Original Research Article

Assessment of Genetic Diversity in Plantain (*Musa paradisiaca L*) using Yield-Related ~~Agro-morphological~~ Traits and Microsatellite markers

ABSTRACT

Background and Objective: Plantain (*Musa paradisiaca* L) remains one of themost important staple food crop and perhaps, one of the oldest cultivated fruit tree crop in the humid tropics of Africa, Central Asia, South America and the West Indies.Fourteen (14) elite plantain cultivars were evaluated for genetic diversity using agro-morphological yield related attributes and simple sequence repeat (SSR) markers.

Materials and Methods: Six (6) microsatellite markers that showed distinct fragments varying from 50 bp to 3.0 Kbp in size of polymorphic bands were selected and used for molecular characterization and fingerprinting, while agro-morphological (yield–related) attributes assessed included bunch weight, number of hands/bunch, number of fingers/hands, number of fingers/bunch, harvest interval, length of plant cycle, pulp hardness and pulp to skin weight ratio of the elite plantain cultivars.

Results: The total number of amplified bands (TNB), mean percentage polymorphism (%P), mean polymorphic information content (PIC), average marker index (MI) and mean gene diversity for the SSR assay were 59, 70.24%, 0.79, 3.74 and 0.832 respectively. Results of agro-morphological fingerprint study revealed a significant variations in terms of the bunch weight, number of finger per hands/bunch, number of fingers per hand, number of fingers /bunch, harvest interval, length of crop cycle, pulp hardness and pulp/wt. ratio all showed significant variations among the cultivars. The distribution of the elite cultivars along with the principal components showed cluster pattern of distribution within the study location. Principal component analysis revealed four principal components contributing 99.91% to the observed morphological variations while analysis of molecular variance revealed 96.00% contributed by molecular characteristics to observed variations. The yield displayed revealed significant contributions of bunch weight, fingers/hand and fingers/bunch as the main indices for plantain yield. The dendrograms for both morphological and molecular characteristics delineated the cultivars into four distinct cluster groups and subgroups each varying in genetic distance.

Conclusion: These good cultivars can ~~exploited~~be exploited for the improvement of low yielding cultivars in other region to increase and improve plantain yield, promote food security and income generation especially under the present COVID -19 pandemic where food security is threatened by the global pandemic.

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Key words: Plantain cultivars, Yield display, Gene diversity, PCA, microsatellite fingerprint.

1.1 INTRODUCTION:

Plantain (*Musa paradisiaca* L) is a giant perennial herb of the genus *Musa*. It is one of the most versatile food crops in the tropics, where it is seen as a very important component of food security and provides a substantial amount of income to local farming communities through internal trade[1]. The crop is of extraordinary significance with a bright economic horizon in West and Central Africa and Latin America, which are the predominant plantain growing regions of the world. It is one of the few most important suppliers of dietary energy in parts of the humid forests and mid-altitude agro-ecologies of tropical regions of the world, where it is cultivated and utilized as a major starchy staple and consumed by nearly half a billion people in different parts of the world[2]. Plantain *(Musa paradisiaca L)* is undoubtedly one of the most important staple food crop and perhaps, one of the oldest cultivated fruit tree crop in the humid tropics of Africa, Central Asia, South America and the West Indies[3].

Nigeria is one of the major ~~plantain~~plantains producing countries[4]; propelled in part by the tremendous rate of consumption in the country as a result of the rapidly increasing urbanization rate and the great demand for easy and convenient foods by the non-farming urban proletariat[5].Nigeria is the largest producer of plantain in West Africa with an estimated annual production of about 12.4 million metric ~~tonnes~~tons. Most part of the country is characterized by a tropical rain forest, even rainfall distribution and soil conditions that favours the cultivation and production of the crop[6]. Plantains differs greatly and show unique phenotypic variability regarding plant size, bunch type, bunch and fruit orientation, fruit shape, pseudo-stem and pulp colour. However, biodiversity in the elite plantain cultivars is complex and is represented by cultivars that are distinguishable by variation in bunch phenotypes, bunch size, fruits and hands per bunch and degree of pulp hardness[7].

The development and application of molecular markers provide powerful tools to reveal polymorphism, and are also robust to detect genetic variability[8], and are not influenced by environment or developmental stages of the plant, thus making them an ideal tool for the assessment of genetic diversity[8]. However, the potential usefulness of molecular techniques in identifying genetic relationships among species varies greatly because of the uniqueness of each genome. The microsatellite marker is a DNA-based fingerprint technique that has been successfully used to distinguish diverse *Musa* germplasm[9,10]. The present study reports the use of SSR analysis for the assessment of genetic variability among selected elite plantain cultivars grown in Nigeria.

Molecular markers like Simple Sequence Repeats markers ~~has~~have proved an efficient tool in detecting genetic variations and genetic relationships among germplasm of many plants, including plantain[11]. It is relatively quick and inexpensive, and requires a small amount of DNA and no prior sequence information of the target genome[12], and have the advantage of high reproducibility. The microsatellite markers as a polymerase chain reaction (PCR)- based technique, is a reliable marker system for many organisms, especially plants[13], because of its simplicity, speed, high stability, no prior requirement of sequence information, cost effectiveness, and versatility of markers that amplify DNA repeat sequences using single primers in addition to having high reproducibility. It involves amplification of the DNA segment present at an amplifiable distance between 2 identical microsatellite repeat regions

oriented in opposite directions[14]. Therefore, SSR has been widely used for varietal fingerprinting or genetic diversity analysis[15]. This co-dominant marker can sample multiple loci from across different genomes and have been widely useful in resolving genetic diversity and phylogenetic relationships[16]. Although application of morphological studies in variability analysis of plantain (*Musa* AAB) has been carried out by various researchers, the use of SSR for variability analysis of *Musa* spp. (AAB) has not received wide attention especially for the available elite cultivars of this agro-ecology[17]. The present study, therefore, tries to comparatively assess the attributes of the elite cultivars of plantain in this agro-ecology using agro-morphological and microsatellite molecular markers to compare their effectiveness and to assess the levels of genetic diversity and similarity among the plantain (*Musa paradisiaca* L.) elite cultivars[18]. This is to ascertain the attributes and the cultivars that can be selected and used for the genetic improvement of the others using conventional breeding or modern biotechnological tools.

This study was therefore design to assess and evaluate the genetic diversity using agro-morphological characteristics and microsatellite markers as markers in 14 elite plantain cultivars out of the numerous cultivars farmed in the study area which would serve as a basi for molecular breeding, conservation effort, marker assisted breeding and selection and t ensure the sustenance of biodiversity of these elite cultivars. These 14 are the prominen cultivars which are easily identified by the local names in the study area. The study will also provide a platform for the development of gene bank for plantain and improvement of available cultivars of plantain for value addition and food security in Nigeria and beyond.

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2.1. Materials and Methods

Elite Plantain Cultivars used for the study and their coordinates

Table 1

Latitudes, Longitudes and Altitudes of 14 elite plantain cultivars used for the study.

Elite Cultivars	Latitude (N)	Longitude (E)	Altitude elevation (m a.s.l)
1. Enugu plantain	06° 02.835’	008° 41. 104’	210m
2. Egome plantain	05° 56.540’	008° 50. 457’	131.98m
3. Ogoni Red plantain	06° 54.583’	009° 17. 799’	178m
4. Kigwa Brown plantain	06° 48.617’	009° 15. 301’	183m
5. Ejorgom plantain	06° 30.723’	009° 10. 687’	119m

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6. ~~Bakpri plantain~~Bakpri plantain	04° 97.778’	008° 36. 013’	54m
7. Owomoh plantain	05° 55.882’	008° 26. 391’	175m
8. Kainjen plantain	05° 58.200’	008° 63. 520’	181m
9. Ikpobata plantain	06° 28.427’	009° 08. 845’	97m
10. Mgbeghe plantain	05° 38.710’	008° 46. 024’	119m
11. Kenkwa plantain	06° 04.445’	008° 54. 776’	129.6m
12. Uhom plantain	05° 42.188’	008° 03. 233’	56m
13. Ekunkwam plantain	06° 33.462’	008° 52. 290’	110m
14. Ingwam plantain	06° 39.995’	008° 51. 607’	92

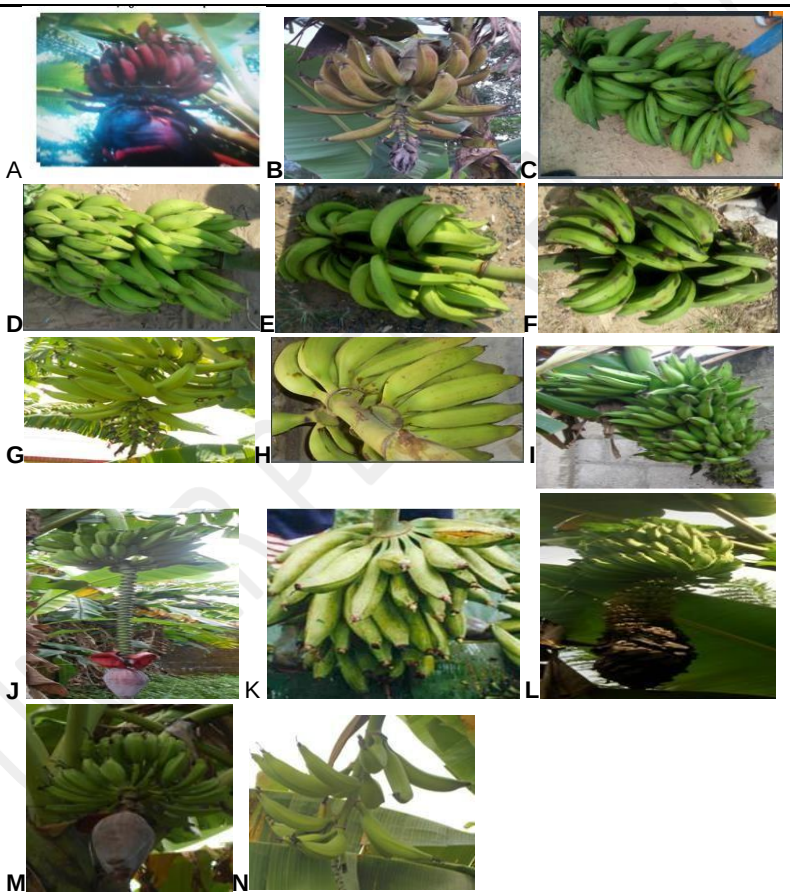

Figure 1A – 1N showing 14 elite plantain cultivars
A=1 = Ogoni Red French Plantain
B=2= Kigwa Brown False Horn Plantain
C=3= Enugu Black False Horn Plantain
D=4= Ebi Egome False Horn Plantain
E=5= Owomoh True Horn Plantain
F=6= kenkwa False Horn Plantain

G=7= Kainjen False Horn Plantain
H=8 = Uhom False Horn Plantain
I=9= Ekumkwam French Plantain
J=10= Ikpobata French plantain
K=11= Mgbeghe False Horn Plantain
L=12= Ingwam French Plantain
M=13= Bakpri French Plantain
N=14= Ejorgom True Horn Plantain

2.1.1 Morphological Studies

Morphological studies for the 14 elite plantain cultivars ~~was~~were conducted for the only quantitative attributes of the cultivars ~~*in situ*~~ in farmers field using relevant data collection instruments. This morphological study lasted for a year and six months when the last cultivar under investigation was matured. The quantitative attributes evaluated included:

i. Length of cycle (months)
ii. Bunch weight per plant (kg)
iii. Number of fingers per hand of plantain
iv. Number of fingers per bunch of plantain
v. Number of hands per bunch of plantain
vi. Harvest interval (days)
vii. Pulp hardness (kg/m^2)
viii. Pulp to finger weight ratio

Determination of pulp hardness in kg/cm^3

Hardness of the pulp is measured at the median transverse cut of fingers using a crossbow penetrometer (GMBH 2345, Cali, USA) of Cosse type model with 6mm diameter nozzle[19]. This equipment was used to determine the pulp hardness in kg/cm^3. Pulp hardness determination was done in triplicates for each elite plantain and the mean hardness was recorded.

Determination of pulp/skin weight ratios

An electronic weighing balance was used to measure the weight of skin (peel) in kg and the weight of the pulp in kg before dividing to get the pulp/skin weight ratios for each of the 14 elite plantain cultivars. Pulp/skin weights ratio determination was done in triplicates for each elite plantain cultivar and the mean was recorded.

The quantitative data generated from the studies was used to generate and develop the following

i. Dendrogram
ii. Dissimilarity ~~Eucledean~~Euclidean distance
iii. Principal component analysis for traits and cultivars
iv. Scattergram
v. Boxplot for yield display and evaluation
vi. Relative abundance of different quantitative attributes within the cultivars and
vii. Quantitative attributes K- dominance within the cultivars plot

Statistical analysis for morphological data

Data generated from the study were collated and subjected to statistical analysis using the GENSTAT V 12, DARwin 5.0, and Minitab 17 software. All analyses were performed at 5 % level of significance.

Molecular studies

2.2 Collection of Plant Materials

This study used fourteen Elite plantain cultivars comprising of 14 cultivars collected from different locations of Nigeria.
The apical leaf samples of less than 2 day old were collected from all the 14 elite cultivars from the different areas dried with silica gel using method for sampling as described by Ubi et al.[19]. The leaf samples were outsourced to South Africa for DNA extraction and PCR electrophoresis for SSR fingerprinting analyses;

2.3 DNA extraction

The Cetyl Trimethyl Ammonium Bromide (CTAB) method was adopted for the extraction of sample total genomic DNAs. Young and tender leaf tissue (10g) was weighed and ground in liquid Nitrogen using mortar and pestle along with 50 µl of B – mercapto-ethanol (BME) and a pinch of polyvinylpyrrolidone (PVP). The sample was further ground using excess of liquid Nitrogen and 4 mL of extraction modified CTAB buffer (2X) and the powder was transferred to a sterile 50-mL centrifuge tube containing 3 mL of pre-warmed extraction buffer. The homogenate was incubated for 30 minutes at 65°C with intermittent mixing or ~~votexing~~vertexing.
Equal volumes of ~~chlorofoam~~chloroform and isoamyl alcohol at (24:1) was added and centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected. A 0.6 volume amount of chilled isopropanol was added to enable for the collection of the supernatant. This was followed by incubation at – 20°C for 30 min. The precipitated DNA was pelleted by centrifugation at 10,000 rpm for 10 min at 4°C.
The DNA pellet was harvested and washed with 5mM ammonium acetate in 70% ethanol and later with 100% ethanol. The DNA samples were air dried for 30 min at room temperature and dissolve in 100 µl of TE buffer. The DNA sample was further treated with RNase-A at 37°C for 1 hour to remove any RNA contamination. The quantity and quality of genomic DNA were estimated using a Nano Drop ND 1000 spectrophotometer (NanoDrop Technologies Inc., USA) and the samples was diluted to a concentration of 50ng/µl. Isolated DNA was visualized for its quantity and quality by running them in 1% agarose gel electrophoresis as described by Ubi et al.[20], Lakshmanan et al.[21] and De Langhe[22].

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2.4 DNA amplification

PCR reaction was carried out in an Ependorf Master Thermocycler (Ependorf, USA). Each reaction tube was made of 100ng of template DNA~~m~~ 2.5mM of MgCL2, 100 µM of dNTPs, 1X *Taq* buffer, 20 pM of 10-mer Primer, and 1 unit of *Taq* DNA polymerase made to a final volume of 20µL.
The thermocycler profile was an initial denaturation temperature of 94°C for 3 mins, followed by 30 cycles of denaturation temperature at 94°C for 1 min, annealing at 37°C for 1 min, extension at 72°C for 1 minute; final extension at 72°C for 10 minutes where the reactions were terminated.

2.5 Primers selected and for the study

A total of 10 microsatellite markers (Table 2) previously used by Crouch et al.[1]for simple sequence repeats studies were screened and used for the study. The selected primers were screened for polymorphism against plantain cultivars. Primers that produced distinct bands and patterns with good quality of amplification and reproducibility were selected and used for markers study. The attributes of the selected primers such as the motif and annealing temperatures with their references are presented in Table 2.

Table 2.

Some microsatellite markers used and selected for the 14 Elite plantain (*Musa paradiasiaca L)* markers

S/N	Primer	Motif	Reference	Annealing temp.
1	Ma 1 – 32	$(GA)_{17}AA(GA)_8AA(GA)_2$	Crouch *et al.*[4]	~~58^{0}C~~58^{0}C
2	Ma 3 -90	$(CT)_{11}$	Crouch *et al.*[4]	53^{0}C
3	mMaCIR 307	$(CA)_6$	Hippolyte *et al.*[5], 2010	54^{0}C
4	mMaCIR 264	$(CT)_{17}$	Hippolyte *et al.*,[5] 2010	53^{0}C
5	mMaCIR 260	$(TA)_8$	Hippolyte *et al.*,[5]	55^{0}C
6	mMaCIR 39	$(CA)_5GATA(GA)_5$	Lagoda *et al.*,[6]	52^{0}C
7	mMaCIR 196	$(TA)_4 (TC)_{17} (TC)_3$	Hippolyte *et al.*,[5]	55^{0}C
8	mMaCIR 214	$(AC)_7$	Hippolyte *et al.*,[5]	53^{0}C
9	mMaCIR 01	$(GA)_{20}$	Lagoda *et al.*,[6]	55^{0}C
10	mMaCIR 03	$(GA)_{13}$	Lagoda *et al.*,[6]	53$_0$C

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2.6 Data scoring and analysis

Each band in the SSR fingerprinting pattern was considered as a separate locus. Only distinct, reproducible, well-resolved fragments were selected and scored for presence (1) and absence (0) of a band. The binary matrix of SSR phenotypes was then assembled for analyses. A similarity matrix was constructed and subjected to cluster analysis following the un-weighted pair group method with arithmetical averages (UPGMA) of the computer program DARwin version 5.0. Measurement of genetic distance for pair-wise accessions was based on Nei's unbiased genetic distances[23] using DARwin 5.0 genetic software.

The Gene diversity for each selected primer was calculated as [1 - the ratio of number of bands per primer to the total bands informativeness of all the bands produced by the primers][24]. Gene diversity of a cultivar = [1 – NPB/TNB], where NPBis the number of polymorphic bands or band informativeness and TNB is the total number of bands produced by the 14 genotypes (Elite plantain cultivars evaluated) that contains the bands. The polymorphic information content (PIC) value was calculated using the formula of Smith et al.,[25][$1 - \Sigma\, pi^2$], where *pi* is the frequency of the *nth* allele. The number of alleles here refers to the number of scored bands and the frequency of an allele was obtained by dividing the number of elite cultivars where it refers to the total number of elite cultivars present. Marker index (MI) for a primer was calculated as the product of PIC and the number of polymorphic bands obtained per primer[26]. The Analysis of molecular variance (AMOVA) was ascertained for the generated molecular data using the Genetic analysis in excel (Genailex) software.

3.1. Results and Discussion

3.1.1 Morphological markers of 14 elite plantain cultivars

The results of yield related agro-morphological traits generated from field data in this study are presented in Table 3. The results show that the length of crop cycle among the elite cultivars varies from 13 months in Owomoh, Uhom and Ikpobata cultivars to 17 months in Ingwam. The study further revealed that bunch weight varied ($p<0.05$) significantly among the elite cultivars ranging from 5.11kg in Bakpri cultivar to 24.79 kg in Mgbeghe cultivar. The number of hands per bunch also differed ($p<0.05$) significantly among the cultivars and ranged from 3.00 in Bakpri cultivar to 9.00 in Ebi Egome and Ekunkwam cultivars respectively. The number of fingers per hand varied from 3.00 in Bakpri cultivar to 9.00 in Ebi Egome cultivar while the number of fingers per bunch varied from 9.00 in Bakpri cultivar to 81.00 in Ebi Egome cultivar. The harvest interval for the elite cultivars was 61 days in Bakpri cultivar to 74 days in Ebi Egome cultivar. The results of pulp hardness among the different cultivars indicates that this morphological attribute did not differ ($p>0.05$) significantly among cultivars but slightly varied from 1.00 kg/cm^3 in Ekunkwam cultivar to 1.80 kg/cm^3 in Ogoni red, Ikpobata and Mgbeghe cultivars respectively. The evaluated pulp to skin weight ratio for the cultivars showed that this attribute ranged from 1.09 in Owomoh cultivar to 1.50 in Mgbeghe and Ejorgom cultivars respectively (Table 3).

Table 3: Morphological attributes of Elite plantain accessions cultivated in Cross River State

Elite Plantain accessions	Length of Cycle (Months	Shape	Bun weight (kg)	No. of hands/ Bunch	No. of fingers /hand	No. of fingers /bunch	Finger skin colour	Pulp colour	Harvest interval (days)	Fingers cross section	Pseudostem colour	Bunch pheno-type	Hardness (kg/cm^3)	Weight ratio
Ogoni red french	15	Medium /curve	13.23	6	4	24	Red	Brown	69	Triangular	purple	French type	1.9 – 1.7	1.3-1.5
Kigwa brown false horn	14	Medium/ curve	12.01	4	4	16	Brown	Milky white	62	Quadri Lateral	Gray	False horn	1.8-1.6	1.3-1.5
Enugu black false horn	15	Big /curve	23.54	8	8	64	Dark green	Creamy	71	Pentagonal	Green	False horn	1.4 -1.1	1.2-1.3
Ebi egome false horn	14	Big /flat	22.13	9	9	81	Pale green	Creamy	74	Quadri Lateral	Green	False horn	1.4-1.1	1.2-1.3
Owomoh true horn	13	Medium /flat	15.79	6	5	30	Pale green	Milky white	65	Triangular	Gray	True horn	1.7-1.5	1.3-1.3
kenkwa false horn	14	Medium /curve	16.23	7	6	42	Pale green	Creamy	66	Pentagonal	Brown	False horn	1.3-1.0	1.02-1.15
Kainjen false horn	15	Big /flat	19.88	8	6	48	Olive green	Whitish	62	Quadri Lateral	Brown	False horn	1.7-1.5	1.3-1.4
Uhom false horn	13	Medium /curve	14.23	7	5	35	Pale green	Creamy	67	Quadri Lateral	Green	False horn	1.7-1.5	1.3-1.4
Ekumkwam French	14	Medium /flat	16.22	9	8	72	Dark green	Creamy	63	Triangular	Green	French type	1.1-0.9	1.14-1.3
Ikpobata French (cooking bananas)	13	Small /curve	7.21	4	4	16	Dark green	Milky white	69	Triangular	Brown	French type	1.9-1.7	1.5-1.7
Mgbeghe false horn	14	Big /flat	24.97	6	7	42	Pale green	Creamy	73	quadrilateral	Green	False horn	1.9-1.7	1.4-1.6
Ingwam French	17	Medium /curve	15.64	6	5	30	Pale green	Creamy	65	Quadrilateral	Brown	French type	1.7-1.5	1.2-1.4
Bakpri French (dwarf mutant)	16	Small /curve	5.11	3	3	9	Dark green	Milky white	61	Triangular	Green	French type	1.8 -1.6	1.4-1.6
Ejorgom true Horn	15	Medium /curve	13.80	6	5	30	Pale green	Creamy	68	Pentagonal	Brown	True horn	1.7 -1.5	1.3-1.5

The results of distribution of elite plantain cultivars on the principal axes is presented in Fig 2. As shown, cultivars 1 (Ogoni red), 14 (Ejorgom), 4 (Ebi Egome) and 10 (Ikpobata) lies with the Y – X (positive axes). Cultivars 11(Mgbeghe), 3 (Enugu black), 5 (Owomoh) and 12 (Ingwam) are distributed along the Y (positive) and X (negative) axes.In the Y– X (negative axes) lies the cultivars 6 (Kenkwa), 2 (Kigwa brown) and 7 (Kainjen). The cultivars 8 (Uhom), 13 (Bakpri) and 9 (Ekunkwam)also lies in the negative axis of the X - Y principal axis..

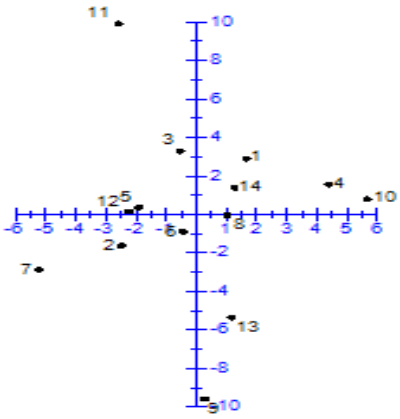


Figure ~~2:Scattergram~~2: Scattergram showing distribution of 14 elite cultivars along the principal axes

The network analysis of the elite plantain cultivars places the cultivars into an evolutionary relationship comprising of four network clade each separated by a genetic distance. All the four network groups comprising of 5 members and 3 members each for the remaining three are separated from each other by a genetic distance of 24. The network grouping is based on the genetic and morphological similarity among cultivars of same network. For instance, the cutlivars 14 (Ejorgom), 12 (Ingwam) and 6 (Kenkwa) in the first group all have a creamy pulp and a brown pseudostem colourations. Cultivars 7(Kainjen), 8(Uhom) and 11(Mgbeghe) belonging to second network group are all of the falsehorn plantain types.Cultivars in the third network group 10(Ikpobata), 2(Kigwa brown) and 13(Bakpri) all have a milky white pulp coloration while the cultivars in network group one such as 3 (Enugu black), 4(Ebi Egome) and 9(Ekunkwam) all show dark finger skin colourations (Table 3 and Figure 3).

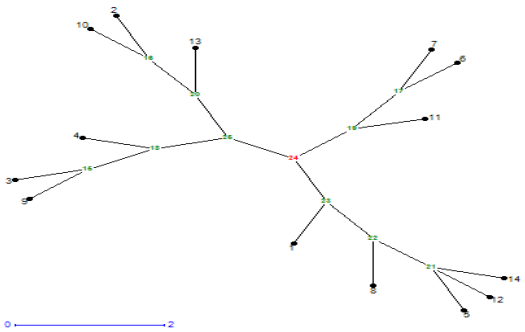


Figure 3: Network analysis showing the distribution of the 14 elite cultivars into four different clusters

The results of principal component analysis for the eigen values and the percentage contribution of the individual attributes and cultivars to the total observable phenotypic variations in the elite plantain cultivars was explained by four principal components in this study and presented in Table 4. The results indicated that all quantitative attributes and cultivars evaluated in the four principal components contributed a total of 99.91% of all the total phenotypic variations observed among and within the plantain population.

Principal component (PC-1) had an eigen value of 6379 and contributed the highest percentage of 94.31 % to the total observed variations. The principal and major loading attribute to this component is fingers / bunch with a loading value of 0.9723. The second principal component (PC-2) showed an eigen value of 255 and a percentage contribution of 3.77 % to the overall phenotypic variations observed among the attributes evaluated and with the principal and major loading values for this principal component coming from harvest interval (0.76391) and bunch weight (0.61348). Third principal component (PC-3) showed an eigen value of 108 and a percentage contribution of 1.59 % to the overall observed attributes variability with principal and major loading value for this component coming from bunch weight (0.75681). The fourth principal component (PC-4) had an eigen value of 16 and the least percentage contribution of 0.24 % to the total observed morphological variations in the attributes with the principal and major loading value of 0.98960 coming from length of plant cycle.

Principal component analysis was also evaluated for the cultivars on four principal component axes. Principal component (PC-1) with an eigen value of 6379 contributed 94.31 % to the total observed variations with the principal component score of 43.556 contributed from Ebi egome cultivar alone (Table 4). Second principal component (PC-2) had an eigen value of 255 and contributed 3.77 % to the overall phenotypic variations observed among the cultivars with the principal component score of 9.911 contributed by Mgbeghe cultivar. Kainjen cultivar contributed the highest principal component score of 5.226 in the third principal component (PC-3) which also had an eigen value of 108 and percentage of 1.59 % in the overall observed variations in the cultivars (Table 4).The last principal component showed an eigen value of 16 and contributed the least percentage of 0.24 % to the overall observed variations among the cultivars and with the least principal component score of 0.793 was contributed by Enugu black plantain cultivar.

Table 4:
Principal Component Analysis for 14 elite plantain cultivars

PCA AXIS	**PC-1**	**PC-2**	**PC-3**	**PC-4**
Eigen Values	**6379**	**255**	**108**	**16**
% Variations	**94.31**	**3.77**	**1.59**	**0.24**
Cum. % Variation	**94.31**	**98.08**	**99.67**	**99.91**

Latent vectors (loadings)

Traits	**PC-I**	**PC-2**	**PC-3**	**PC-4**
Bunch weight	0.20144	0.61348	0.75681	-0.06189
Fingers/bunch	0.97023	-0.18918	-0.11288	0.02165
Fingers/hand	0.07883	0.04730	0.01220	-0.02984
Hands/bunch	0.07696	-0.02538	0.06353	-0.08690
Harvest/interval	0.07629	0.76391	-0.63052	0.08771
Length of cycle	-0.00618	-.02620	0.11186	0.98960

Pulp to wt. Ratio	-0.00292	0.00688	-0.01519	0.01540
Pulp hardness	-0.00873	0.02595	0.00352	0.00340

Principal component scores

Cultivars	**PC-1**	**PC-2**	**PC-3**	**PC-4**
Ogoni red	-14.557	2.889	-1.663	0.687
Kigwa Brown	-23.246	-1.619	2.492	-0.840
Enugu black	26.956	3.299	0.540	0.793
Ebi Egome	43.556	1.558	-4.374	0.406
Owomoh	-.8.431	0.361	1.912	-1.707
Kenkwa	3.529	-0.889	0.442	-0.511
Kainjen	9.847	-2.881	5.226	-0.054
Uhom	-3.666	-0.037	-1.034	-1.409
Ekunkwam	32.642	-9.587	-0.277	-0.448
Ikpobata	-23.674	0.814	-5.669	-0.916
Mgbeghe	5.819	9.911	2.592	-0.376
Ingwam	-8.487	0.166	2.243	2.264
Bakpri	-31.672	-5.365	-1.163	1.445
Ejorgom	-8.617	1.382	-1.266	0.664

Table 5shows the dissimilarity matrix for the morphological attributes observed among the 14 elite plantain cultivars evaluated. As shown, the highest dissimilarity among the evaluated cultivars using the normal euclidean distance was 75.62 units (Table 5) andthis was observed between cultivar 4 (Ebi Egome) and cultivar 13 (Bakri). The results also revealed that the least dissimilarity among the evaluated cultivars using the normal Euclidean distance 4.00 units and this was observed between cultivar 5 (Owomoh) and cultivar 12 (ingwam). This was also shown in the Fig.1.

Table 5:
Dissimilarity matrix for morphological attributes of elite plantain cultivars

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**C13**
C2	10.93												
C3	41.60	50.50											
C4	58.21	67.24	17.40										
C5	7.98	15.01	35.62	52.42									
C6	18.66	26.89	23.84	40.40	12.18								
C7	26.02	33.27	18.82	35.34	18.94	8.21							
C8	11.49	20.04	30.94	47.41	5.70	7.49	15.22						
C9	48.87	56.52	14.16	16.51	42.33	30.39	24.40	37.58					

C10	10.40	8.54	51.09	67.26	17.05	27.94	35.46	20.60	57.52				
C11	22.08	31.27	22.27	39.28	17.24	11.30	13.74	14.36	33.29	31.95			
C12	7.93	15.23	35.65	52.51	4.00	12.51	18.97	6.92	42.43	17.44	17.55		
C13	19.12	10.17	59.29	75.62	24.35	35.59	42.13	28.70	64.49	11.33	40.60	24.12	
C14	6.19	15.53	35.67	52.27	4.12	12.53	20.04	5.58	42.72	15.79	17.28	4.04	24.07

Figure 4 shows the clusters and groupings based on morphological quantitative attributes. The dendrogram revealed that morphologically, the 14 elite plantain cultivars are grouped into 4 major clusters and 9 subclusters. Cluster 1 had five cultivars with 3 subclusters. Subcluster 1.1 had cultivars 14(Ejorgom), 12(Ingwam) and 5(Owomoh). Subcluster 1.2 had cultivars 12(Ingwam), 5(Owomoh) and 3 (Enugu black) as major cultivars while subcluster 1.3 had cultivars 5(Owomoh), 3(Enugu black) and 1 (Ogoni red) as main cultivars. However, cultivars in these subclusters differ slightly in genetic distances (Fig. 4) within the cluster 1.
Cluster 2 had 2 subclusters with 3 cultivars. Subcluster 2.1 consist of cultivars 7 (Kainjen) and cultivar 6 (Kenkwa) with same genetic distance as subcluster 1.2. Subcluster 2.2 had a higher genetic distance and made up of cultivars 7(Kainjen),6(Kenkwa) and 11(Mgbeghe).
Cluster 3 showed 2 subclusters with 3 cultivars in all. Subcluster 3.1 had cultivar 10 (Ikpobata) and cultivar 2(Kigwa brown) but with same genetic distance as subcluster 2.1. Cultivars 10 (Ikpobata), 2(Kigwa brown) and 13 (Bakpri) are the major cultivars in subcluster 3.2 and with similar genetic distance as subcluster 1.3.Cluster 4 had 2 subclusters with 3 cultivars in all. Subclusters 4.1 had cultivars 9 (Ekunkwam) and 3 (Enugu black) with same genetic distance as subcluster 2.2. Subcluster 4.2 had cultivars 9 (Ekunkwam), cultivar 3 (Enugu black) and cultivar 4 (Ebi Egome) with higher genetic distance than all other subclusters in the dendrogram (Fig. 4).

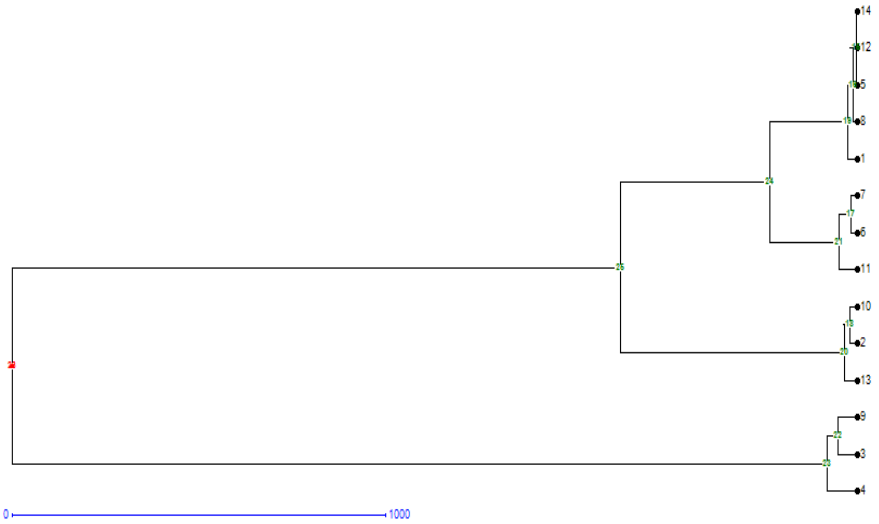


Figure 4; Dendrogram for morphological attributes of elite plantain showing 4 major clusters and 9 subclusters

Table 6 presents the results of correlation analysis for quantitative attributes of the elite plantain cultivars. A strong, positive and significant correlation was observed(r=0.7455**; 0.8377**; 0.7631**; 0.5381*; P<0.001) between bunch weight with hands/bunch, fingers/hand, fingers/bunch and harvest interval respectively. Hands/bunch also positively and significantly correlated with fingers/hand (r=0.8718**; p<0.001), fingers/bunch (r=0.9346***, p<0.001) but negatively and non-significantly correlated with pulp hardness (r= -0.7310,p>0.001), pulp/wt. ratio (r= -0.6020,p>0.001). Fingers/hand positively and significantly correlated with fingers/bunch (r=0.9751***,p<0.001) and negatively, weakly and non-significantly correlated with pulp hardness (r= -0.7735,p>0.001). Pulp hardness was found to be correlated positively and significantly with pulp/wt. ratio (r=0.5700*,p<0.001).

Commented [MF8]: PCA is a good representative of correlation of the genotypes and there is no need to have simple correlation coefficient

Table 6
Correlation matrix for agro-morphological yield related attributes of plantain

Attributes	Length of cycle	Bunch weight	Hands/bunch	Fingers/hand	Fingers/bunch	Harvest interval	Pulp hardness
Bunch weight	-0.0460						
Hands/bunch	-0.1145	0.7455**					
Fingers/hand	-0.1439	0.8377**	0.8716**				
Fingers/bunch	-0.1174	0.7631**	0.9346***	0.9751***			
Harvest interval	-0.2262	0.5381*	0.2859	0.4795	0.3776		
Pulp hardness	0.0679	-0.3650	-0.7310	-0.7123	-0.7735	0.0413	
Pulp/wt. ratio	0.0585	-0.4154	-0.6020	-0.4417	-0.5076	0.1361	0.5700*

*Strength of correlation

3.1.2 Box plot for Yield display and evaluation

The yield attributes of the different cultivars of elite plantain was evaluated using a boxplot approach. The result as presented in fig.5 showed that the yield displayed was a function of the bunch weight (kg), the number of fingers per hand and the number of fingers per bunch. These attributes are the major contributors to the yield (Fig. 5). The number offingers per hand was plotted along the vertical (y) axis while the number of fingers/bunch was plotted on the horizontal (X) axis. The yield display pattern revealed a significant contribution of the quantitative attributes toward the realization of productive potentials of the evaluated cultivars. Thus it is an index for selection of the cultivars for further breeding and development.

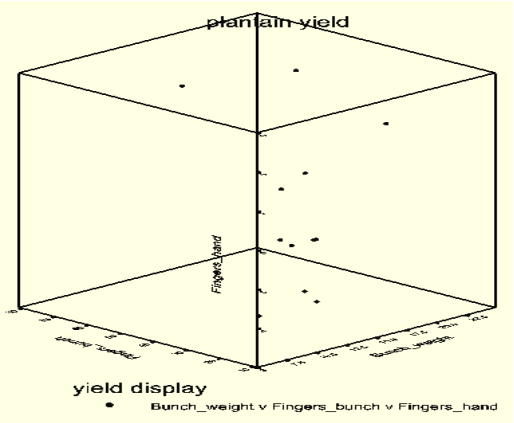


Figure 5: Boxplot showing yield display and major contributing attributes to yield

3.1.3 Species (traits) Relative abundance of traits within the elite plantain cultivars

Figure 6 presents the relative abundance and rank/ plot abundance of species in terms of the evaluated quantitative attributes. The results revealed that the relative abundance of the species in terms of the attributes showed a downward trend with attributes abundance decreasing with increasing plot abundance. Fingers per bunch as one of the yield determining attribute was the most abundant attribute per plot at the onset of production but showed a declining trend with time as does all other attributes (Fig.6). This may be due to declining soil fertility or failure in routine agronomic practices.

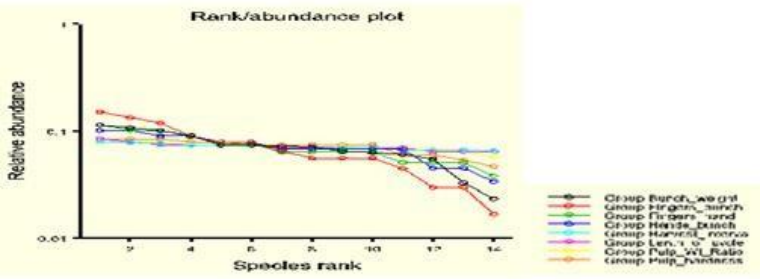


Figure 6: Relative abundance of traits among the elite plantain cultivars

3.1.4 Species (traits) diversity ranking/dominance within the elite plantain cultivars

The attributes diversity ranking and dominance among the elite plantain cultivars is presented in Fig. 7. The upward trend observed in all the attributes of all the evaluated cultivars revealed that there is no much differences or diversity among the attributes and the K-dominance (that is the attribute dominating each cultivar) was mostly observed in fruits per bunch for all the cultivars. However, all the attributes showed dominance in all the cultivars as time progresses (Fig.7).

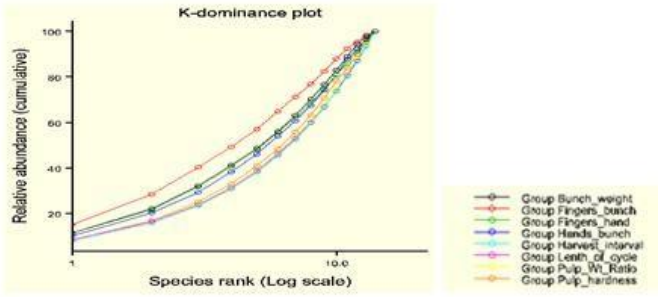


Figure 7: Traits diversity and ranking among the elite plantain cultivars

3.2. Molecular (SSR) Markers for 14 elite plantain cultivars

Results of DNA amplification further showed that the 14 elite plantain cultivars produced a wide array of strong and weak bands using the simple sequence repeats markers. However, only distinct, reproducible, well-resolved fragments were scored as present or absent band for each of the SSR primers with 14 elite cultivars. Since the sequences are very short, this may have eased the higher degree of fragments amplification. Figure 8 shows the typical level of polymorphisms and ~~electrophoregram~~electropherogram among the 14 elite plantain cultivars in 6 SSR primers that revealed distinct bands and amplification.

The DNA amplification produced 59 bands, of which two primers mMaCIR-39 and Ma1-32 were monomorphic while mMaCIR-03 showed 71.42% polymorphism. Among the selected primers mMaCIR-39 and Ma-1-32 produced the highest number of 14 polymorphic bands, while Ma3-90 produced the lowest number of 6 polymorphic bands. Size of DNA bands varied from 300 bp to 2.0Kbp, Ma-3-90 being the highest range of DNA size (300bp-2.0Kbp) and Ma1-32 is the lowest (Table 7).

The SSR profiles indicated that each primer could generate polymorphisms among the accessions. The polymorphism may be due to mutation at priming sites and/or insertion/deletion event within the SSR region; and the extent of polymorphism also varies with the nature and the sequence repeat of the primer used[27].

A total of 6 primers showed good quality bands and distinct polymorphism among the 14 elite plantain cultivars using the Simple Sequence Repeat (SSR) markers. The number of bands produced by the polymorphic primers varied from 6 to 14 and the molecular weight of bands from 300 bp to 2.0 Kbp. The total number of polymorphic bands and the mean percentage of polymorphism was 59 and 70.24%, respectively. The Marker Index was lowest with the primer Ma1-32 and mMaCIR-39 (1.00) and highest with the primer mMaCIR-196 (5.36) with an average of 3.74. The TNB, NPB, %P, PIC, and MI obtained by SSR primers are summarized in the Table 7.

The PIC values derived from allelic diversity and frequency among the genotypes were also not uniform for all the SSR loci tested. The PIC values for the 6 selected primers varied between 0.49 (mMaCIR-03) and 1.00 (Ma1-32) with an average of 0.79 (Table 7).

Figure 3 shows the typical level of polymorphisms generated by the various amplicons fragment polymorphism in the ~~electrophoregram~~electropherogram. Number of DNA amplification bands depended on how primer attached to its homolog at DNA template. RAPD polymorphism are the result of either a nucleotide base change that alters the primer binding site, or an insertion or deletion within the amplified region[28], polymorphism usually noted by the presence or absence of an amplification product from a single locus[29]. The differences in polymorphism may be due to the differences in amount of genetic variation that exist among the different accessions.

Simple Sequence Repeats Markers of 14 Elite plantain cultivars

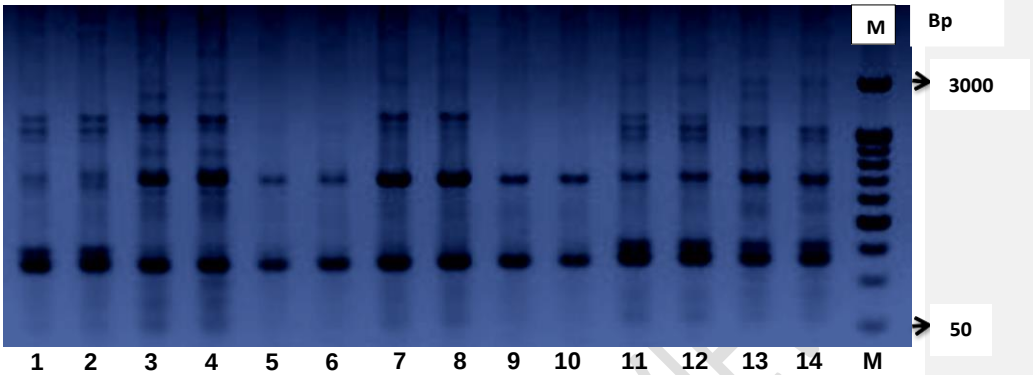


Figure 8: ~~Electrophoregram~~Electropherogram showing amplified fragments bands of 14 elite cultivars amplicons in Agarose gel using SSR primers

Table 7:
Molecular characteristics and gene diversity of cultivars from SSR fingerprint

SSR primers	NPB	% polymorphism	PIC	Marker index	Gene Diversity
Ma 1- 32	7	50.00	0.75	5.25	0.881
Ma -3-90	8	57.14	0.67	5.36	0.864
mMaCIR-39	10	71.42	0.49	4.96	0.830
mMaCIR-03	14	100.00	1.00	1.00	0.762
mMaCIR-196	6	42.85	0.82	4.92	0.898
mMaCIR-260	14	100.00	1.00	1.00	0.762
/Mean/Total	59	70.24	0.79	3.74	0.832

Table 8 presents the results of analysis of molecular variance (AMOVA) for the 14 elite plantain cultivars. The AMOVA results showed that 96.00 % of the total variations observed within the elite cultivars were due to variations in their genetic molecules controlling the different traits evaluated (Table 8). The remaining 4 % may be attributable to environment and other factors. The estimated population fixation and genetic differentiation (Phqt) showed significant effect of the genetic component on the expressed phenotypes.

Table 8:
AMOVA table for simple sequence repeats analysis of 14 accessions of Plantain (*Musa paradisiaca* L)

SOV	DF	TSS	MSS	Estimated variance	% Molecular Variance	P-Value	Phot

Among Pop.	3	192.3	64.10	3.143	04	0.010	0.141
Within Pop.	10	203.89	20.39	9.665	96		
Total Pop.	13	295.11		13.005	100		

Key: phqt = estimate of the population genetic differentiation based on Permutation.

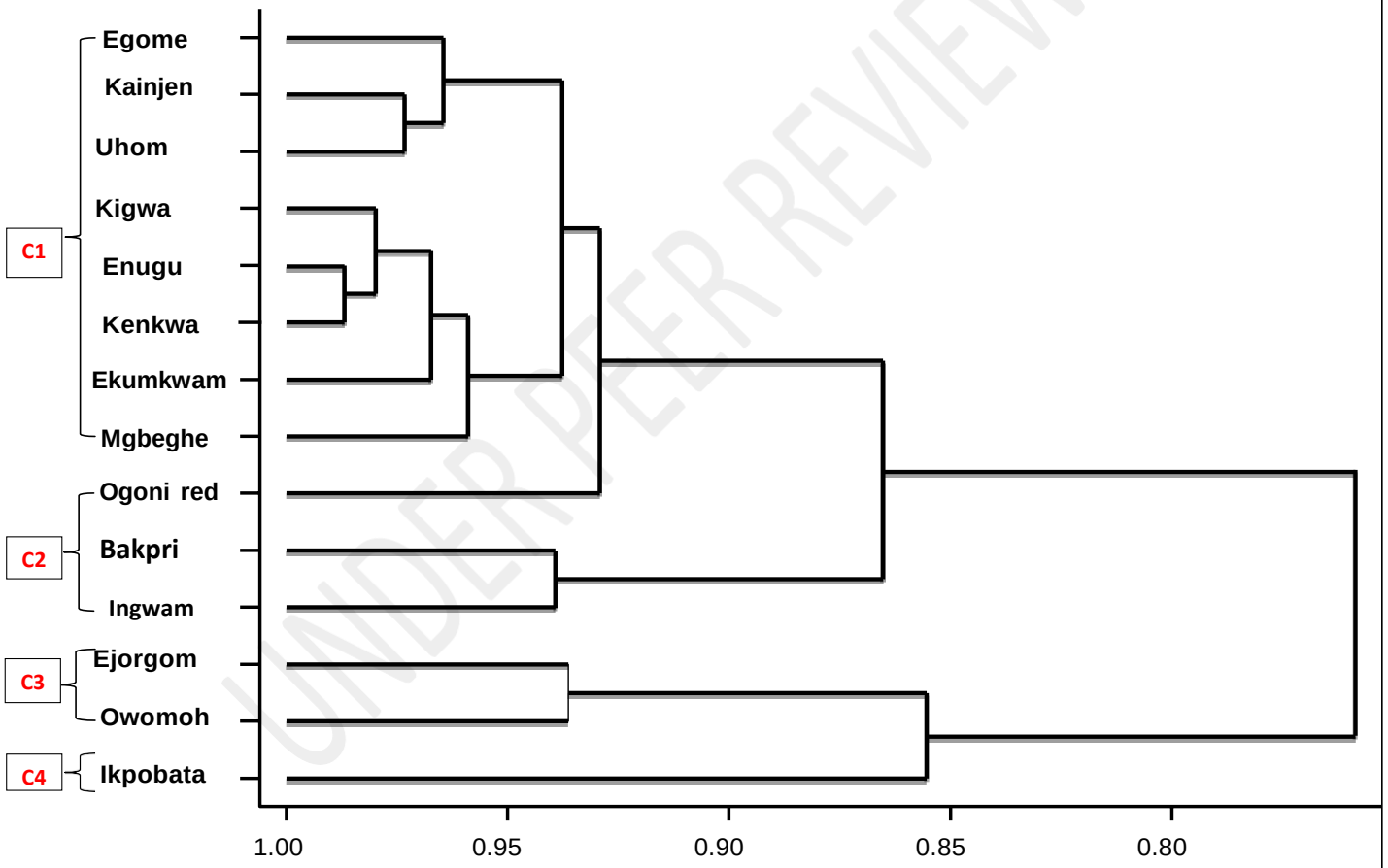
Table 9 shows the genetic similarity matrix among the elite plantain cultivars. The results showed that the closest relatives with similarity index of 0.91 or 91 percent was evident from Bakpri and Ikpobata cultivar while the most genetically dissimilar cultivars was observed between Ogoni red and Enugu black with similarity index of 0.09 0r 9 percent (Table 9).

The dendrogram (Fig. 9) generated from the binary or molecular data also delineated the 14 elite plantain cultivars into four clusters like does the one generated with morphological data. Cluster 1 had 2 subclusters with a total of 8 cultivars. Subcluster 1.1 had 3 cultivars Ebi Egome, Kainjen and Uhom while subcluster 1.2 had cultivars like kigwa brown, Enugu black, Kenkwa, Ekunwkwamand Mgbeghe. The 2 subclusters however differ in their genetic distance (Fig.9). Cluster 2 had 2 cultivars which include Bakpri and Ingwam while cluster 3 also ahd 2 cultivars which are Ejorgom and owomoh. Cluster 4 had just a single cultivar called Ikpobata. All the clusters (1-4) differ in their genetic distance and molecular attributes which probably have grouped the cultivars into the different clades which is beyond the phenotypic evidence.

TABLE 9:
Similarity matrix showing genetic distance among 14 elite cultivars of plantain

Elite cultivars	Ogoni red	Ebi egome	Kenkwa	Kain Jen	Mgbe Ghe	Bakpri	Uhom	Ekum Kwam	Ingwam	Ejorgom	Ikpobata	Kigwa brown	Enugu black	Owomoh
Ogoni red	0.00													
Ebi egome	0.39	0.00												
Kenkwa	0.26	0.25	0.00											
Kainjen	0.38	0.23	0.25	0.00										
Mgbeghe	0.35	0.28	0.29	0.21	0.00									
Bakpri	0.29	0.80	0.86	0.88	0.89	0.00								
Uhom	0.35	0.26	0.27	0.25	0.26	0.23	0.00							
Ekumkwm	0.41	0.23	0.29	0.37	0.29	0.32	0.39	0.00						
Ingwam	0.39	0.70	0.51	0.43	0.44	0.34	0.44	0.45	0.00					
Ejorgom	0.30	0.69	0.60	0.62	0.62	0.28	0.56	0.64	0.44	0.00				
Ikpobata	0.52	0.86	0.85	0.83	0.83	**0.91**	0.82	0.84	0.73	0.75	0.00			
Kigwa brown	0.76	0.38	0.33	0.35	0.37	0.44	0.26	0.39	0.58	0.68	0.86	0.00		
Enugu black	0.09	0.74	**0.11**	0.38	0.25	0.37	0.30	0.26	0.55	0.67	0.84	0.31	0.00	
Owomoh	0.34	0.53	0.62	0.44	0.35	0.51	0.61	0.47	0.63	0.18	0.57	0.62	0.73	0.00

Figure 9: Dendrogram based on UPGMA showing 4 cluster groups andgenetic diversity among 14 elite cultivars of plantain



Pulp hardness depends on age of plantain fruit and moisture content of fruit at the time of harvest. Physiologically matured plantain fruits will show lower pulp hardness than fully matured fruits. This attribute contributes significantly to the horticultural and market value of the cultivars. Also pulp to skin weight ratio is another important agro-morphological attribute that determines selection for value addition. This horticultural attributes also depends on the stage of growth and age of plantain fruit at harvest. Fully matured fruits may show higher ratios than physiologically matured fruits. This report is in tandem with the findings of Ubi et al., [22] and Simmonds and Shepherd[28]who studied the organoleptic properties of elite plantain cultivars.

The findings had revealed that the relative abundance of the species in terms of the attributes shows a downward trend with attributes abundance decreasing with increasing plot abundance. Fingers per bunch as one of the yield determining attribute was the most abundant attribute per plot at the onset of production but showed a declining trend with time as does all other attributes. This may be due to declining soil fertility or failure in routine agronomic practices. This calls for a more deliberate response and proactiveness on the part of plantain farmers in this area to adopt modern and routine agronomic practices to sustain increasing fruits per bunch.

The yield display as revealed in the boxplot showed the sole dependence of plantain yield on bunch weight, fingers per hand and fingers per bunch. Cultivars with promising potentials for these attributes should be selected and used for the improvement of others. Yield is the basis for plantain farming by farmers in this area. This makes the yield display study very important as it has revealed the most important attributes responsible for yield which molecular study could not reveal. ~~Thus~~Thus, the combine use of agro-morphological and molecular studies as basic markers will complement our resolve to select and improve these local cultivars. The yield display pattern revealed a significant contribution of the quantitative attributes towards the realization of productive potentials of the evaluated cultivars. Thus it is an index for selection of the cultivars for further breeding and development as previously reported by Ray*et al.*[29].

The yield displayed pattern also affirms the results of the principal component analysis for the attributes where the numbers of fingers per bunch contributed a higher percentage of 94.31% to the total observed variations of 99.91% among the cultivars. It is very interesting to note that comparatively, the agro-morphological attributes influenced the total observed variations by 99.91%, the genetic and molecular attributes of the cultivars influenced the total observed variations by 96.00%. This shows that the use of both morphological and molecular markers in the study was ideal and complementary towards a better understanding of the selectable markers in sample species.

The study had also revealed that the 14 elite cultivars were morphologically and genetically delineated into four distinct cluster groups as evident in the dendrogram (Fig. 2), network analysis (Fig.3), principal components (Table 4) and dendrogram (Fig.9). ~~These evidence~~This evidence also shows the complementarity and relevance of the use of the morphological and molecular markers for this study which also indicates the genetic relatedness, thus revealing desirable attributes and forming the basis for selection by farmers. This was earlier posited by Venkatachalam*et al.*[30]who reported the expression of desirable traits in plantain as a basis for selection and improvement by farmers and mandate institutions.

Polymorphism in a given population is often due to the existence of genetic variants represented by the number of alleles at a locus and their frequency distribution in a population[11,30]. ~~Heterozygousity~~Heterozygosity corresponds to a probability that 2 alleles taken at random from a population can be distinguished using the markers in question. Thus a convenient quantitative estimate of marker utility and the polymorphism detected can be given in terms of Nei's genetic diversity, polymorphic information content (PIC), Marker indices (MI), percentage polymorphism and number of reproducible polymorphic ~~bands among~~bands among the population as well as the agro-morphological indices evaluated[27,31].

The value for total genotype diversity among the population was calculated as 0.1269. The polymorphic information content indicates good informativeness of both morphological and molecular markers. Since 13 out of the 14 elite plantain cultivars were from a common genetic base (plantain AAB), Nei's genetic diversity and total variability were low. Genetic divergence was found to be quite low within the elite plantain cultivars gene pool, which is in line with the proposed development of their germplasm through somatic mutagenesis and conventional breeding[14,32]. These results are in tandem with those obtained bySwennen*et al*.[33] in analysis of some economically important species, varieties, and cultivars of the genus Musa using ISSR markers, where they assumed higher values.

Polymorphic information content and Nei's genetic diversity indicate high informativeness in SSR and complemented by the results of morphological markers. SSR studies conducted by Tingey*et al.*[34] revealed higher mean value for polymorphic information content and Nei's genetic diversity among populations of *Podophyllum hexandrum* Royle. The reason for this could be the longer nucleotide sequences and occurrence of these microsatellites which occur more frequently throughout the entire *Musa* genome.

The high genetic similarity (Table 9) between Ikpobata, a French plantain with ABB genome and Bakpri (Dwarf mutant) an elite cultivar with AAB which is also a French plantain and belong to a different cluster may be attributable to genetic indices and molecular characteristics. High genetic distance was also observed and recorded between and among all members of the different clusters with members of cluster one having small or low genetic distances among them showing high or wide genetic differences with members of the other clusters (Fig. 4).

Morphologically, (Fig. 4) cultivars within each cluster group and subclusters shared similar phenotypic characters and similar molecular attributes (Fig.9) with other members in the same cluster or subclusters. There was a close relationship between some of the elite plantain cultivars used in this study, as these cultivars may have been derived from the same pedigree or the amplification of the same nucleotide sequence present between the simple sequence repeats. Similar results have been found by De Langhe[22]Ubi et al.[35]Venkatachalam et al.[36] for banana accessions with an estimated 46% genetic similarity based on ISSR marker data. ISSR markers have also been used for genetic diversity analysis in *Musa* spp.[28,31,37]. These data reports indicated that SSR and ISSR markers were effective for diversity studies in plantain cultivars[38,39].With the uniqueness and dominance of these selected elite cultivars in this agro-ecological region of Nigeria, present study had unveiled important attributes of staple food using phenotypic and genotyping approaches. However, the study was limited by the non-availability of other cultivars from other regions in the study area.A future study that also will sequence the genome of these cultivars will be necessary to store the germplasm in form of sequences in the gene bank and databases to forestall genetic erosion, germplasm extinction and loss of plantain biodiversity in this agro-ecology.

5.0 Conclusion

Morphological and Molecular markers were adopted in the study of genetic diversity in 14 elite cultivars of plantain in the rain forest ecology of Nigeria. Results of agro-morphological fingerprint study revealed significant variations in terms of the bunch weight, number of finger of hands/bunch, number of fingers per hand, number of fingers /bunch, harvest interval, length of crop cycle, pulp hardness and pulp/wt. ratio. The distribution of the elite cultivars along with the principal components showed cluster pattern of distribution within the study location. Principal component analysis revealed four principal components contributing 99.91% to the observed morphological variations while analysis of molecular variance revealed 96.00% contributed by molecular characteristics to observed variations. The yield displayed revealed the significant contributions of bunch weight, fingers/hand and fingers/bunch as the main indices for plantain yield and hence an attribute for improvement.

The dendrograms for both morphological and molecular characteristics delineated the cultivars into four distinct cluster groups and subgroups each varying in genetic distance.

The study revealed the existence of a sufficient amount of genetic variability among the elite cultivars of plantain, which could be exploited further in breeding programs. However, knowledge of the extent of the genetic relationship between these elite cultivars will be important for germplasm collection, in situ conservation, and *Musa* breeding programs. The results of the present study will be useful in plantain DNA fingerprinting and in determining genetic diversity among the elite plantain cultivars.

Commented [MF9]: It is only six SSR primer and with this number cannot be decides fully on the molecular characterization of the genotypes. It is only a prediction

Significance Statement

This study discoveredthat there exist large genetic pool and polymorphism among the plantain cultivars (*Musa* spp., AAB group) by using agronomic and molecular markers. These studies revealed that the agro-morphological and SSR markers can be effectively used in the determination of genetic relationships among plantain cultivars. The study further showed that the two approaches adopted complemented the results obtained from each individual study. Hence, the study will help future researchers to uncover the critical traits of importance needed for the genetic improvement of the low yielding cultivars which are yet to be exploited in these cultivars and which many researchers were yet to explore. Thus, an ideal way of complimenting agro-morphological and molecular studies on these local elite cultivars of plantains and possibly others may be achievable.

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