *Mangifera indica* L. (Mango) Varieties Using Simple Sequence Repeat (SSR) Marker

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Authors' contributions

This work was carried out in collaboration among all authors. All Authors designed the study. Author IIA wrote the protocol and the first draft of the manuscript. Author OJO performed the statistical analysis and interpreted the analyses of the study. All authors managed the literature searches, read and approved the final manuscript.

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ABSTRACT

This study established phylogenetic relationships among mango varieties collected from NIHORT, Ogbomosho, Saki, Oyo, Isehin and Ibadan using Simple Sequence Repeat (SSR) markers with a view of determining their polymorphism, gene and allelic diversities. Sweet Mango UI Acc-3 had the highest total genomic DNA of 1379.00µl, while OYOM ACC-5 had the lowest concentration of 0.9 gl from total genomic DNA of 0.25. The number of alleles ranged from 2 to 4 with an average of 2.50 alleles per locus in which the highest allelic frequency of 0.97 was recorded for EF 592217 and EF 59210 primers. However, Primer SSR20 had the highest information of polymorphic at 57.57% and highest gene diversity of 0.64. The result from the dendrogram showed that out of the three major clusters generated, the second delineated the highest number of 12 varieties in which Ogbomosho Mango Acc-2 (OGBM ACC-2) branched out at a distance of 0.15 from other varieties. Sweet Mango UI 3, Ogbomosho Mango Acc-2 (OGBM ACC-2), Julie Mango are potential future breeding accessions while Primer SSR20 could therefore be considered for further molecular breeding of other mango varieties and other tree crops in the Mangifera family.

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Keywords: Mangifera indica; genetic diversity; genomic DNA; phylogenetic; SSR marker.

1. INTRODUCTION

Mangifera indica L. (Mango) is a popular perennial edible fruit crop, known as the 'king of fruits' due to its rich taste, flavor, color, production volume and diverse end usage. It's cultivated in most ecological zones particularly in Africa [1] and in the tropical world [2]. Mango is a member of the family Anacardiaceae in the Order Sapindales [3,4]. Mango is believed to have originated in the Indo-Burma region [5-8,1] during the earlier period of the Cretaceous era [9] and gradually spread to the tropical and subtropical regions of the world [10]. Presently, India represent the biggest mango germplasm in the world and harbors more than 1000 mango cultivars which represents the biggest mango germ pool in the world [11,12].

Mango came to Nigeria in the 20th Century through itinerant of indigenous cropping systems as reported by [13,14,15]. Mango cultivars spread to other parts of Nigeria and became highly adapted to Nigerian condition. The following mango varieties sourced from National Horticultural Research Institute (NIHORT), Ibadan Nigeria, have been found promising and are recommended for Production: Alphonso, Zill, Julie, Palmer, Keitt, Lippens, Saigon, Edward, Haden and Early gold. These mature within 3–4 years after been transplanted or grafted. Therefore, this necessitated the improvement of more varieties using marker assisted approach.

Characterization of crop diversity is a necessary requirement for crop improvement, use and conservation of plant genetic resources [2]. It can also be used extensively as a tool for identification and differentiation of cultivars, since published descriptors lists are readily available for most major crop species including mango [3,16-29]. A universally accepted procedure had been developed for characterization of mango varieties by the International Plant Genetic Resources Institute (IPGRI) and the list of descriptors of morphological traits of plants include; leaves, flowers, seeds and fruits [30,31].

The interpretation of genetic diversity on the basis of morphological characters have several limitations such as complex inheritance pattern, often limited in number, vulnerability to environmental conditions. Molecular studies (using genetic markers) have provided an effective tool to conventional phenotypic diversity, identification of plants and estimation of their genetic relatedness [32,33]. A Marker must be heritable, discriminate between accessions, easy and cost-effective to measure, evaluate, provide reliable repeatable results in the absence of environmental influences [34,35,36]. There are numerous examples of the application of different molecular markers in the genetic diversity analysis of crops; these include; Random Amplified Polymorphic DNA (RAPDs), Variable Number of Tandem Repeats (VNTRs), Restriction Fragment Length Polymorphism (RFLPs), Amplified Fragment Length Polymorphism (AFLPs), Microsatellites or Simple Sequence Repeats (SSRs), Inter-Simple Sequence Repeats (ISSRs) and Directed Amplified Mini Satellite DNA (DAMD). In all the above markers, microsatellite markers are of particular importance to study genetic relatedness and distinctiveness of mango germplasm [30]. Microsatellites are efficient type of molecular markers based on tandem repeats of short (2–6 nucleotides) DNA sequence [37] and have advantages over other types of molecular markers. Simple Sequence Repeats (SSRs) had gained considerable importance in genetic studies due to their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage and being free of selection bias [38]. SSR analysis shows great potential for mango improvement, can be performed for varied identification, validation of parentages and estimation of genetic variation in existing mango populations [39].

Also, the description of mango varieties in Nigeria has been largely on morphological characters variation in leaf, fruits, stem and root) leading to assigning of common names which are generally misleading and may not be universally acceptable. Limited work had been documented on its genetic improvement. Therefore, there is need for genetic characterization using SSR marker with a view to enhancing efficient breeding programs and the establishment of phylogenetic relationship among mango varieties. This study aimed at establishing molecular relationships among mango varieties.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Ten (10) ripe mango seeds and fruits were randomly collected from 35 trees sourced from

the mango orchard of the National Horticultural Research Institute (NIHORT), Ibadan, Oyo, Saki, Ipapo, Isehin in Oyo State during the mango harvest season from February to May, 2018, according to the method described by Biodiversity International (formerly International Plant Genetic Resource Institute [40]. The geographic location of each of the sampled trees was recorded using a hand-held Global Positioning System (GPS) along with location information and local names of the surveyed trees as shown in Table 1.

Table 1. The mango varieties collected, locations and their coordinates

Varieties	Local names	Locations	Coordinates
Tommy harkins	Tommy harkins	NIHORT, Ibadan	N 07º 24´ 35 6 E 003º 51´ 16 8
Johnbull	Johnbull	NIHORT, Ibadan	N 07º 24´ 33 6 E 003º 51´ 16 4
Julie	Julie	NIHORT, Ibadan	N 07º 24´ 33 4 E 003º 51´ 09 8
Edward	Edward	NIHORT, Ibadan	N 07º 24´ 33 5 E 003º 51´ 14 8
Saigon	Saigon	NIHORT, Ibadan	N 07º 24´ 33 2 E 003º 51´ 11 2
Ogbomosho nihort	OGBOMOSHO	NIHORT, Ibadan	N 07º 24´ 35 1 E 003º 51´ 17 1
Palmer	Palmer	NIHORT, Ibadan	N 07º 24´ 37 3 E 003º 51´ 18 7
Harden	Harden	NIHORT, Ibadan	N 07º 24´ 35 0 E 003º 51´ 17 5
Keint	Keint	NIHORT, Ibadan	N 07º 24´ 36 3 E 003º 51´ 16 8
OGBM Acc -2	Ogbomosho	Lautech	N 08º 10´ 09 2 E 004º 16´ 52 9
OGBM Acc -3	Ogbomosho	Lautech	N 08º 10´ 09 4 E 004º 17´ 53 2
OGBM Acc -4	Ogbomosho	Lautech	N 08º 10´ 06 0 E 004º 16´ 50 9
OGBM Acc -8	Ogbomosho	Surulere LGA	N 08º 12´ 39 0 E 00º 18´ 23 1
OGBM Acc -9	Ogbomosho	Surulere LGA	N 08º 14´ 49 0 E 004º 23´ 18 1
OGBM Acc -10	Ogbomosho Mango	Ogbomosho South	N 08º 06´ 40 4 E 004º 13´ 59 8
OGBM Acc -12	Butter Mango-Ajuwa	Ogbomosho South	N 08º 03´ 10 5 E 004º 08´ 51 7
OGBM Acc -13	Ogbomosho Mango	Atiba South	N 08º 05´ 18 2 E 004º 12´ 58 4
BUTM Acc-1	Butter	OGO Oluwa	N 08º 12´ 42 1 E 004º 25 ´ 15 9
BIGMCB Acc-1	Big Mango	MCB DEPT. UI, Ibadan	N 07º 26´ 37 7 E 003º 53´ 47 5
SWM UI Acc-1	Sweet Mango	UI, Ibadan	N 07º 21´ 28 9 E 003º 50 ´ 11 7
SWM UI Acc-3	Sweet Mango	UI, Ibadan	N 07º 26´ 23 3 E 003º 53´ 11 4
SWM UI Acc-4	Sweet Mango	AWBA DAM UI, Ibadan	N 07º 26´ 40 0 E 003º 52´ 22 9
SWM UI Acc-5	Sweet Mango	UI, Ibadan	N 07º 24´ 29 3

Varieties	Local names	Locations	Coordinates
SWM Acc-6	Sweet mango	Ibadan	E 003º 50´ 14 5 N 07º 25´ 22 6
SWM Acc-7	Sweet Mango	Ibadan	E 003º 51´ 14 7 N 07º 29´ 21 9
SWM Acc-4	Sweet mango	Ibadan	E 003º 57´ 11 8 N 07º 29´ 20 7
OROM Acc-1	ORO mango	Oke Adagba, Saki west	E 003º 58´ 14 6 N 08º 40´ 30 8
OROM Acc-2	ORO mango	BSH, Saki west	E 003º 23´ 02 0 N 08º 19´ 37 5
OROM Acc-4	ORO mango	Saki	E 003º 23´ 53 4 N 08º 13´ 13 1
SHRIM Acc-2	Sheri Mango	African baptish church	E 003º 27´ 27 1 N 08º 39´ 33 4
OYOM Acc-1	OYO	OYO	E 003º 23´ 43 1 N 08º 10´ 41 9
OYOM Acc-2	OYO	OYO	E 004º 23 ´ 17 6 N 08º 15´ 45 1
OYOM Acc-3	OYO	OYO	E 004º 29 ´ 19 9 N 08º 16´ 44 4
OYOM Acc-5	OYO	OYO	E 004º 26 ´ 18 6 N 08º 11´ 39 3
OGBSHEM Acc-1	Ogbomosho sheri	SAKI	E 004º 23 ´ 12 8 N 08º 40´ 40 8
SAKM Acc-2	SAKI	SAKI	E 003º 23´ 43 3 N 08º 40´ 13 8
SAKM Acc-3	SAKI	SAKI	E 003º 24 ´ 43 8 N 08º 35´ 33 5
SAKM Acc-4	SAKI	SAKI	E 003º 46 ´ 48 6 N 08º 07 ´ 36 5
BIGM Acc-2	Big Mango	IPAPO, ITESIWAJU	E 003º 30´ 30 2 N 08º 26´ 18 3
SWMUI IDIA-1	Sweet Mango	IDIA UI, Ibadan	E 003º 23´ 50 7 N 07º 26 18 3
SWMUI IDIA-2	Sweet Mango	IDIA UI, Ibadan	E 003º 53 47 9 N 07º 26 18 3
			E 003º 53 47 9

2.2 Study Locations, Experimental Design, Seed Processing and Planting Procedure

The molecular studies were carried out at the molecular laboratory of the Department of Virology, University College Hospital, Ibadan, Oyo State Nigeria. Fresh young apical leaves were collected after two weeks of planting into ice bags, and taken to the laboratory where they were stored at -80ºC prior molecular studies.

The field experiment was laid out in a Completely Randomized Design (CRD) in three replicates with 1.0 m spacing within the row and column at the open field of the nursery farm of the Department of Botany, University of Ibadan, Nigeria.

The planting of mango seeds was done using the procedure described by Verheiji [41] during the raining season from May to September, 2018. The mango pulp was removed from the mango husk, and the hairs were scraped away with a knife and set in the sun for 1-2 days to dry completely dry. A dull knife was carefully used to open the seeds on the round end so as not to damage it. The husks were gently opened with bare hands to speed germination, avoid cramping of roots and also permits detection and removal of the larva of the seed weevil. After the husk was opened, the umbilical cord attached to

the bean shaped seed was detached from the husk, the seeds inside looked tan and fresh. The seeds were wrapped with a paper towel moistened, to avoid rot and wrapped seeds were then placed in sandwiched bags and sealed tightly. The sandwiched bags (labeled and dated) were placed inside a dark drawer to avoid disturbance. The seeds were checked once in two days to ensure they remained moist. After about 7-9 days, the mango seeds started geminating, and were transplanted to the field at 8cm depth inside perforated polythene bags filled with 8 kg dried sandy-loam soil. Planting was done early in the morning and the plants were watered every three days throughout the course of the experiment. Weeding of the plots was done within the first ten days and subsequently as the weeds appeared.

2.3 DNA Extraction and Quantification

Plant genomic DNA was extracted from the leaves of mango varieties using Jena Bioscience easy Plant Mini Kit (Jena Bioscience web site) protocol. Fresh young sample leaf of 100 mg was grinded with liquid nitrogen using mortar and pestle, and placed in a 1.5 ml micro tube, 300ul of cell lysis solution was added to the tissue and incubated at 65ºC heat block for 60 minutes. The tubes were inverted once in every 10 minutes to properly homogenize the tissue with extraction buffer. 1.5ul of RNAse solution was added to the cell lyses and mixed with the sample by inverting the tube 25 times and incubated at 37ºC for 15-60 min. The sample was cooled at room temperature (25ºC) and 100ul of protein precipitation solution was added to cell lysate, the solution was mixed by vortexing. The solution was centrifuged at 15,000rpm for 3 minutes. The precipitant formed a tight, green pellet. The DNA containing in the supernatant was transferred into 1.5 ml micro-tube containing 300ul of isopropanol, it was then mixed by inverting gently 50 times and later centrifuged for 15,000rpm for 1 minute. The DNA was visible as pellets with white/light green colour. The supernatant was discarded and the tube was drained briefly on a clean absorbent paper, 500ul of washing buffer was added and the tube was severally inverted to wash the DNA pellet. The solution was centrifuge at 15,000rpm for 1minute. The ethanol was discarded and air dried at room temperature for 10-15 minutes. 50ul of DNA Hydration solution was added to the dried DNA pellets and then incubated at 65ºC for 60 minutes. The DNA was stored at -80ºC.

The absorbance of the total genomic DNA was quantified by measuring optical density (OD) at A 260 and 280 with a Nanodrop Spectrophotometer (ND 2000) according to the manufacturer protocol. The concentration of DNA was calculated from the absorbance at 260 nm. The ratio of nucleic acids to protein in the genomic DNA sample was evaluated by the ratio of absorbance at 260 and 280 nm (A260/A280 ratio) [42]. The presence and quality of gDNA were also evaluated by electrophoresis on 1% agarose gel at 100V for 120 min in 1X TAE (Tris-base, glacial acetic acid, EDTA). The gel was stained with 0.25ug/Ml ethiduim bromide for DNA visualization. Gel was viewed under UV trans-illuminator light.

2.4 PCR Amplifications

The amplification reaction were prepared in 10 µl PCR cocktail reaction mix consisting of 10 × PCR buffer of 1ul, 0.8 mM dNTPs, 0.4mM $MgCl_2$, 0.06 unit of Taq polymerase, 0.8ul of DMSO, 1.94 µl, PCR grade H_2O, 1 µl of each primer and 3 µl of DNA. Amplification were performed in thermocycler programmed for a touch-down (TDSSR) protocol at the initial denaturing of 94ºC for 2 minutes, 9 cycles of denaturing was done for 20 seconds at 93ºC, annealing for 35 seconds at 65ºC and extension at 72ºC for 45 seconds. At 24 cycles, Denaturing was done at 93ºC for 20 seconds, Annealing was done at 55ºC for 35 seconds, and extension at 72ºC for 45 seconds the final extension was done at 72ºC for 5 minutes and held at temperature of 10ºC infinity (-10ºC).

2.5 Agarose Gel Electrophoresis and Scoring of Bands of SSR Primers

The Agarose Gel Electrophoresis was conducted on a quick check for the presence of DNA in the extracted mango plant samples. One gram (1g) of agarose was dissolved in 100 ml of 0.5 × TBE buffer in a microwave oven for 5 minutes. This was placed under a running tap water for 1 minute to allow it to cool. Afterward, 2 µl of cyber stain was added to the agarose gel solution as a staining dye. The agarose gel solution was cast into the agarose gel plate and the comb was set on the rack. Finally, casted gel was placed in the electrophoresis tank which contained the loaded 3 µl of the dye extracted DNA and was allowed to run for 30 minutes at 100ºC. A negative control lacking a DNA template was used. Visualization of the separated amplified fragments (stained alongside the 3kb plus mid-

range DNA ladder from Jena Bioscience) was observed under UV trans illuminator light for the formation of bands.

A set of 12 Simple Sequence Repeat (SSR) primers comprising of forward and reverse oligonucleotide sequences as shown in Table 2 were optimized and later used for the genetic diversity study. These primers were used based on previously published data [43]. The bands produced were scored as discrete variables unto an excel sheet. Bands were scored as 1 for presence and 0 absence.

2.6 Statistical Analysis

Molecular data generated based on binary similarity matrix were analysed using Numerical Taxonomical System of Statistics (NTSYS) version 2.02e package and Power marker version 3.25 software [44]. Jaccard Coefficient of similarity was used to estimate the genetic distance while construction of dendogram was done by Unweighted Pair Group Method of Arithmetic Means (UPGMA) as described by Sneath and Sokal [45] to reveal phylogenetic relationship among mango varieties.

3. RESULTS

The result in Table 3 shows that Sweet Mango UI 3 had the highest total genomic DNA extracted with 1379.00µl while OYOM ACC-5 had the lowest DNA concentration of 0.91gl from total genomic DNA of 0.25. Sweet Mango UI 3 had the highest total genomic DNA extracted with

Table 2. Simple sequence repeats and their oligonucleotide sequences

S/N	Primers	Sequence 5'-3'	
1	SSR 20	F: CGCTCTGTGAGAATCAAATGGT	R:GGACTCTTATTAGCCAATGGGATG
2	SSR 16	F: GCTTTATCCACATCAATATCC	R: TCCTACAATAATAACTTGCC
3	SSR 19	F: AATTATCCTATCCCTCGTATC	R: AGAAACATGATGTGAACC
4	SSR 8	F: TTGATGCAACTTTCTGCC	R: ATGTGATTGTTAGAATGAACTT
5	EF592203	F: TCTGACCCAACAAAGAACCA	R: TCCTCCTCGTCCTCATCATC
6	EF592206	F: GCGAAAGAGGAGAGTGCAAG	R: TCTATAAGTGCCCCCTCACG
7	EF592216	F: TCTATAAGTGCCCCCTCACG	R: ACTGCCACCGTGGAAGTAG
8	EF592214	F: CTGAGTTTGGCTAGGGAGAG	R: TTGATCCTTCACCACCATCA
9	EF59210	F: AGCTATCGCCACAGCAAATC	R: GTCTTCTTCTGGTCGCCAAC
10	EF592198	F: TCTGACGTCACCTCCTTTCA	R: ATACTCGTGCCTCGTCCTGT
11	EF592211	F: TTCTGTTAGTGGCGGTGTTG	R: CACCTCCTCCTCCTCCTCTT
12	EF592197	F: GCTTGCTTCCAACTGAGACC	R: GCAAAATGCTCGGAGAAGAC

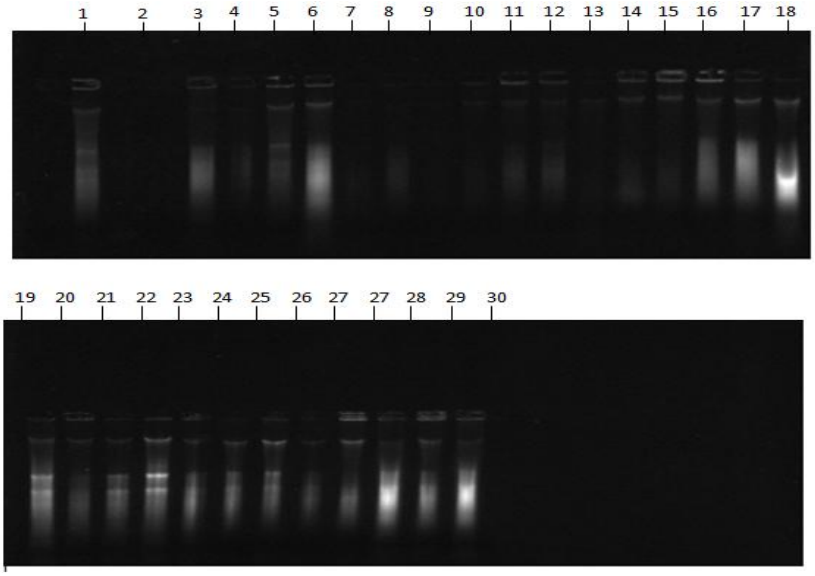


Plate 1. Photograph showing the gel from PCR Amplification of genomic DNA of *Mangifera indica* varieties

1379.00µl while OYOM ACC-5 had the lowest DNA concentration of 0.91 gl from total genomic DNA of 0.25 as shown in Table 3. In the present study, Twelve (12) SSR markers were used to reveal genetic diversity among 30 mango varieties, out of which 10 were polymorphic, with SSR 20 being the most polymorphic (Table 4). The result in Table 4 also shows the number of alleles which ranged from 2 to 4 with an average of 2.50 alleles per locus while Gene diversity

Table 3. Nanodrop and DNA concentration of *Mangifera indica*

S/N	Varieties	Total genomic DNA extracted (µl)	DNA concentration (260/280 gl)
1	Tommy Harkin	28.84	1.00
2	Johnbull	449.10	1.39
3	Julie	62.99	1.35
4	Edward	75.08	1.05
5	Saigon	411.20	1.63
6	Palmer	173.1	1.73
7	Harden	231.4	2.01
8	OYOMAcc-5	0.25	0.91
9	OGBMAcc-8	15.40	1.60
10	OGBMAcc-10	98.65	2.56
11	BIGMCBAcc-1	94.31	1.61
12	OGBMAcc-3	166.80	1.40
13	OYOM Acc-3	54.61	2.14
14	OGBMAcc-5	405.30	4.03
15	SAKMAcc- 2	262.20	1.43
16	SAKMAcc- 4	956.40	2.42
17	OROMAcc- 4	1263.00	1.97
18	SWMUI IdiaAcc- 2	1229.00	1.91
19	OYOM Acc-1	176.00	1.74
20	OROMAcc -2	833.90	1.67
21	GRAFE	437.80	1.88
22	ISEHIN MANGO	209.70	1.86
23	GERMAN Acc- 1	214.30	1.88
24	FEDMINAcc- 2	232.10	1.90
25	BUTMAcc-1	286.70	1.76
26	BIGM (Ipapo)	171.40	1.89
27	SWMUIAcc- 1	275.50	1.82
28	SWMUIAcc- 3	1379.00	1.77
29	FEDMINAcc- 1	507.50	1.94
30	SHRIMAcc- 2	89.07	1.60

Table 4. Frequency, diversity of alleles and Polymorphic Information Content (PIC) of *Mangifera indica* varieties

Markers	Major allele frequency	Sample size	Allele number	Gene diversity	PIC (%)
SSR 20	0.50	30.00	4.00	0.64	57.57
EF 592216	0.90	30.00	2.00	0.18	16.38
EF 592211	0.73	30.00	2.00	0.39	31.46
EF 592206	0.73	30.00	3.00	0.42	38.33
EF 592217	0.97	30.00	2.00	0.07	6.56
EF 59210	0.97	30.00	2.00	0.07	6.56
SSR 16	0.57	30.00	4.00	0.57	45.28
EF 592197	1.00	30.00	2.00	0.00	0.00
SSR 8	1.00	30.00	2.00	0.00	0.00
SSR 19	0.93	30.00	2.00	0.11	5.22
EF 592203	0.43	30.00	3.00	0.61	56.22
EF 592198	0.87	30.00	2.00	0.23	24.89
Mean	0.80	30.00	2.50	0.27	2.88

values ranged from 0.00 to 0.64 with a mean of 0.27. Results obtained as shown in dendrogram in Fig. 1 indicated the genetic diversity of varieties from different locations. Three major clusters were generated, Clusters 2 had the highest number of varieties with Ogbomosho Mango Acc-2 (OGBM ACC-2) branched-out at a distance of 0.15 from other varieties. However, Primer SSR20 produced the highest values for allele diversity and Polymorphic Information Content at 0.5757 (57.57%) followed by EF 592203 with 0.5622 (56.22) as shown in Table 4. However, the photograph in Plate 1 shows the gel of Polymerase Chain Reaction (PCR) amplification from genomic DNA in Mangifera Indica. The photographs in Plate 2-13 are gels showing bands obtained with SSR primers. The photographs revealing the variation in M. indica collected from selected locations in OyoState, Nigeria are shown in Plate 14.

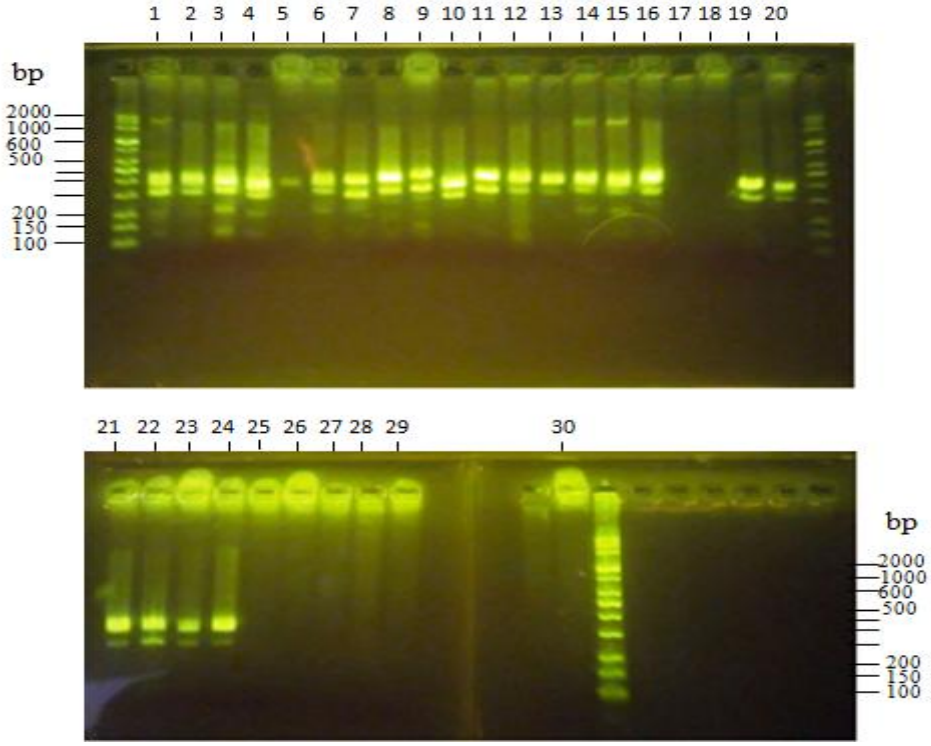
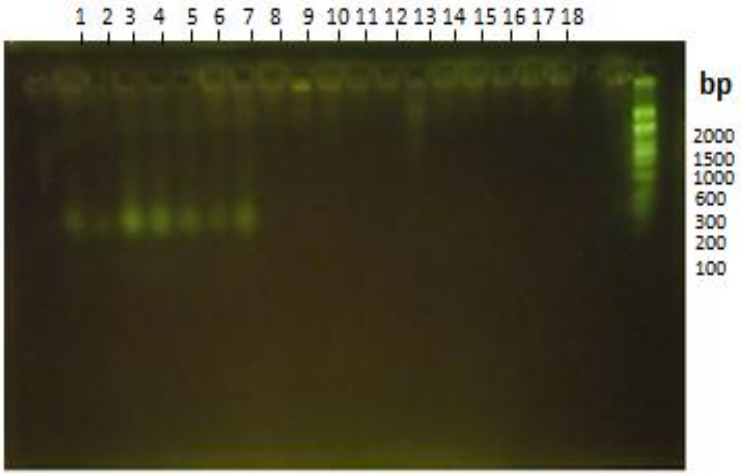


Plate 2. Photograph showing bands obtained with Primer EF 592216



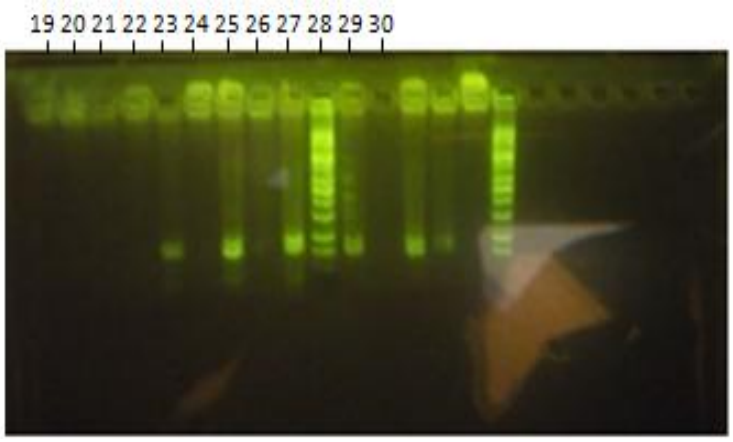


Plate 3. Photograph showing bands obtained with Primer SSR 20

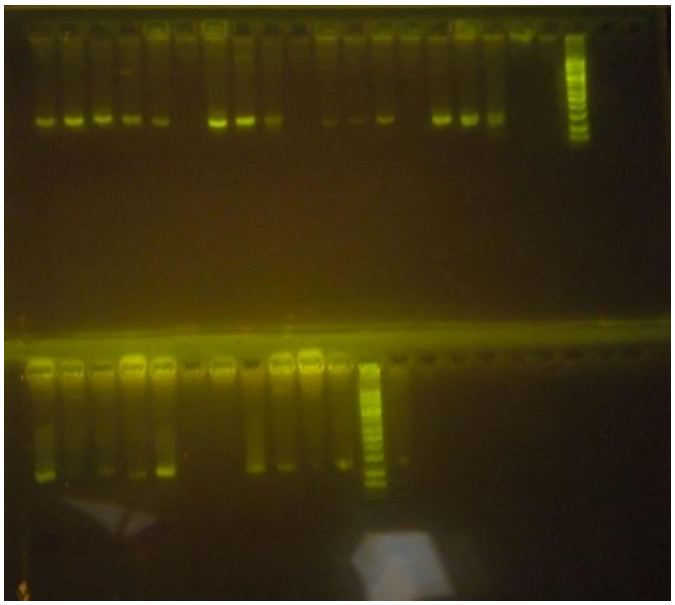


Plate 4. Photograph showing bands obtained with Primer EF 592211

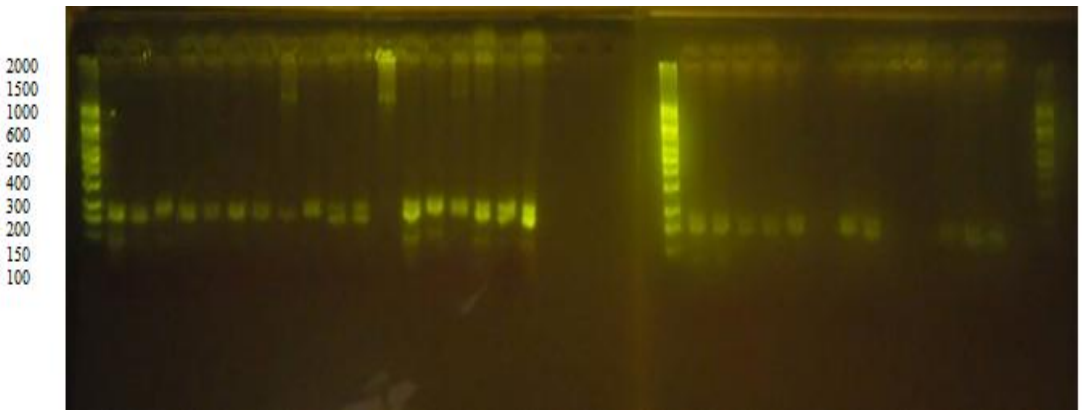


Plate 5. Photograph showing bands obtained with Primer EF 592206

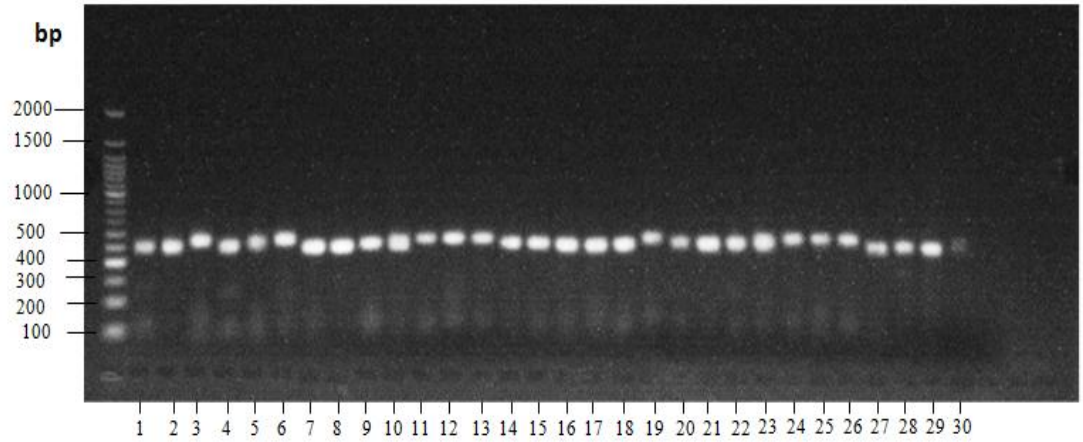


Plate 6. Photograph showing bands obtained with Primer EF 592197

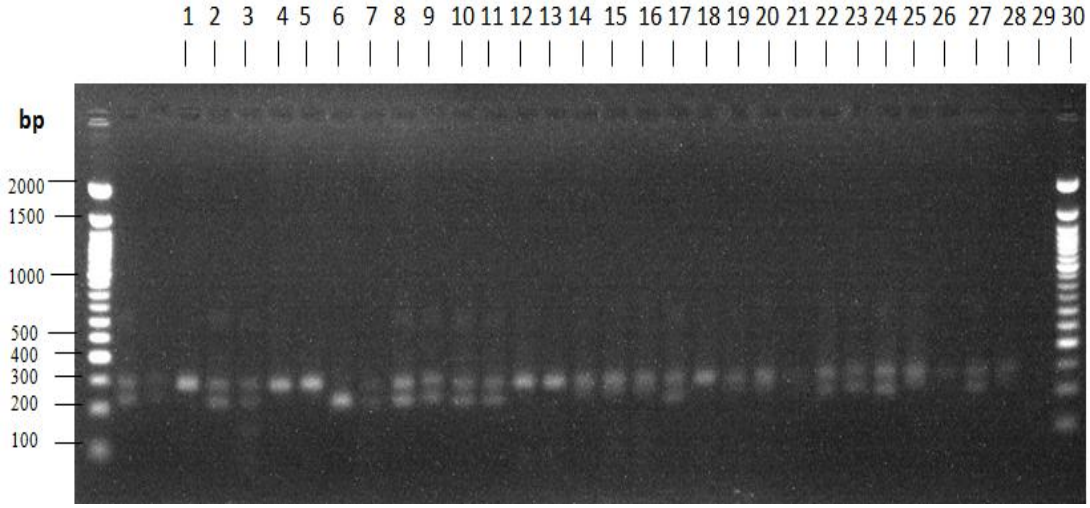


Plate 7. Photograph showing bands obtained with Primer EF 592203

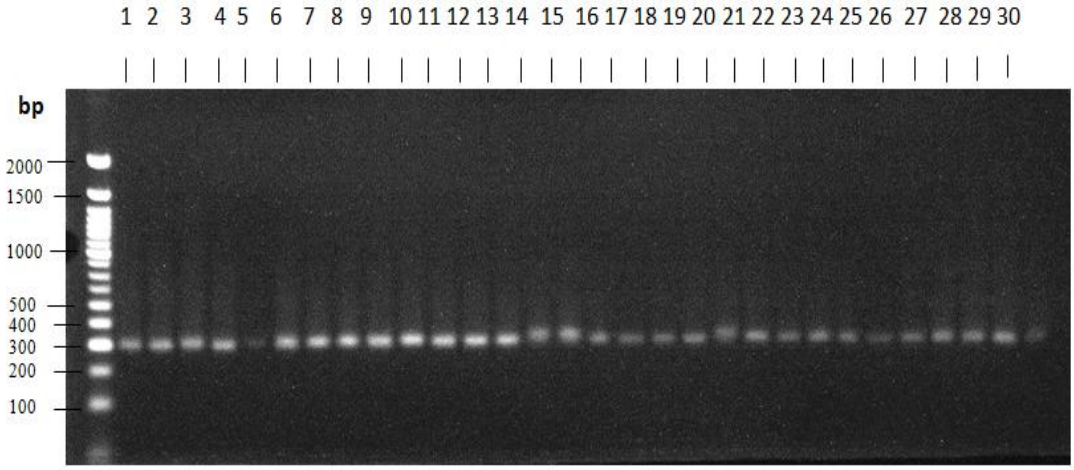


Plate 8. Photograph showing bands obtained with Primer SSR 8

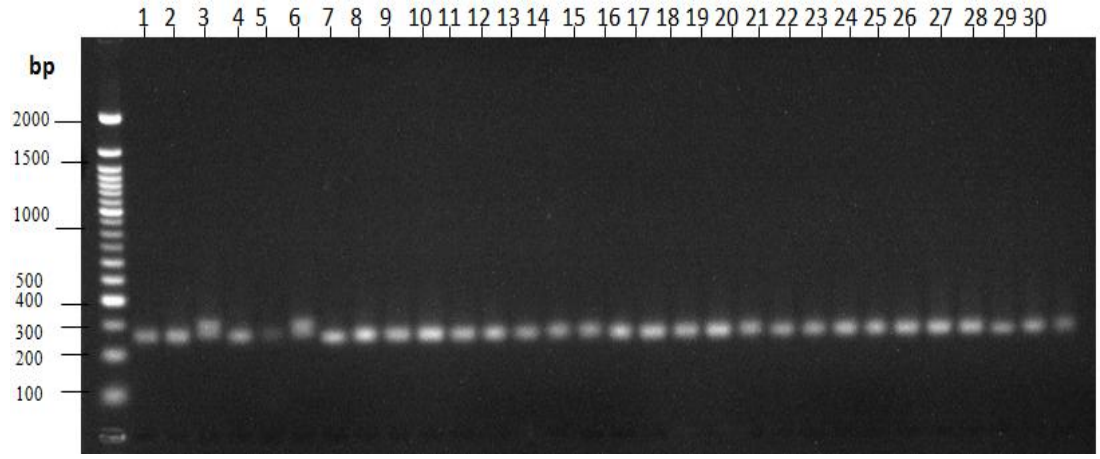


Plate 9. Photograph showing bands obtained with Primer SSR19

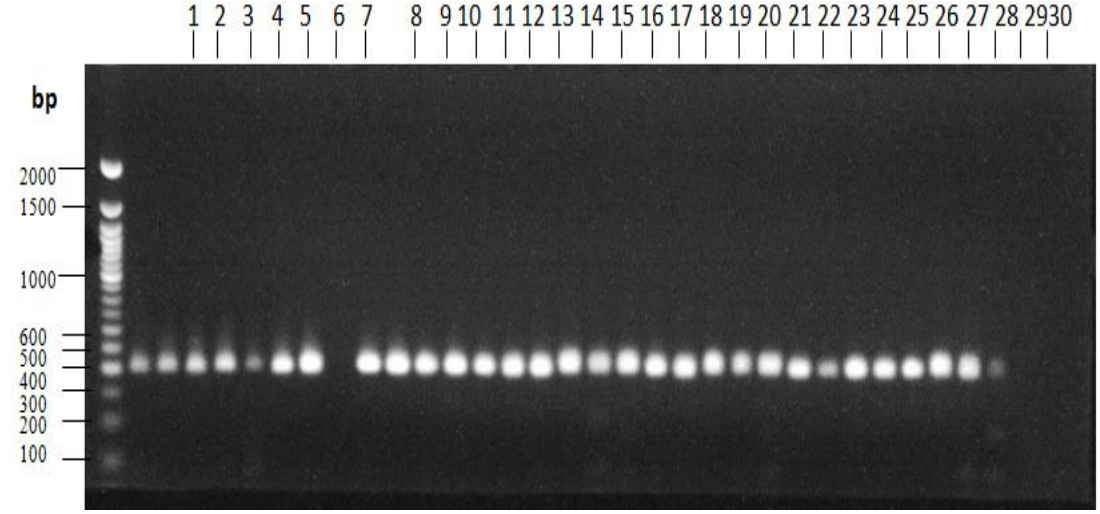


Plate 10. Photograph showing bands obtained with Primer EF59210

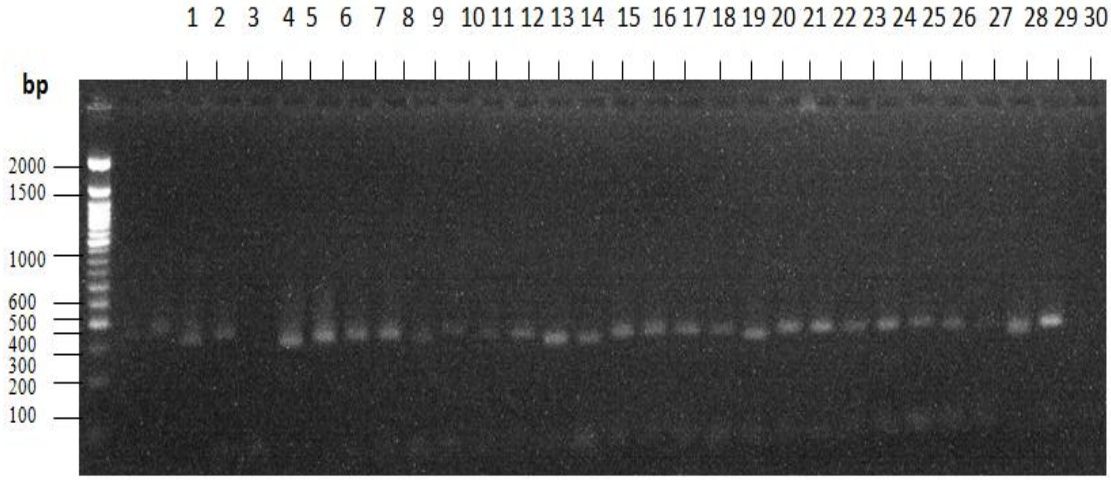


Plate 11. Photograph showing bands obtained with Primer EF592198

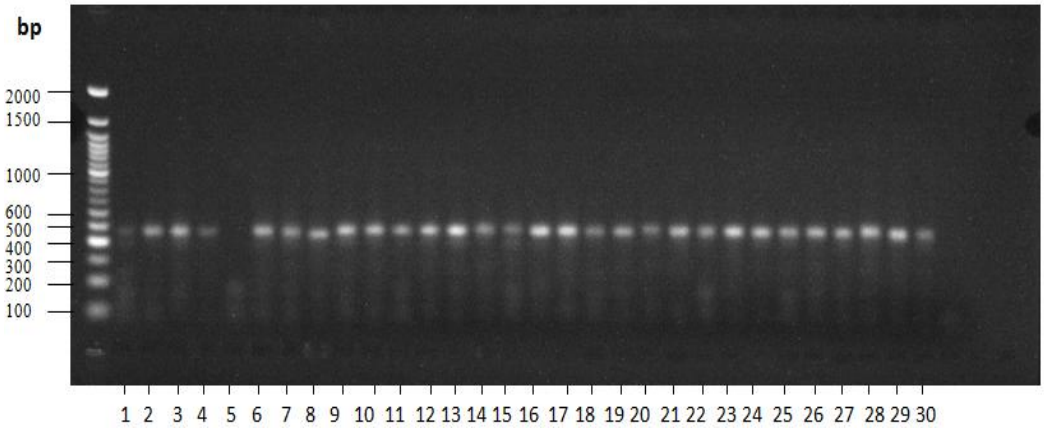


Plate 12. Photograph showing bands obtained with Primer EF592217

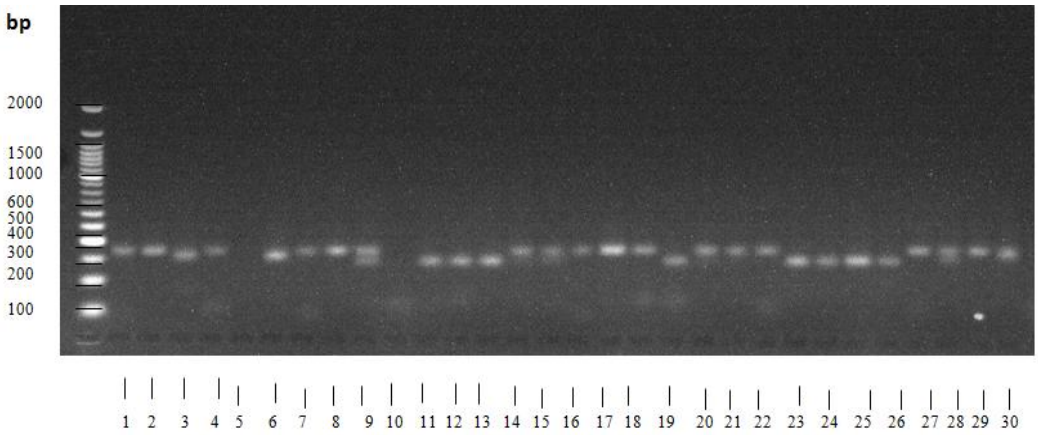


Plate 13. Photograph showing bands obtained with Primer SSR16

Plate 14. Photographs of the mango fruits collected from NIHORT, Ogbomosho, Saki, Oyo, Isehin and other locations in Oyo State

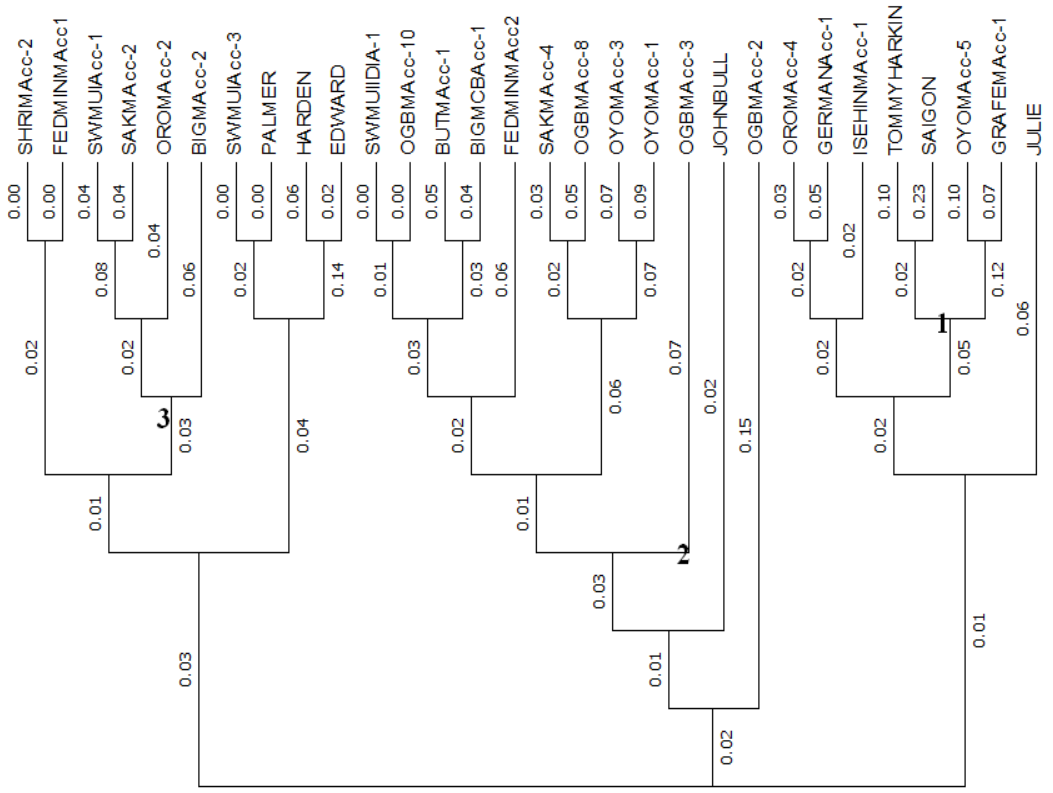


Fig. 1. Dendrogram showing the phylogenetic relationships between 30 varieties of *Mangifera indica*

4. DISCUSSION

Molecular characterization is more sensitive and unaffected by environmental conditions unlike morphological characterization that may have resulted from undefined variation in leaf, fruits, stem and root of Mangifera indica. Molecular characterization methods provide a more detailed and clear description of both existing and new germplasm in plants such as *Mangifera indica*. In particular, SSR markers (high reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage markers) are very useful for variety identification in plant genetics. It has the ability to reveal additional insights when morphological descriptors are insufficient to distinguish between varieties even when genetically close [46,47].

The small range of PIC value in this study disagrees with the high PIC values and alleles number obtained in the finding of Ravishankar, et al. [47]. PIC value helps to predict the potential utility of DNA markers for germplasm assessment in molecular breeding. Markers with higher PIC values have greater potential in showing allelic variation according to the findings by Spandana, [48]. Moreso, Low level of genetic diversity observed in the present study may have resulted from the frequent use of few parents in breeding and a narrow genetic base among selected Mango varieties in Oyo State. This supports the findings of Kumar, et al. [49] who opined that low level of genetic diversity depicts the frequent use of only few parents in breeding among selected cultivars.

5. CONCLUSION AND RECOMMENDATION

Molecular studies on mango could be considered in breeding of mango varieties, so as to broaden the genetic base of cultivated mango for enhancement of proper nomenclature and yield of different mango varieties. Sweet Mango UI 3, Ogbomosho Mango Acc-2 (OGBM ACC-2), Julie Mango are potential accessions for further breeding studies. Primers SSR20 and EF 592203 were more polymorphic and should be

considered for sequencing of mango genes in order to assess genetic variability and order of nucleotide bases along its genome.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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