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Genetic assessment of *Mangifera indica* Linn. (Mango) from selected locations in Oyo State, Nigeria

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Abstract

This study characterized five (5) varieties of mango comprising 15 accessions collected from Ogbomosho, Saki, Ibadan and other locations in Oyo State. The field experiment was laid out in a Complete Randomized Design (CRD) with three replicates. Morphological characters were assessed on the stem, leaf and fruit. Also, Molecular studies (DNA amplification and sequencing) were carried out on 15 accessions of mango. The edited sequences were blasted in the National Center of Biotechnology Information (NCBI) data website. The Results showed variability in morphological characters of Mango. Ogbomosho Acc-2 performed best for leaf width (4.53cm) and lamina length (16.25cm) while Isehin Acc-1 had the highest number of leaves per seedling (7.76cm), leaf length (17.06cm), leaf area (38.84cm), petiole length (2.27cm), plant height (24.07cm) respectively. The Number of leaves had positive correlation with Leaf length (r=0.53), Leaf Area (r=0.59), Internodal Length (r= 0.55) and strong positive correlation with plant height (r=0.73) at p≤0.05. The success rate of amplified DNA products and sequencing was 77.78%. The query coverage of 99% and 100% confirmed positive amplification and sequencing of *rbcL* gene in the mango varieties. The sequences blasted in the NCBI data website were identified to be similar to accession KX871231.1. Sequences of *rbcL* marker showed genetic differences among samples; Grafe and OGBM Acc -1. Genetic distance between varieties from the same location was most often lower with Grafe mango being the most distant variety with genetic distance of 0.114-0.117. There were morphological and molecular variations in mango varieties and accessions. Isehin Acc-1, Saki Acc-1 and OGBM Acc-6 accessions had better growth performance.

Keywords: Mango, marker, sequences, morphological, phylogenetic.

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INTRODUCTION

Mango (*Mangifera indica* L.) is a favorite diploid fruit tree with 20 pairs of chromosomes (2n = 40) and 439 Mbp genome size (Roy and Visweswaraiya, 1995; Mukherjee SK, and Litz, 2009). A perennial fruit crop, rich source of vitamins, β-carotene, minerals, and antioxidants, often called "king of fruits" for its unmatchable taste and flavor (a native of Southern Asian countries (Begum *et al.,* 2014; Kaur *et al.,* 2014). India is the largest producer in the world (18.0 million tons per year) (FAO, 2015), More than a thousand varieties of Mango have been identified all over the world (Rymbai *et al.,* 2014). Mango was introduced to West Africa in the 16th century by the Portuguese and since then it has become highly diversified and accepted fruit in Nigeria and other African countries (Okigbo, 2001; Fowomola, 2010). About 63 countries account for more than 1000 million tons of mango fruit production annually with India as the leading producer (FAOSTAT, 2015).

Morphological characterization is an important traditionally tools used to study variation in different crops (Gonzalez *et al*., 2002) including mango (Subedi *et al*., 2009). Morphological characteristics are still extremely useful for identification and or differentiation of cultivars, since mango published descriptors, lists are readily available (Hoogendijk and Williams, 2001; IPGRI, 2006). Also, being an important fruit crop with huge diversity, the plant portends an important genetic resource that may be explored by breeders for improvement purposes especially the fruit characters (IITA, 2015). Genetic variation plays a key role in successful breeding programs of plants (Olawuyi *et al.,* 2015).

DNA extraction is one of the methods used in molecular analysis of plants and the use of Sodium Dodecyl Sulfate (SDS) and Proteinase K procedure described by Goldenberger *et al.,* (1995) has been found promising in DNA extraction with high rate of efficiency. The Sodium Dodecyl Sulfate (SDS) is strong anionic detergent that can solubilize the proteins and lipids that form the membranes to removes the negative ions from the protein and destroys its confirmation (Goldenberger *et al.,* 2005). Recently, the necessity of DNA sequencing became eminent as described by Francis Crick's theory that the sequence of nucleotides within a DNA molecule directly influenced the amino acid sequences of proteins (Mussane *et al.,* 2010; Azim, *et al.,* 2014).

Several studies on characterization of mango focused on morphology and use of molecular markers. There is need to provide more information on molecular sequence of mango. Hence, this study investigated the variability and relationship among the mango varieties and accessions evaluated in this study.

MATERIALS AND METHODS

Plant collections and study location

Five (5) mango fruit varieties comprising of 15 accessions were collected between March to May 2018 following the method described by IPGRI, (2006) (Table 1). The geographic location of each of the sampled trees was recorded using a hand-held Global Positioning System (GPS) as shown in Table 1.

Experimental design and planting procedure

The field experiment was in Completely Randomized Design (CRD) in three replicates. The Mango seeds were processed using the procedure described by Verheij (2004).
The planting was done in an open field using 1.0 m spacing within the row and column at the research farm of the Department of Botany, University of Ibadan, Nigeria.

Determination of morphological characters

The morphological characters of all accessions were carried out from the first week to the twelfth week using the method described by IPGRI (2006).

Molecular studies

The Molecular experiments (DNA extraction, Amplification, purification and Sequencing) using rbcL with HIf and Fofana primers were carried out for all accessions at Bioscience Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

DNA extraction

Fresh leaf samples were harvested from each accession early in the morning and lyophilized at −80˚C. DNA extraction was carried out using Sodium Dodecyl Sulfate (SDS) and Proteinase K procedure described by Goldenberger (1995). Each sample (100mg) of frozen dried leaves and two steel balls was added into each extraction tube and grind into fine powder using Genogrinder-2000. Pre-heated plant extraction buffer of 450µl was added and incubated at 65^0c for 20 minutes, by inverting the tubes to homogenize the sample.

Table 1: List of mango accessions collected from different locations with their coordinates

s/n	Accessions	Local names	Locations	Coordinates
1	OYOM Acc-2	Oyo mango	Oyo	N 08^0 26 12 7 E 003^0 29 22 5
2	SAKM Acc-1	Saki	Saki	N 08^0 38 39 0 E 003^0 24 01 9
3	ISEM Acc-1	Iseyin Oro mango	Isehin	N 08^0 40 13 8 E 003^0 23 43 6
4	OGBM Acc -11	Mango	South	N 08^0 06 40 6 E 004^0 13 58 7
5	OYOM Acc-4	Oyo mango	Oyo	N 08^0 26 01 4 E 003^0 29 23 4
6	SHRIM Acc-1	Sheri mango	Agunrere- Atisbo	N 08^0 24 01 3 E 003^0 23 32 7
7	OGBM Acc -1	Mango	LAUTECH	N 08^0 10 07 4 E 004^0 16 52 4
8	GERMAN Acc-2	German mango	South	N 08^0 03 12 5 E 004^0 08 35 7
9	GERMAN Acc-3	German mango	South	N 08^0 03 12 8 E 004^0 05 32 2
10	OROM Acc-3	Oro mango	Agoare, Saki	N 08^0 37 55 9 E 003^0 24 21 7
11	OGBM Acc -5	Mango	LAUTECH	N 08^0 15 07 0 E 004^0 18 50 2
12	OGBM Acc -6	Mango	LAUTECH	N 08^0 10 06 3 E 004^0 16 49 7
13	OGBM Acc -7	Kerosene mango	Surulere LGA	N 08^0 11 39 0 E 004^0 16 15 1
14	GRAFEM Acc-1	Grafe mango	Saki	N 08^0 40 13 8 E 003^0 23 43 7
15	SWMUI IDIA-2	Sweet mango	Idia UI	N 07^0 26 18 3 E 003^0 53 47 9

KEY: Oyo Mango Variety **(**OYOM Acc-2, OYOM Acc-4, OROM Acc-3 and GRAFEM Acc-1 accessions), Ogbomosho 1 variety (OGBM Acc -1, OGBM Acc-5, OGBM Acc-6, OGBM Acc-7, OGBM Acc -11 accessions), Ogbomosho 2 variety (GERMAN Acc-2, GERMAN Acc-3 and SHRIM Acc-1 accessions), SAKI variety (SAKM Acc-1 and ISEM Acc-1 accessions), Ibadan Variety (SWMUI IDIA-2 accession), LAUTECH (Ladoke Akintola University of Technology, Ogbomosho, Oyo State), UI (University of Ibadan, Nigeria).

The tubes were later removed and allow to cool for 2 minutes before adding 200µl of ice-cold 5M Potassium acetate and incubated on ice for 20 minutes to precipitate protein later centrifuged at 10000rpm for 10 minutes and then the supernatant was transferred into freshly labeled tubes. Ice-cold Isopropanol of 2/3 volume was added, mixed gently and incubated at -80^{o}c for 15mins, centrifuged at 100000rpm for 10 minutes to precipitate the DNA. The supernatant was decanted until the last drop was released and 400µl of 70% ethanol was added to wash the DNA pellet and centrifuged at 10000rpm for 10minutes. The supernatant was decanted until the last drop and air dry the pellet.

Also, 60µl of ultra-pure water or low salt TE was added to re-suspend the DNA with 2ul of RNase and incubated at 37^{o}C for 30-40 minutes. Agarose gel of 0.8% was prepared for checking DNA quality and

removal of RNA (boil 0.8 gram of agarose in 100ml of 1X TBE) from the extracted DNA. The gel was cooled to about 60^{o}c then 5µl ethidium bromide was added and gently mixed, later poured into the gel tray before it polymerizes. Air bubbles was avoided in the middle of the gel. DNA of 3µl was mixed with 3µl of loading dye was pipetted into 0.8% agarose gel and run at 80 volts for about 60 minutes. The gel picture was saved.

DNA amplification and primers

The amplification reaction were prepared for 25µL PCR Reaction volume containing 2.0 µl of 100ng/µl DNA, 2.5 µl of 10× PCR buffer, 1.5 µl of 50mM $Mgcl_2$, 1.0 µl of 5pMol forward primer, 1.0 µl of 5pMol reverse primer, 1.0 µl of DMSO, 2.0 µl of 2.5Mm DNTPs, Taq 5µ/µl 0.15, 13.85 µl of H_2O for 25µL. Amplification were performed in thermocycler programmed for a touch-down (TDSSR) protocol at the initial step of denaturation for 5 minutes at 94°C followed by 9 cycles and later 35 cycles each consisting of denaturation step of 15 seconds at 94°C, an annealing step of 20 seconds at 65°C and an extension step of 30 seconds at 72°C. Seven minutes will be given after the last cycle of the extension step at 72°C to ensure the completion of primer extension reaction followed by cold temperature at 10°C lasting for infinity. The primers considered are H1f F: CCACAAACAGAGACTAAAGC and Fofana R**:** GTAAAATCAAGTCCACCGCG.

PCR purification process and sequencing

The PCR product was purified by adding 20 µl of absolute ethanol to the PCR product and incubated at room temperature for 15minutes later spined down at 10000rpm for 15minutes, the supernatant was decanted, spined again at 10000rpm for 15minutes then 40ul of 70% ethanol was added, the supernatant was later decanted, air dry. The amplicon was checked on 1.5% agarose (Zeugin and Hartley, 1985).

Purified samples were sequenced by Genetic analyser 3130×1 sequencer from Applied Biosystem using manufacturer's manual, the sequencing kit of Big Dye terminator V3.1 cycle sequencing kit was considered while Bio edit software (Mega 6) was used for sequence editing.

Statistical analyses

Morphological Data were subjected to Analysis of Variance (ANOVA) using SAS 9.1 software 2003 version. The Differences in means were separated using Duncan Multiple Range Test (DMRT) at $p<0.05$. Variation trends among the quantitative traits were established using Pearson Correlation Coefficient and Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

The five (5) varieties of mango and their accessions are listed in Table 1. The varieties include Oyo Mango Variety **(**OYOM Acc-2, OYOM Acc-4, OROM Acc-3 and GRAFEM Acc-1 accessions), Ogbomosho 1 variety (OGBM Acc-5, OGBM Acc-6, OGBM Acc-7, OGBM Acc -11 and OGBM Acc-7 accessions), Ogbomosho 2 variety (GERMAN Acc-2, GERMAN Acc-3 and SHRIM Acc-1 accessions), SAKI variety (SAKM Acc-1 and ISEM Acc-1 accessions), Ibadan Variety (SWMUI IDIA-2 accession).

The result in Table 2 shows the growth performance of five Mango varieties. The variety of mango from Oyo is significantly ($p<0.05$) higher for spouting days (0.58). The number of leaves per seedling (7.76), leaf area (34.86 cm^2), leaf length (17.06cm), Plant height (24.07cm) and lamina length (15.34cm) are higher in Ibadan variety. The Ogbomosho 2 variety was higher for leaf length (16.32cm) and lamina length (16.25cm), while varieties from Ogbomosho 2 and Ibadan are significantly higher in leaf width at 4.53cm and 4.44cm respectively**.** The leaf area in varieties from Ogbomosho 2, Ibadan and Saki were higher, while the petiole length in varieties from Ogbomosho 1, Ogbomosho 2, Ibadan, Oyo and Saki were significantly higher. The Ogbomosho 1, Oyo and Saki varieties were significantly higher for lamina length.

The growth performance of Mango based on locations revealed significant difference in table 3. The mangoes from Ogbomosho had the highest mean of 0.08 for Sprouting Days, leaf length (15.30cm) and lamina length (14.74cm), while Saki produced the highest mean number of leaves per seedling (9.09), leaf area (32.57 cm^2), internodal length (26.74cm) and plant height (26.16 cm). The leaf width (4.63cm) and petiole length (2.12cm) had the highest for Ibadan accession. Ogbomosho, Saki and Ibadan varieties were significantly higher for sprouting days (0.80), number of leaves per seedling (9.09), leaf length (15.30cm), leaf width (4.63), leaf area (32.57cm^2). The lamina length (14.74cm) in mango variety from Ogbomosho is significantly higher than other varieties.

The result in Table 4, shows the effect of mean square interaction of location, replicate, varieties and weeks on growth related characters of Mango. The locations, accessions, weeks, first order interaction (location x accessions, location x week) and second order interaction (location x accessions x week) had

higher significant ($p<0.001$) effect on Sprouting days. The location, replicate, accessions and weeks, first order interaction (location x replicate, location x accessions, location x week, accessions x weeks) and second order (location x accessions x replicate, location x accessions x week) significantly affected the number of leaves per seedling. The leaf length, leaf width, leaf area, plant height and lamina length had higher significant effect for accessions, week, first order interaction (location x replicate, location x accessions, accessions x replicate, accessions x week) and second order interaction (location x accessions x replicate). The petiole length produced high significant effect on location, replicate, accessions and first order interaction (location x accessions, location x week) (Table 4).

The result from PCA of growth-related character in Mango (Table 5). Mango varieties in Six (6) Principal Component Axes, Prin 1, Prin 2, Prin 3, Prin 4, Prin 5 and Prin 6. Prin 1 accounts for the highest Eigen value of 2.62 with proportion of 29.00% while Prin 6, had the least eigen value 0.66 with proportion of 7% (Table 5). It was observed in Prin 1 that leaf length (0.57cm) and lamina length (0.55cm) are positively closely related. In Prin 2, Number of leaves per seeding (0.55), leaf area (0.48cm^2) and plant height (0.47cm) were positively closely related. Prin 3, leaf length (-0.08cm), leaf width (-0.03), leaf area (-0.06cm^2) and lamina length (-0.06cm) are negatively closely related while petiole length (0.67cm) and intermodal length (0.57cm) are positively closely related. Prin 4, Number of leaves per seedling (-0.01cm), leaf area (-0.02cm^2) are negative closely related while leaf length (0.15cm) and petiole length (0.16cm) are positively related. Prin 5 shows that leaf length (-0.06cm), leaf width (-0.03), lamina length (0.08cm) were negatively closely related sprouting days (0.05), internodal length (0.09cm) are closely related as shown in Prin 6.

Correlation coefficient among the growth-related characters of Mango varieties at 5% level of significance ($P\geq0.05$). The result of table 6 shows that the No of leaves per seedlings had a positive correlation with leaf length (r=0.53), leaf area (r=0.59), internodal length (r= 0.55) and strong positive correlation with plant height (r=0.73). Leaf length produced a strong positive association with leaf width (r=0.73), lamina length (r=0.99) has a positive correlation with plant height (r=0.53). Leaf width produced strong positive correlation of leaf length (r= 0.74); Leaf area produce positive correlation with plant height of (r=0.52).

The result in Table 7 shows the genetic distance among mango accessions. OROM Acc-3 (0.002), SHRIM Acc-1 (0.002) and OGBM Acc- 6 with (0.002) genetic distance are closely related than OGBM Acc-1 (0.046) and GRAFE Acc-1 (0.114), while German Acc-2, OROM Acc-3, German Acc-3, SWMUI IDIA-2 and OYOM Acc-1 (0.000) are genetically related. Also, German Acc- 3, OROM Acc-3, OGBM Acc-5, SWMUI IDIA-2 and OYOM Acc-2 (0.002) are closely related than OGBM Acc-1 (0.048) and Grafe (0.117). The GRAFE Acc-1 had higher genetic distance of 0.114 to 0.117 as compared to other mango accessions. Studies of genetic diversity based on molecular markers in the selected mango varieties revealed that location also played an important role in diversity. Genetic distance between varieties from the same location was most often lower. Sánchez-Guillén *et al.,* (2011) had indicated the influence of location in genetic diversity studies, this might be responsible for the close relationship between members originated from close locations.The success rate of amplified DNA products and sequencing was 77.78%, and DNA sequencing showed 100% query cover which is identical to the mango on the Michigan Center for Biological Information (MCBI) as similarly reported by Iquebal *et al.* (2017). Edited sequences were blasted in the NCBI data website and were identified to be similar to Mango accession KX871231.1, indicating the closeness of all varieties tested as shown in Table 8. However, the result from sequence analysis shows that sequencing region of amplified gene revealed genome size of 439Mbp, and this agrees with the reports of Singh (2016). The result of each *rbcL* sequence from NCBI database shows that all the sequences of *rbcL* loci were identified as *rbcL* sequences of *Mangifera indica* in which most of them had identity of 99% and 100% coverage confirming positive amplification and sequencing of the *rbcL* gene in mango varieties (Table 8).

Sequences of *rbcL* marker shows several genetic differences among accessions especially in GRAFE Acc-1 and OGBM Acc-1 (LAUTECH 1) as they didn't cluster close to the other varieties (Figures 1 and 3). The result in figure 1 is the Dendrogram showing the relationships among accessions based on quantitative characters in fruit. All accessions in the same clusters are similar or closely related to each other. Ogbomosho 11 (OGBM Acc-11) is more closely related to German 1 and related to German 2 as shown in cluster 1. Cluster 2 had 5 accessions with Oro Mango 3 more closely related to Cherry Mango (SHRIM Acc-1). Also, Lautech 1 (OGBM Acc-1) and Lautech 5 (OGBM Acc-5) are closely related to each other in sub clusters of 4.

Table 2: Growth Performance of Mango Varieties

Mango Varieties	Sprouting days	No. of leaves per seedlings	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)	Petiole Length (cm)	Internodal Length (cm)	Plant Height (cm)	Lamina Length (cm)
OGBOMOSHO 1	0.36^{b}	6.63^{c}	14.85^{b}	3.87^{b}	14.83^{d}	2.08^{ab}	2.89^{b}	20.30^{b}	4.79^{b}
OGBOMOSHO 2	0.54^{a}	7.33^{b}	16.32^{a}	4.53^{a}	16.27^{c}	1.88^{bc}	2.24^{c}	23.39^{a}	16.25^{a}
OYO	0.58^{a}	6.94^{c}	13.36^{c}	3.47^{c}	13.78^{d}	1.77^{c}	2.79^{b}	20.95^{b}	13.31^{c}
IBADAN	0.28^{c}	7.76^{a}	17.06^{a}	4.44^{a}	34.86^{a}	2.27^{a}	2.79^{b}	24.07^{a}	15.34^{a}
SAKM	0.56^{a}	7.76^{a1}	13.08^{c}	3.87^{b}	32.31^{b}	1.96^{bc}	3.10^{ab}	20.62^{b}	11.82^{ab}

Mean with the different letters in the same column are significant at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT)

Table 3: Growth Characters of Mango Varieties from different locations

Locations	Sprouting Days	No. of Leaves per Seedlings	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)	Petiole Length (cm)	Internodal Length (cm)	Plant Height (cm)	Lamina Length (cm)
Ogbomosho	0.80^{a}	7.10^{b}	15.30^{a}	3.79^{b}	20.41^{b}	1.85^{b}	2.29^{a}	21.36^{b}	14.74^{a}
Saki	0.25^{c}	9.09^{a}	14.79^{ab}	3.65^{c}	32.57^{a}	2.01^{a}	26.74^{b}	26.16^{a}	13.98^{b}
Ibadan	0.33^{b}	5.81^{c}	14.66^{b}	4.63^{a}	14.91^{c}	2.12^{a}	2.73^{b}	18.18^{c}	14.70^{a}

Mean with the different letters in the same column are significant at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT). Locations: Ogbomosho (OGBM Acc -1, OGBM Acc-5, OGBM Acc-6, OGBM Acc-7, OGBM Acc -11, GERMAN Acc-2, GERMAN Acc-3, SHRIM Acc-1, OYOM |Acc-2, OYOM Acc-4, OROM Acc-3 and GRAFEM Acc-1 accessions). Saki (SAKM Acc-1 and ISEM Acc1 accessions). Ibadan (SWMUI IDIA-2 accession)

The result in figure 2 is the Dendrogram showing the relationships among accessions based on quantitative characters in seed and pulp. It consists of two main clusters. Cluster 1 had 4 accessions while cluster 2 had 11 accessions. German 1 and Oyo Mango 3 are more closely related to each other and related to Oyo Mango 3 in sub cluster of 1. In Cluster 2, cherry mango (SHRIM) 1 and Oro Mango 3 (OROMAcc-3) are more closely related. Also, Surulere 7 (OGBM Acc-7) and Sweet Mango UI 2 are more closely related in different sub cluster of 2.

The dendrogram showing the relatedness between the 15 accessions of Mango is shown in Figure 3. The dendogram showed that the plant produced a close cluster with their most identical sequence in the NCBI except for LAUTECH 6 (OGBM Acc-6), LAUTECH 1 (OGBM Acc-1) and Grafe Acc-1 which formed an out grouped. This implies that they were the most distantly related but closer to sweet mango UI (SWMUI IDIA-2), LAUTECH 5 (OGBM Acc-5) and Oyo mango 1 (OYOM Acc-1) this agrees with the observation made by Hartana (2010). The main group formed 2 major cluster with Oro Mango, German 3 mango, sweet mango and Surulere mango clustering together and closely related to the reference mango sequence while Oro Mango Acc-2, Oyo mango Acc-1, Sweet Mango UI, LAUTECH 5 and German Mango Acc-1 clustering together. Plate 1 photograph shows the gel obtained with Primer which reveals variation in mango accessions

Table 4: Mean Square Interaction of Location, Accessions and Growth stages of Mango

Source of Variation	Df	Sprouting Days	No. of Leaves per Seedling	Leaf Length	Leaf width	Leaf area	Petiole Length	Intermodal Length	Plant length	Lamina Length
Location	2	15.88**	666.58**	16.82^{ns}	62.81**	17983.15**	4.48**	2.63*	3724.50**	23.72*
Replicate	3	0.13^{ns}	16.38**	184.03**	10.72**	489.79**	1.84*	10.49**	279.33**	167.75**
Accessions	4	2.28**	28.52**	430.54**	27.30**	14093.79**	5.15**	14.39**	42.11**	543.91**
Weeks	11	129.09**	511.5**	1197.79**	69.12**	4263.66**	13.49	79.01**	3722.78**	1164.05**
Location *Replicate	6	0.13^{ns}	6.27**	46.08**	1.77**	183.67**	0.63^{ns}	1.32*	238.48**	53.54**
Location * Accessions	8	8.03**	167.15**	245.99**	23.83**	7933.43**	2.15*	13.99*	1067.01**	345.26**
Location *Weeks	22	15.08**	21.92**	5.20^{ns}	0.88**	438.13**	1.19*	2.65*	314.35**	5.61*
Accessions*Replicate	12	0.08^{ns}	8.55**	51.35**	1.98**	163.39**	0.52^{ns}	1.54*	160.96**	52.47**
Weeks *Replicate	33	0.12^{ns}	1.48^{ns}	8.22^{ns}	0.38**	21.24**	0.75^{ns}	1.72*	48.01**	7.81*
Accessions *Week	44	2.18**	8.39**	31.91**	1.38**	365.84**	0.92^{ns}	1.29*	64.81**	33.44**
Location*Accessions* Replicate	24	0.08^{ns}	11.87**	37.46**	1.28*	212.03**	0.81^{ns}	2.58*	143.38**	37.33**
Location*Weeks* Replicate	66	0.11^{ns}	0.95^{ns}	11.32^{ns}	0.23^{ns}	31.86	0.83^{ns}	1.03*	34.49**	11.99**
Location*Accessions* Weeks	87	6.96**	13.06**	17.54*	0.94^{ns}	261.61	0.99^{ns}	1.47*	78.36**	16.98**
Accessions*Weeks* Replicate	132	0.08^{ns}	1.54^{ns}	10.41	0.20^{ns}	25.64	0.82^{ns}	0.74^{ns}	17.04**	10.85**

Note: * P<0.05 significant, ** P<0.01 highly significant, *** P<0.001 highly significant.

Table 5: Principal component axis showing the growth characters of mango

Characters	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Sprouting days	-0.15	0.02	-0.44	0.83	0.24	0.05
No of Leaves per seedling	0.22	0.55	-0.69	-0.01	0.05	0.12
Leaf length (cm)	0.57	-0.18	-0.08	0.15	-0.06	-0.21
Leaf width (cm)	0.39	-0.33	-0.03	-0.12	-0.03	0.46
Leaf Area (cm^2)	0.26	0.48	-0.06	-0.02	0.14	-0.58
Petiole length (cm)	0.12	-0.06	0.67	0.16	0.70	0.00
Intermodal length (cm)	0.04	0.21	0.57	0.45	-0.63	0.09
Plant height	0.20	0.47	-0.12	-0.13	0.07	0.58
Lamina length (cm)	0.55	-0.25	-0.08	0.15	-0.08	-0.14
Eigen Value	2.62	1.85	1.12	0.96	0.80	0.66
Proportion (%)	0.29	0.21	0.12	0.11	0.09	0.07

Table 6: Correlation coefficients among the growth-related characters of Mango

Character	Sprouting days	No of leaf per seedling	Leaf length	Leaf width	Leaf area	Petiole length	Intermodal length	Plant height	Laminal length	Location	Weeks	Variety	Replicate
No of Leaves per seedling	-0.26												
Leaf length (cm)	-0.31	0.53*											
Leaf width (cm)	0.37	0.39	0.73**										
Leaf Area (cm^2)	-0.18	0.59*	0.41	0.21									
Petiole length (cm)	0.23	0.26	0.36	0.36	0.19								
Intermodal length (cm)	-0.27	0.55*	0.48	0.41	0.38	0.35							
Plant height	0.28	0.73**	0.53*	0.43	0.52*	0.25	0.49						
Lamina length (cm)	-0.31	0.47	0.99**	0.74**	0.29	0.35	0.45	0.49					
Location	-0.10	-0.14	-0.05	0.23	-0.12	0.09	-0.68	-0.12	-0.01				
Weeks	-0.37	0.69	0.68	0.66	0.40	0.41	0.68	0.65	0.66	-0.01			
Samples	0.05	0.08	-0.71	-0.02	0.38	0.01	0.09	0.01	-0.14	-0.01	-0.07		
Replicate	0.02	0.04	-0.14	-0.15	-0.07	-0.84	-0.10	0.04	0.13	0.00	-0.01	0.01	

Note: * P<0.05 significant, ** P<0.01 highly significant, *** P<0.001 highly significant.

Table 7: Genetic distance comparing the relationship among the mango varieties

Mangifera_indica														
OROM Acc-3	0.002													
SHRIM Acc-1	0.002	0.000												
GERMAN Acc- 3	0.000	0.002	0.002											
OROM Acc-3	0.000	0.002	0.002	0.000										
OGBM Acc- 1	0.046	0.048	0.048	0.046	0.046									
OYOM Acc-2	0.000	0.002	0.002	0.000	0.000	0.046								
GERMAN Acc-2	0.000	0.002	0.002	0.000	0.000	0.046	0.000							
OYOM Acc -1	0.000	0.002	0.002	0.000	0.000	0.046	0.000	0.000						
OGBM Acc – 6	0.002	0.004	0.004	0.002	0.002	0.046	0.002	0.002	0.002					
OGBM Acc-5	0.000	0.002	0.002	0.000	0.000	0.046	0.000	0.000	0.000	0.002				
SWM U.1 Acc-1	0.000	0.002	0.002	0.000	0.000	0.046	0.000	0.000	0.000	0.002	0.000			
GRAFE Acc-1	0.114	0.117	0.117	0.114	0.114	0.141	0.114	0.114	0.114	0.114	0.114	0.114		
OGBM Acc-7	0.000	0.002	0.002	0.000	0.000	0.046	0.000	0.000	0.000	0.002	0.000	0.000	0.114	
OGBM Acc-11	0.000	0.002	0.002	0.000	0.000	0.046	0.000	0.000	0.000	0.002	0.000	0.000	0.114	0.000

Table 8: NCBI blasted result showing the level of similarities among the mango accessions and established sequences in the data base

		Max score	Total score	Query cover	E value	Ident	Accession
OROM Acc-3	*Mangifera indica*	1286	1286	100%	0	99%	KX871231.1
SHRIM Acc-1	*Mangifera indica*	1291	1291	100%	0	99%	KX871231.1
ISEM Acc-1	*Mangifera indica*	1247	1247	96%	0	99%	KX871231.1
GERMAN Acc- 2	*Mangifera indica*	1295	1295	99%	0	99%	KX871231.1
GERMAN Acc-3	*Mangifera indica*	876	876	83%	0	95%	KX871231.1
SAKM Acc-1	*Mangifera indica*	1291	1291	98%	0	99%	KX871231.1
OROM Acc-3	*Mangifera indica*	1288	1288	100%	0	99%	KX871231.1
OGBM Acc-1	*Mangifera indica*	1284	1284	98%	0	99%	KX871231.1
OYOM Acc-2	*Mangifera indica*	1273	1273	98%	0	99%	KX871231.1
OGBM Acc- 6	*Mangifera indica*	1275	1275	100%	0	99%	KX871231.1
OGBM Acc-5	*Mangifera indica*	1288	1288	100%	0	99%	KX871231.1
OGBM Acc-7	*Mangifera indica*	658	658	98%	0	90%	KX871231.1
SWMUI Acc-2	*Mangifera indica*	1299	1299	99%	0	99%	KX871231.1
GRAFE MANGO	*Mangifera indica*	1303	1303	100%	0	99%	KX871231.1

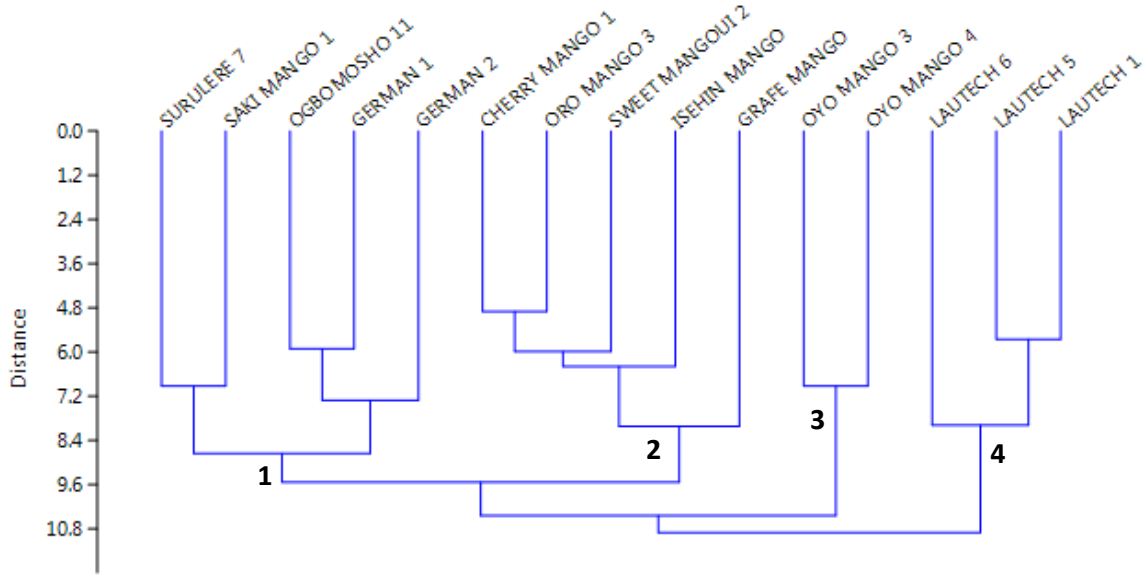

Figure 1: Dendrogram of showing the fruit characters of mango

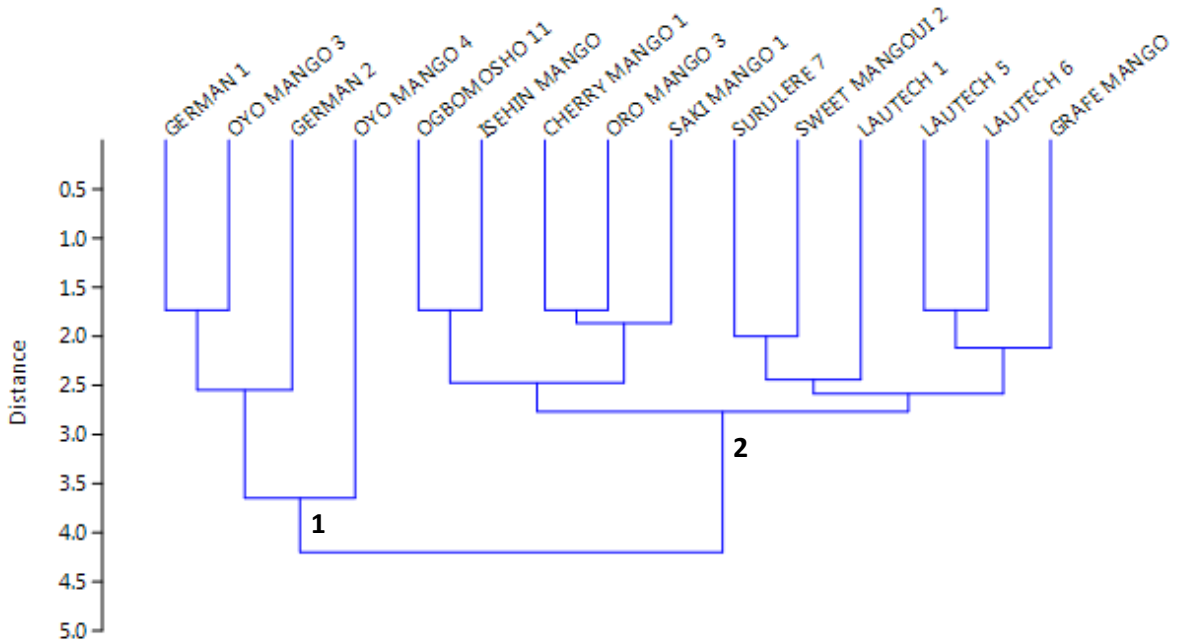

Figure 2: Dendrogram of showing the seed and pulp characters of mango

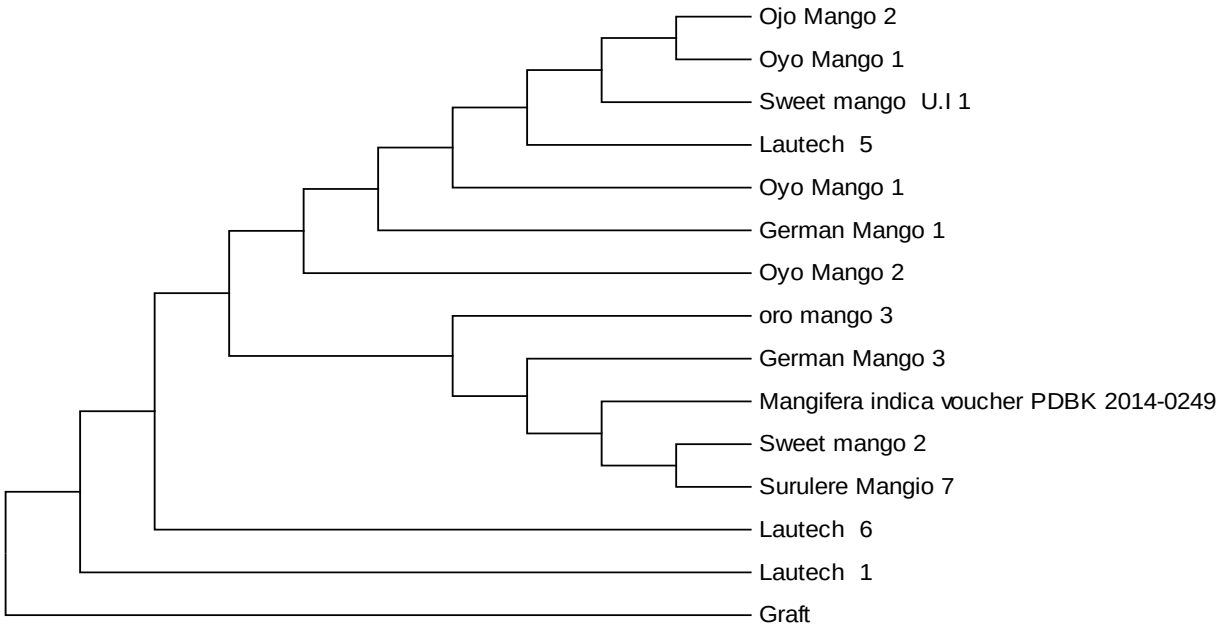

Figure 3: Dendrogram showing relationship among the mango accessions

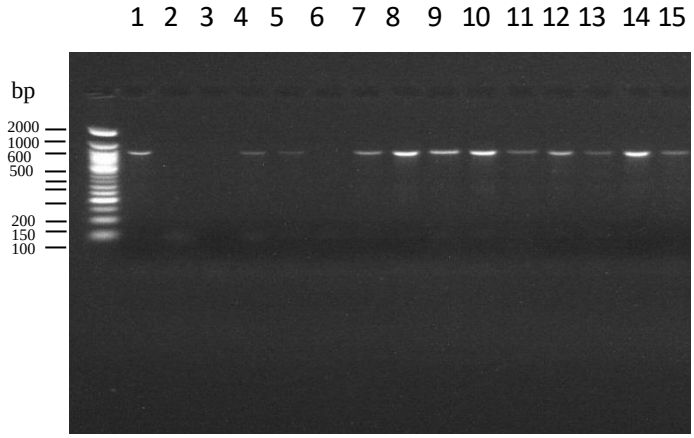

Plate 1: Gel Picture obtained showing 15 accessions of Mango

CONCLUSION

There were morphological and molecular variations in mango varieties and accessions. Isehin Acc-1, Saki Acc-1 and OGBM Acc-6 accessions had better growth performance. The mango from Ogbomoso and Saki locations had higher growth characters. The leaf length, leaf area, internodal length, plant height and number of leaves per seeding were best characters to be selected for further breeding of mango. Hif and Fofana were promising genes for molecular analysis of mango.

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AUTHOR CONTRIBUTIONS

The experiments were carried out by EPC and AII and supervised by OOJ and AAE. OOJ and AAE designed the study and wrote the protocol, while authors EPC and AII performed the literature search and wrote the first draft of the manuscript. All authors performed and interpreted the statistical analyses. All authors read and approved the final draft of the manuscript.

COMPETING INTERESTS

Authors have no competing interest to declare.

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