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Genetic diversity of conserved West African plantain (*Musa* spp.) using simple sequence repeat markers

Original paper

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Abstract: Assessment of the genetic diversity of germplasm is essential for sound germplasm management and its successful utilization in breeding programs. This study aimed to estimate genetic diversity among plantain accessions and establish relationships among the genotypes using simple sequence repeat (SSR) markers. SSR markers amplified 21 alleles, 3.50 alleles per locus, and major allele frequency (mean ± SD, 0.80 ± 0.34) across the 20 plantain accessions. The polymorphic information content (PIC) and Shannon's diversity index ranged from 0.054 to 0.919 and 0.000 to 1.864, respectively. Analysis of molecular variance (AMOVA) showed that 88% genetic variation occurred among genotypes within populations, with minimal variation observed between populations. This resulted in Nei genetic distance and FST values being negligible when distinguishing the populations. The gene flow rate unequivocally demonstrated the efficacy of employing co-dominant markers, as evidenced by both the Principal Coordinates Analysis (PCoA) and the dendrogram. This study revealed a clear-cut genetic variation among the 20 plantain accessions across plantain populations and established new cluster groups, providing valuable insights for future use in breeding programs.

Keywords: *Musa* spp., SSR markers, genetic diversity, genetic relationship, gene flow rate, heterozygosity.

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Introduction

Plantains, a staple crop for many Nigerians, are a significant food source in tropical regions, particularly in Africa, Latin America, and the Caribbean (Lescot, 2004; Ekunwe and Ajayi, 2010). They are a good source of nutritional energy and nutrients and can be cooked in various ways, such as boiling, frying, grilling, or roasting (Elayabalan et al., 2017). In 2020, plantains were grown in 54 countries, with global production exceeding 43 million metric tons (Data, 2020). This crop is critical for food security and revenue generation for small-scale farmers in tropical countries (Kahane et al., 2013). In Nigeria, plantain output is projected to reach 2.4 million metric tons, with the most production coming from the country's southern states (Folayan and Bifarin, 2011). However, plantains face various challenges that may reduce productivity and availability. Pests and diseases, such as plant-parasitic nematodes, can reduce nutrient and water transport to the main plant system, causing plant toppling and yield loss (Gowen et al., 2005; Godefroid et al., 2017). Synthetic pesticides are commonly used to control or manage these pests, but they have harmful effects on other beneficial soil-living species (Lafont et al., 2007; Carrascosa et al., 2015). Efforts to address these flaws have resulted in the creation of new hybrid types through breeding programs to establish lines with resistance or tolerance (Quain et al., 2018). However, over the years, their resistance or tolerance to pests and diseases has been genetically compromised by locals' activities in mixed agriculture systems involving hybrids, landraces, and other aspects of farming practices (Quain et al., 2018). Therefore, understanding the current alignment among plantain genotypes in the population would provide the initial knowledge needed to develop better-adapted varieties for sustainable agriculture in plantain production.

Simple sequence repeats (SSRs), also known as microsatellites, are suitable targets and methods for analyzing genetic variation and genotype relationships due to their genome-wide coverage, robustness, excellent reproducibility, co-dominant inheritance, high polymorphism with multiple alleles per locus, transferability between species, and low requirements for knowledge and instruments (Park et al., 2009; Foster et al., 2020; Bhattarai et al., 2021; Cusaro et al., 2021). SSR markers are comparatively low-cost for plant genotyping and can be employed in small laboratories. Fingerprinting, genetic diversity research, population structure analysis, association mapping, and linkage mapping have all used SSR markers, contributing to the expansion of fundamental genetic research and plant breeding efforts (Bhattarai et al., 2021). Several crops and plant diseases are being targeted with SSR markers, including genetic diversity studies based on genetic distance, gene flow and crossing over rate, evolutionary studies, building linkage maps, mapping loci involved in quantitative traits (QTL), marker-assisted selection, and defining cultivar DNA fingerprints (Jonah et al., 2011; Kalia et al., 2011). Several studies have shown the extent to which SSR markers have been utilized, such as those by (Tenkouano, 2008; Wang, 2016; Yamasaki, 2016; Leal, 2018; Wu, 2018; Jiang, 2020). These studies have shown that SSR markers are an effective technique for identifying genomic regions linked with significant traits or diseases in plants and have been used in genome-wide association studies (GWAS). Therefore the purpose of the study was to assess the genetic diversity among plantain accessions in West Africa and establish relationships among the genotypes using SSR markers.

Materials and Methods

Experimental site

This experiment was conducted at the Genetic Resources Center and Bioscience Center located at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

Plant material, experimental design, and treatment

Twenty (20) plantain accessions from the Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA) were used in this study. Table 1 shows the plantain accessions originating in three West African countries: Nigeria, Côte d'Ivoire, and Ghana. The experiment was laid out in a Completely Randomized Design (CRD), utilizing a factorial experiment with three replicates. The twenty (20) plantain accessions

(Table 1) and six SSR markers listed in Table 2 [DN239472 (locus_1), DN240063 (locus_2), Ma513026332 (locus_3), Ma513019043 (locus_4), Ma513036168 (locus_5), and Ma513035997 (locus_6)] were prepared by Inqaba Biotec, South Africa, served as the treatment.

Genomic DNA extraction of the biological sample and SSR primer sequences

Biological samples from 20 plantain accessions were taken from the young leaves of 8-week-old vegetatively propagated plantain seedlings maintained in the greenhouse at IITA, Ibadan. The collected fresh leaf samples (100–110 mg each) of the twenty plantain accessions were further shared in three replicates and placed in 2 ml autoclaved and labeled Eppendorf tubes and frozen for the next 24 h at -20°C. After 24 h, the biological samples were further dried in a freeze dryer for another 24 h and then ground with a Geno Grinder (MM-200, Retsch) for 3 minutes. Genomic DNA was extracted in three replications using the Doyle and Doyle (1987) plant tissue DNA extraction protocol.

DNA quantity and quality were assessed by a Nanodrop 2000 spectrophotometer (Thermo-Scientific, USA) and 1% agarose gel. Samples with high band intensity, purity, and lesser smear were selected and normalized to 50 ng/µl for further PCR analyses.

Six (6) Expressed Sequence Tag–Simple Sequence Repeat (EST–SSR) markers, created by Mbanjo et al. (2012), were used to assess twenty (20) plantain accessions. The SSR patterns of each subject were examined according to the procedure established by Quain et al. (2018), using the automated infrared fluorescence technology of a Li-COR 4300 sequencer (Li-COR, USA). Six SSR loci were amplified using particular primers modified with an extension M13 sequence at the 5'-end (5'-CACGACGTTGTAAAAC GAC-3') of the forward primer shown in Table 2 (Quain et al., 2018).

Table 1. List of plantain accessions sourced from the Genetic Resources Center, IITA.

S/N	Accession No.	Cultivar name	Collection	Origin
1	TMp-20	Apem onniaba	*Musa* collection	Pop_1 – Ghana
2	TMp-39	Apem pa	*Musa* collection	Pop_1 – Ghana
3	TMp-52	Abomienu	*Musa* collection	Pop_1 – Ghana
4	TMp-10	Nselouka	*Musa* collection	Pop_2 - Cote d'Ivoire
5	TMp-46	Diby 2 off-type	*Musa* collection	Pop_2 - Cote d'Ivoire
6	TMp-54	Didiede	*Musa* collection	Pop_2 - Cote d'Ivoire
7	TMp-55	Niangafelo	*Musa* collection	Pop_2 - Cote d'Ivoire
8	TMp-81	Poupoulu	*Musa* collection	Pop_2 - Cote d'Ivoire
9	TMp-5	Pome	*Musa* collection	Pop_3 – Nigeria
10	TMp-7	Mbi egome 1	*Musa* collection	Pop_3 – Nigeria
11	TMp-22	Akpakpak	*Musa* collection	Pop_3 – Nigeria
12	TMp-24	Egjoga	*Musa* collection	Pop_3 – Nigeria
13	TMp-25	Mbi egome 3	*Musa* collection	Pop_3 – Nigeria
14	TMp-26	Ntanga 3	*Musa* collection	Pop_3 – Nigeria
15	TMp-28	26285.0	*Musa* collection	Pop_3 – Nigeria
16	TMp-29	11669.0	*Musa* collection	Pop_3 – Nigeria
17	TMp-30	1199-1	*Musa* collection	Pop_3 – Nigeria
18	TMp-31	1199-6	*Musa* collection	Pop_3 – Nigeria
19	TMp-62	Eberedia ukom	*Musa* collection	Pop_3 – Nigeria
20	TMp-69	Okoyo ukom	*Musa* collection	Pop_3 – Nigeria

TMp – Tropical *Musa* plantain, Pop_1/2/3 – Individual population

Table 2. List of the SSR loci used in this study.

Locus	Primer sequences (5' - 3')	A.S.R.O.	T_a (°C)
DN239472	F : CACGACGTTGTAAAACGACCCGTGTTACAGATTGTATTCCCTTGT	105-126	58
	R : CGGCTGCGTCAACAAGCC		
DN240063	F : CACGACGTTGTAAAACGACCGCGGATGATTCATCGTGGACCG	170-174	58
	R : ACAGCAGCAACAATCTCGTCGT		
Ma513026332	F : CACGACGTTGTAAAACGACCCAACTTTCTCCAAGATCAG	164-170	58
	R : TCCAACAAGCAGCCCGT		
Ma513019043	F : CACGACGTTGTAAAACGACGTTAACGGCCACCTGCATGG	174-180	58
	R : GCCTCTTCACTGTGTTAAGTGCACAA		
Ma513036168	F : CACGACGTTGTAAAACGACCGCAGTAGCAGCAGGCAG	158-169	58
	R : GCCACAGCAGGATCCACC		
Ma513035997	F : CACGACGTTGTAAAACGACGAGGACCAATCTGCGTTCGC	114-124	58
	R : ACGCAGCACAAGTCGTCCA		

F – Forward, R – Reverse, A.S.R.O. – Allele size range observed, Ta (°C) – Annealing temperature

Polymerase chain reaction (PCR) product followed by gel electrophoresis

The six SSR markers, or loci, were used to estimate genetic variation and relationships among plantain accessions. The Gene-Amp® PCR System 9700 thermal cycler was used to conduct the PCR analysis in a total volume of 12.5 µl. Briefly, the recipe contains 6.25 µl of 1x [one *Taq* 2x Master Mix (M04821), New England Bio-Labs USA, with standard buffer, which contains all PCR components: 3 mM $MgCl_2$, 10x PCR buffer, 2.5 mM dNTPs, and 1 U *Taq* DNA polymerase (Fermentas)], 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 0.25 µl of 3% dimethyl sulfoxide (DMSO, v/v), 0.1 µM of M13 primer-fluorescent dye IR700 or IR800 (Biosynthesis, Lewisville, Texas, USA), 2 µl of 20 ng genomic DNA, and 3 µl nuclease-free water. The PCR was programmed with an initial denaturation phase of 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 1 min, and extension at 68°C for 1 min, with a final extension at 68°C for 20 min, and a holding step at 4°C. The PCR products were loaded on a 1% agarose gel (w/v) using a 6x loading dye that contained GelRed. Electrophoresis was carried out in 1x Tris-acetate-EDTA (TAE) buffer for 3 h at 100 constant volts. The gel was stained with GelRed, and a Bio Doc-IT Imaging device (Cambridge, UK) was used to view the gel image under UV light. A 50–1500 bp AMPIGENE DNA ladder was used as a molecular ruler to compare DNA bands and estimate the sizes of the DNA fragments.

Data analysis

In this study, the following variables were computed to analyze the genetic diversity within and among plantain accessions. For each locus, the number of alleles (Na), average number of alleles per locus (A), observed heterozygosity (Ho), expected heterozygosity (He), Shannon's information index (I), and inbreeding coefficient (FIS) were calculated by GenAlEx software 6.5v (Peakall and Smouse, 2006). Then, polymorphic information content (PIC) was calculated to evaluate the informativeness of each marker using Power Marker software 3.25v (Liu and Muse, 2005). An analysis of molecular variance (AMOVA) was conducted to examine the genetic structure of several plantain accessions by comparing within and among population variations. The importance of AMOVA was evaluated using a non-parametric permutation method using 999 permutations. The genetic differentiation index was assessed using FST values among subpopulations. Pairwise genetic distances were calculated using unbiased genetic distance (Nei, 1978). Principal coordinate analysis (PCoA), implemented in GenAlEx software version 6.5, was used to elucidate the genetic structure, relying on the pairwise distance matrix. Additionally, to enhance the examination of genetic structure, a dendrogram was generated using the unweighted neighbor-joining approach, utilizing genetic dissimilarity among the 20 plantain accessions via DARwin software version 6.0.12 (Perrier, 2006).

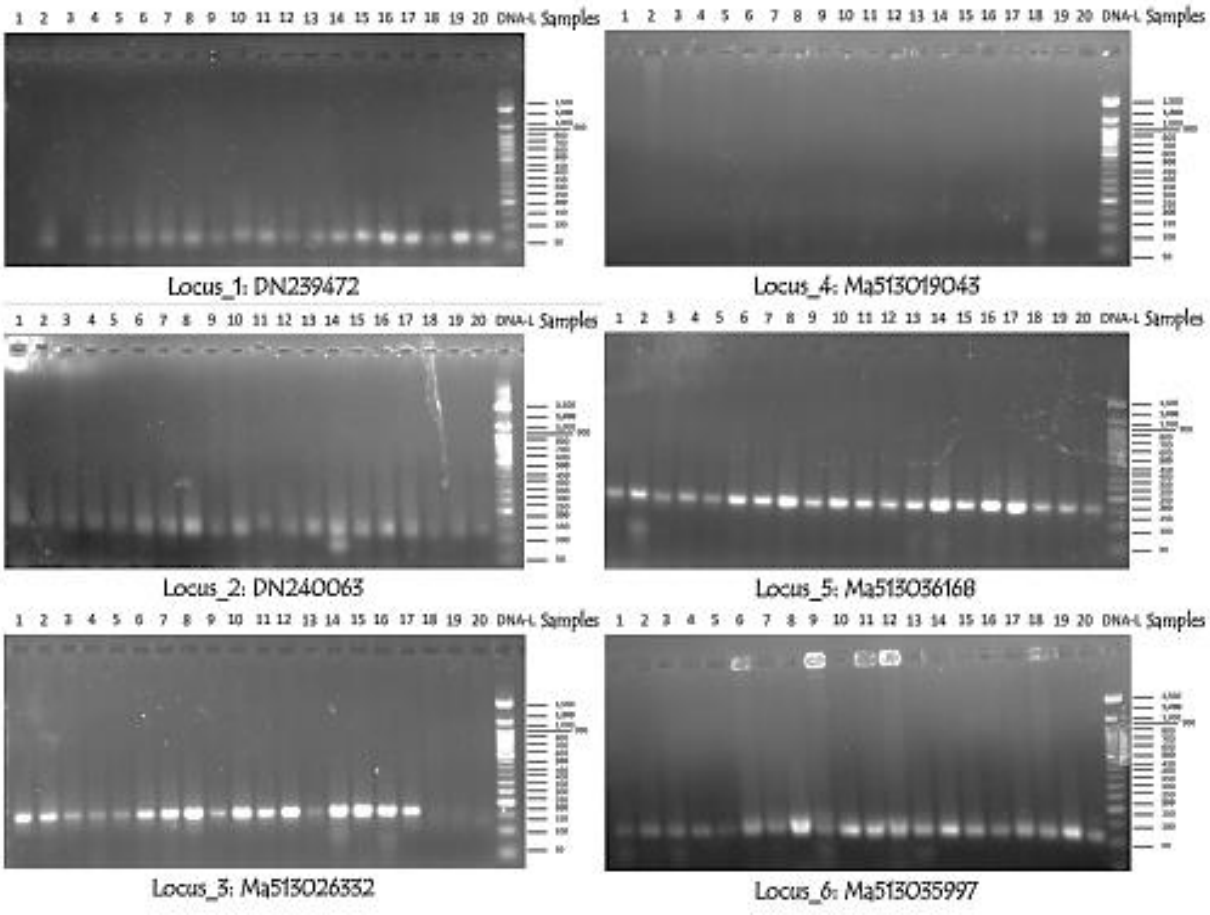

Figure 1. Polymorphisms observed from 20 plantain accessions across the six SSR loci.

Results

Allelic polymorphisms of SSR loci

Six (6) SSR markers were employed to assess the genetic diversity and relationships of twenty (20) plantain accessions available within the locality (Figure 1). The results presented in Table 3 revealed polymorphic profiles for the six SSR markers. A total of 21 alleles were identified across the six loci, resulting in an average of 3.50 alleles per locus. These loci (DN239472, DN240063, Ma513026332, Ma513036168, and Ma513035997) displayed appreciable levels of polymorphisms needed for genetic diversity assessment, with 3, 4, 5, 4, and 4 alleles, respectively. This cascaded on the major allele frequency (mean ± SD, 0.80 ± 0.34), where the following loci (DN240063, Ma513036168, and Ma513035997) assumed full availability (1 each) across the selected twenty (20) plantain accessions. The discriminatory power of each locus was evaluated by polymorphic information content (PIC). The average PIC value was relatively high 0.733 ± 0.307, with a maximum value of 0.919 recorded for the Ma513026332 locus and a minimum value of 0.054 observed for the Ma513019043 locus; the latter showed the most undesirable traits across the measured variables.

SSR markers and genetic diversity analyses

Genetic diversity traits are variables used by co-dominant markers (SSRs, SNPs, etc.) to assess the variation within and among populations. The results revealed the average number of alleles (Na),

as explained in Table 3. The number of effective alleles (Ne) mean values ranged from 1 to 6 (Supplementary Table 1). The results showed that locus_4 (Ma513019043) received the lowest value (mean ± SD) of 1.000 ± 1.523 Ne across the populations, while locus_3 (Ma513026332) had the highest mean value of 6 ± 1.523 Ne present in population_3 (Pop_3). Shannon`s information index (I) illustrated the values range from 0 to 1.864 I, with locus_4 (Ma513019043) was found with the lowest mean value of 0 ± 0.568 I over at least two populations (Pop_1 and Pop_2). However, the highest mean value was recorded for locus_3 (Ma513026332) with 1.864 ± 0.568 I in Pop_3. Observed heterozygosity (Ho) values ranged from 0 to 0.667. The maximum mean value resulting from locus_6 (Ma513035997), with an average of 0.667 Ho. On the other hand, the average expected heterozygosity (He), which reflects genetic diversity ranged from 0 to 0.833 (Supplementary Table 1). Here, locus_4 (Ma513019043) had the lowest mean value (0 ± 0.262, He), and locus_3 (Ma513026332) had the most admirable mean value (0.833 ± 0.262, He).

Table 3. Mean performance of allelic polymorphisms at the studied SSR loci.

S/N	Locus	Number of alleles	Major allele frequency	PIC
1	DN239472	3.00	0.90	0.799
2	DN240063	4.00	1.00	0.890
3	Ma513026332	5.00	0.85	0.919
4	Ma513019043	1.00	0.05	0.054
5	Ma513036168	4.00	1.00	0.906
6	Ma513035997	4.00	1.00	0.832
	Total	21.00	-	-
	Mean	3.50	0.80	0.733
	Standard Deviation	1.26	0.34	0.307

PIC – Polymorphism information content

This trend was seen on the unbiased expected heterozygosity (uHe), which ranged from 0 to 0.889. Same locus_4 (Ma513019043) had the least mean value (0 ± 0.284, uHe), while locus_3 (Ma513026332) showed the highest value across the populations with the highest mean value of 0.889 ± 0.284, uHe. On the fixation index (F), also known as the inbreeding coefficient, negative values denote excess in the heterozygote individuals within a given population, whereas positive values are for homozygotes. Hence, the inbreeding coefficient values ranged from -0.091 to 1. Locus_6 (Ma513035997) was found to have an appreciable mean value for the expected heterozygosity only in Pop_1 (-0.091 ± 0.386, F). On the contrary, loci (DN239472, 1.000 ± 0.386; DN240063, 1.000 ± 0.386; Ma513026332, 1 ± 0.386; Ma513019043, 1.000 ± 0.386; Ma513036168, 1 ± 0.386) had positive mean values for the inbreeding coefficient (F).

Genetic diversity expressed by populations of plantain accessions across the SSR loci

The results presented in Table 4 revealed the gene diversity information over the three populations of plantain accessions at the six SSR loci. The number of individuals per population (N) ranged from 3 to 12, with a grand mean of 6.667 ± 0.936. The number of alleles (Na) ranged from 2.500 ± 0.342 to 5.667 ± 0.882, with a mean value of 3.889 ± 0.471. Similarly, the number of effective alleles (Ne) showed the same trend, with values ranging from 2.324 ± 0.320 to 4.419 ± 0.749 on a grand mean of 3.331 ± 0.369. The

Shannon`s information index (I) ranged from 0.809 ± 0.176 to 1.471 ± 0.253 with a grand mean value of 1.130 ± 0.138. On the contrary, the pattern changed as observed heterozygosity (Ho) had values ranging from 0 to 0.167 ± 0.114 on a general mean value of 0.069 ± 0.041. In contrast, expected heterozygosity (He) recorded values ranged from 0.500 ± 0.105 to 0.688 ± 0.109 on a grand mean value of 0.596 ± 0.064 (Table 4). The results maintained the trend on unbiased expected heterozygosity (uHe), which ranged from 0.600 ± 0.126 to 0.717 ± 0.113 over the general mean value of 0.661 ± 0.069. The fixation index (inbreeding coefficient, F) reported positive values across the populations, indicating fewer heterozygote individuals among the studied populations. The inbreeding coefficient values ranged from 0.673 ± 0.199 to 1, with a grand mean value of 0.878 ± 0.070. Altogether, the findings on percentage polymorphic loci (% P) indicated the levels of genetic diversity or similarity expressed by the 20 plantain accessions. The grand mean value was notably high (88.89% ± 5.56). The lowest mean value among the populations was even on the high side at 83.33%, shared between Pop_1 and Pop_2. The highest recorded percent polymorphism of the six SSR loci studied was found in Pop_3, with a mean value of 100.00%.

Table 4. The mean performance of the three plantain populations on genetic diversity indexes across the SSR markers.

Population	N	Na	Ne	I	Ho	He	uHe	F	%P
Pop_1	3.000 (0.000)	2.500 (0.342)	2.324 (0.320)	0.809 (0.176)	0.167 (0.114)	0.500 (0.105)	0.600 (0.126)	0.673 (0.199)	83.33%
Pop_2	5.000 (0.000)	3.500 (0.563)	3.249 (0.537)	1.110 (0.233)	0.000 (0.000)	0.600 (0.122)	0.667 (0.135)	1.000 (0.000)	83.33%
Pop_3	12.000 (0.000)	5.667 (0.882)	4.419 (0.749)	1.471 (0.253)	0.042 (0.028)	0.688 (0.109)	0.717 (0.113)	0.948 (0.036)	100.00%
Grand M	6.667	3.889	3.331	1.130	0.069	0.596	0.661	0.878	88.89%
SE	(0.936)	(0.471)	(0.369)	(0.138)	(0.041)	(0.064)	(0.069)	(0.070)	(5.56)

Pop_1/2/3 – Individual population, N – Number of individuals per population, Na – Number of different alleles, Ne – Number of effective alleles, I – Shannon`s information index, Ho – Observed heterozygosity, He – Expected heterozygosity, uHe – Unbiased expected heterozygosity, F – Fixation index (inbreeding coefficient), %P – Percent polymorphic loci, GM – Grand mean, SE – Standard error.

Wright's F-statistics for 20 plantain accessions based on the SSR markers

Table 5 presents Wright's F-statistics for 20 plantain accessions across the six SSR markers. Wright's F-statistics include the inbreeding coefficient (FIS), global genetic fixation index (FIT), genetic differentiation index (FST), and estimated gene flow rate (Nm). Regarding the inbreeding coefficient (FIS), the results showed values ranging from 0.607 to 1, with a mean of 0.902 ± 0.064. The global genetic fixation index (FIT), or, in other words, total genetic diversity, revealed high positive values ranging from 0.666 to 1 with a mean value of 0.919 ± 0.054. On the contrary, the genetic differentiation index (FST) among the three plantain populations based on the SSR loci was very low, indicating unity among the populations, with a general mean of 0.168 ± 0.027. The FST values ranged from 0.057 to 0.252. The overall findings indicated that the estimated gene flow rate (Nm) was significant, with values ranging from 0.741 to 4.125 and a mean of 1.614 ± 0.511. Therefore, these results suggest that for every generation among the populations, the highest percent of gene crossing over between populations was approximately 4.13%, it was argued that it is still very low to influence the genetic structure of any population but enough to seek precautions in maintaining pure breeds or lines among the populations.

Genetic differentiation indexes (AMOVA, F_{ST} values, and Nei genetic distance) based on SSR markers for 20 accessions of Musa spp.

The analysis of molecular variance (AMOVA) results indicates that there is little or no genetic variation among populations (6% var.), as shown in Supplementary Table 2. The majority of genetic variance (88% var.) can be attributed to variation among genotypes or individuals across the three populations. However, the percentage variation resulting from within individuals of respective populations was very low, accounting for 6%. This finding suggests a lack of significant population structure destruction, which can be linked to the observed low genetic distances that would amount to a full breakdown of desired traits among the populations. The pairwise population matrix FST values revealed minimal levels of differentiation among the populations, as indicated in Supplementary Table 3 (below the diagonal), ranging from 0.076 to 0.193. Furthermore, the pairwise population matrix based on Nei genetic distance in Supplementary Table 3 (above the diagonal) demonstrated a close genetic relationship within plantain accessions from 0.398 to 0.862. Conversely, Nei genetic distance results revealed wider distances between the populations, which represented the percentage variation found among individuals across the three plantain populations in the AMOVA Table.

Table 5. Wright's F-statistics for 20 plantain accessions based on SSR markers.

Locus	FIS	FIT	FST	Nm
DN239472	1.000	1.000	0.252	0.741
DN240063	0.962	0.969	0.172	1.200
Ma513026332	1.000	1.000	0.165	1.262
Ma513019043	1.000	1.000	0.057	4.125
Ma513036168	0.845	0.877	0.208	0.949
Ma513035997	0.607	0.666	0.151	1.406
Mean ± SE	0.902 ± 0.064	0.919 ± 0.054	0.168 ± 0.027	1.614 ± 0.511

FIS – Inbreeding coefficient, FIT – Global genetic fixation index, FST – Genetic differentiation index, Nm – Estimated gene flow rate, SE – Standard error

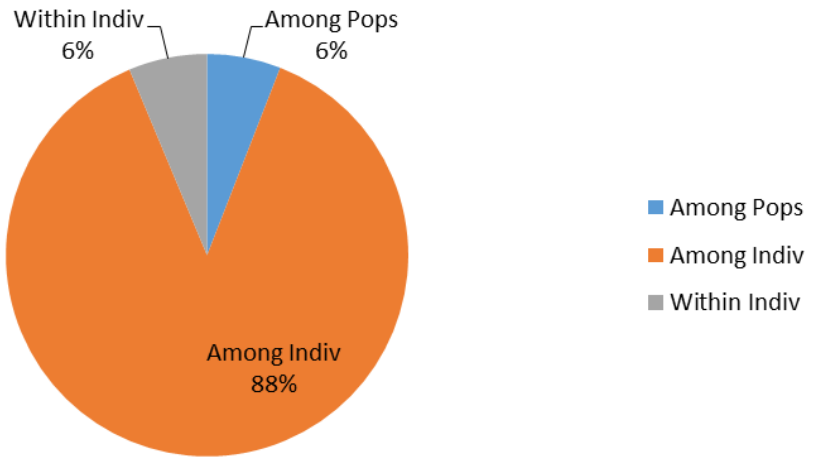

Figure 2. AMOVA for the genetic differentiation indexes on 20 accessions of Musa spp.

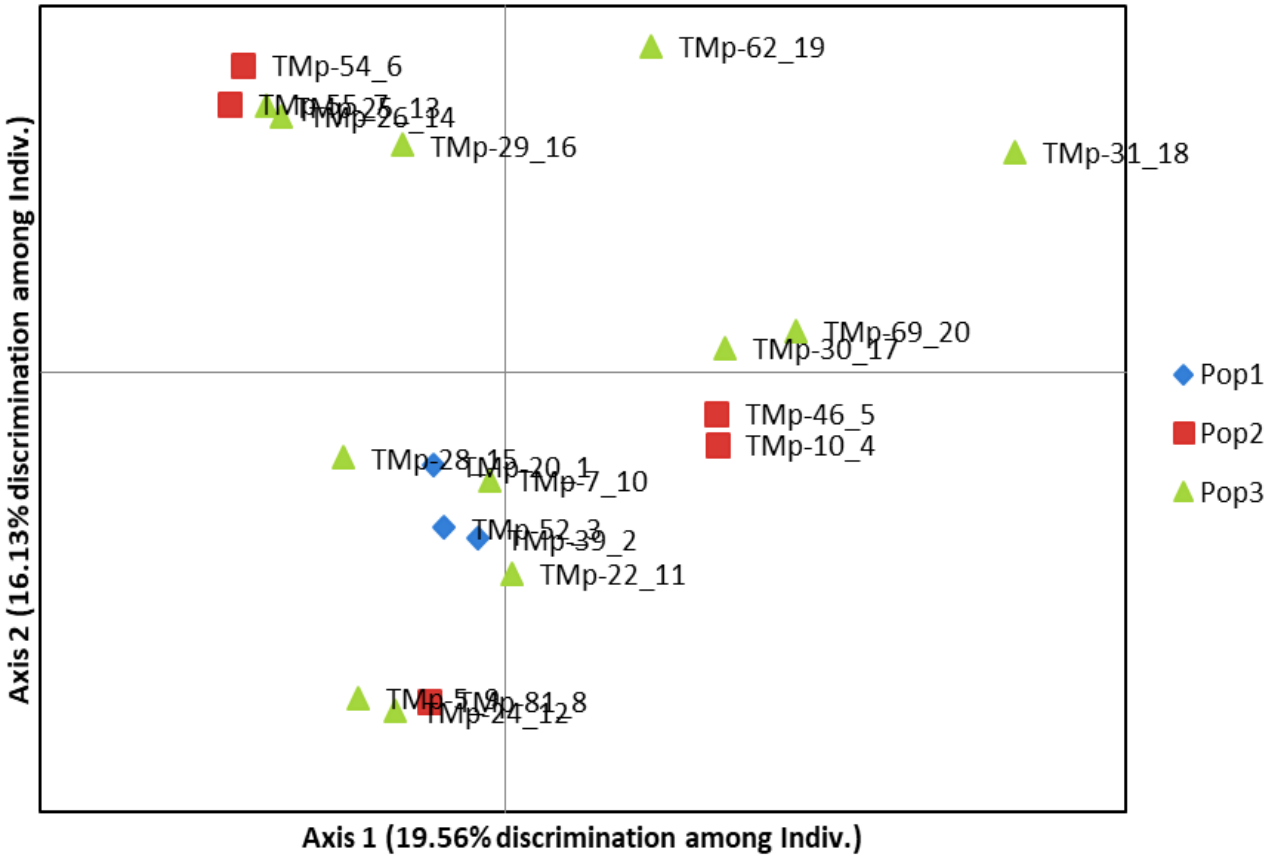

Figure 3. In a Principal Coordinates Analysis (PCoA) scatter plot of 20 plantain accessions based on the genetic distance matrix, the plane of the first, second, and third main PCoA axes accounted for 48.05% of the total variation. The first axis = 19.56% of total information; the second axis = 16.13%.

Genetic structure analysis

The principal coordinates analysis plot presents the genetic relationships among the 20 plantain accessions (Figure 3). The first three axes were the most informative PCoA, accounting for 48.05% of the total variation. The graphic representation of the PCoA showed proficient differentiation among the individuals or genotypes across the three populations based on the Nei genetic distance matrix. However, all three plane axes still did not greatly separate the plantain populations because the total variation was below average.

Though the unweighted neighbor-joining method was employed to justify the dissimilarities among the plantain populations, a dendrogram based on a dissimilarity matrix was generated, which confirmed the relationships and dissimilarities among the genotypes of plantain populations (Figure 4). The bootstrap values of all the nodes were depicted as numbers at the forks of the consensus tree diagram. To this end, three cluster groups were formed (rearranged due to gene flow or crossover effects).

Discussion

The evaluation of genetic diversity in agricultural germplasm is critical for defining a strategic approach to germplasm management and its use in breeding operations. Many studies in West African countries have focused on assessing a range of varieties, from known to unknown, such as the morphological diversity of plantain genotypes, and now using molecular tests. These physical characteristic investigations have usually found extremely minor differences in plantain traits across

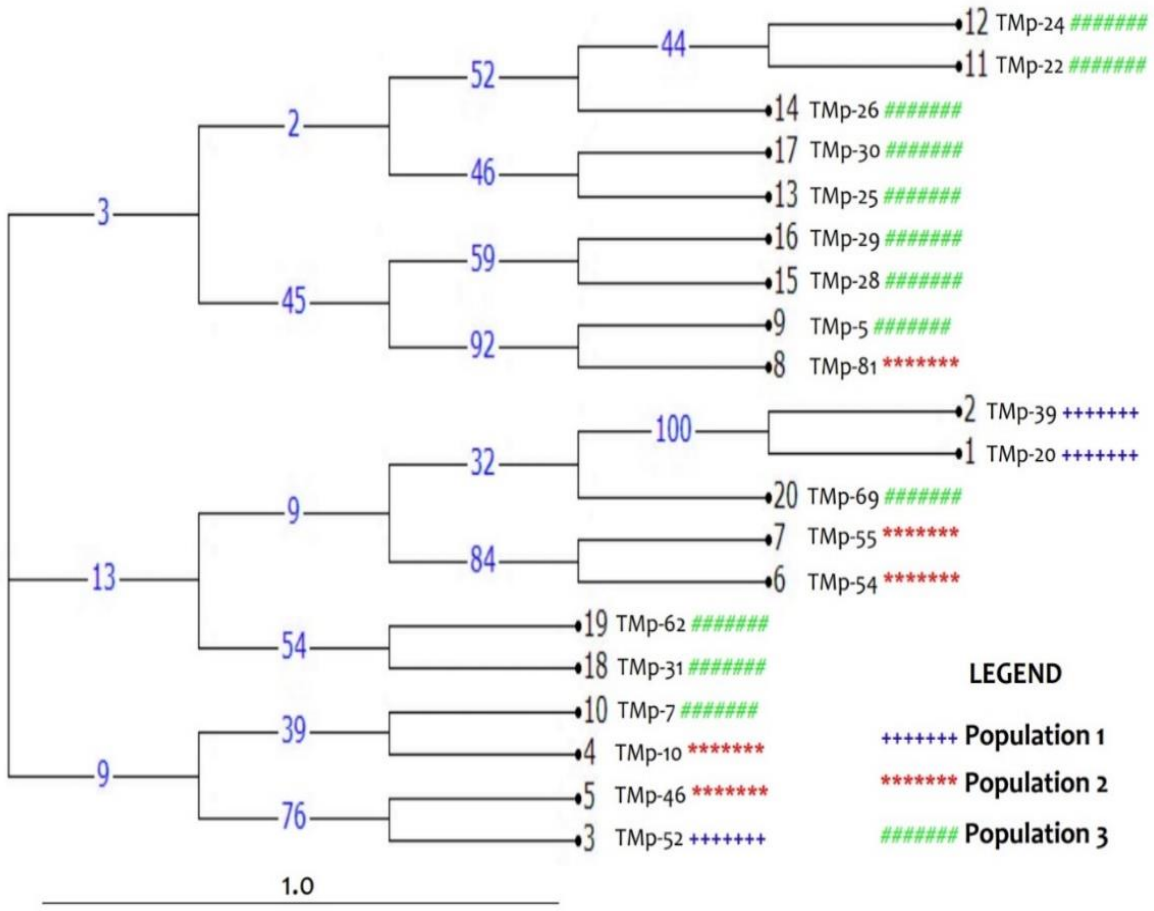

Figure 4. Unweighted neighbor-joining dendrogram depicting genetic relationships and dissimilarities among 20 plantain accessions; numbers along branches denote bootstrap support (shown for all values from 1000 bootstrap replicates).

the region, highlighting the significance of understanding and protecting *Musa* spp. genetic diversity. (Selatsa et al., 2009), for example, carried out a successful study in Cameroon that studied the morphological variety of plantain cultivars collected from several parts of the country. They discovered significant differences in plant height, leaf form, bunch size, and fruit characteristics among the studied genotypes, showing the considerable genetic diversity of plantains in Cameroon. Yet, many phenotypic features are strongly influenced by environmental influences, resulting in continuous variation (Creste et al., 2004). As a consequence, we decided to use molecular method (engaging co-dominant markers, SSRs) to evade the influence of the environment. Therefore, we set out to estimate genetic variation within and among the selected plantain accessions and to establish genetic relationships among the plantain accessions using co-dominant markers, SSRs. In all 20 plantain genotypes, at least 83.33% of the primers successfully produced scoreable amplicons. The microsatellite markers were obtained from diploid genotypes of East African highland banana cultivars, which are known to have substantial genetic variation (Mbanjo et al., 2012). The total number of alleles, average alleles per locus, major alleles frequency, and PIC values were all reported. The six primers amplified a total of 21 alleles, with an average of 3.50 alleles per locus, and expressed a very high frequency of major alleles throughout the 20 plantain accessions. These findings are consistent with previous research by GE

et al. (2005), which claimed an average Na value of 3.32, while Resmi et al. (2011) indicated an average 'Na' value of 2.70. These are values that we also observed. The SSR markers showed extremely high polymorphic information content (PIC) values. The mean PIC value obtained was higher than that reported by Resmi et al. (2011), who analyzed banana cultivars using 15 SSR markers and achieved a mean value of 0.530. In our study SSRs had PIC values greater than 0.500, indicating that they could be used to assess the genetic diversity of any plantain population. PIC values can be classified into three classes: slightly informative (PIC < 0.250), reasonably informative (0.500 > PIC > 0.250), and highly informative (PIC > 0.500) (Hayden et al., 2009). According to Ge et al. (2013), the SSR loci exhibit relatively high polymorphism and discriminative values based on this PIC scale.

Furthermore, the number of effective alleles (Ne, 1.22) was comparable to the report of Quain et al. (2018), who found a Ne value of 1.81 using SSR markers in a study of plantain accessions. In addition, Shannon's diversity index confirmed a substantial amount of genetic variation among plantain genotypes in this investigation. The results revealed that the allelic productivity of SSRs is extremely high. Shannon's information index value of close to one (1.00) indicates a high level of diversity, according to (Costa Tártara, 2014). In terms of heterozygosity traits, the present study found that the observed heterozygosity (Ho) was lower than the average expected heterozygosity (He, which reflects genetic diversity). This pattern was also apparent in unbiased expected heterozygosity (uHe), which had a higher average value than Ho. The fixation index (F) reflected this observation, with a negative value indicating an excess of heterozygote individuals within a given population and a positive value indicating an excess of homozygotes. In contrast, in research on the genetic diversity of plantain landraces from Cote d'Ivoire conducted by Cyrille et al. (2019), the average anticipated heterozygosity (He = 0.506 ± 0.008) was lower than the observed heterozygosity (Ho = 0.897 ± 0.025) across all loci and populations evaluated. Our findings were comparable to those of (Haddoudi et al., 2021).

According to our findings, the average inbreeding coefficient (FIS) value favored low observable heterozygosity. Likewise, the global genetic fixation index (FIT) found a significant positive value, indicating that homozygotes are distributed equally throughout populations. The genetic differentiation index (FST) between plantain populations based on SSR loci was very low, demonstrating unity among the three populations. In a study of B-genome-derived SSR markers in *Musa* spp., Oriero et al. (2006) found negative FIS and FIT values. Additionally, Haddoudi et al. (2021) found substantial population differentiation with an FST of 0.147 by SSR markers. Also, the value was consistent with that reported for *Musa ciliaris* (FST = 0.18) by (Badri et al., 2008). We are confident that the complete set of findings would be sufficient to demonstrate that the results were narrowed down on the predicted gene flow rate (Nm), which was accurate. The values ranged from 0.741 to 4.125, with a mean of 1.614 ± 0.511. This implies that the highest percentage of gene crossing over between populations was roughly 4.13% per generation, which is still too low to impact the genetic structure of any group. Nevertheless, Nm greater than 4.00 suggests substantial gene flow among populations, according to (Ruiz-Erazo et al., 2015).

The results of the analysis of molecular variance (AMOVA) show that there is little genetic diversity both among populations and within individuals in those groups. The primary component of genetic variation detected, on the other hand, was found among individuals (88%) across the three groups. Earlier studies on plantain diversity using SSR markers found similar minor genetic variations between populations (Changadeya et al., 2012; Kitavi, 2015; Quain et al., 2018). These findings confirm the concept that plantain morphological variety emerged predominantly through somatic mutations in a hypervariable region of the genome arising from a small number of botanically distinct clonal origins (Langhe et al., 2005). Still, the results indicate a lack of population structure, which can be attributable to the low genetic distances observed between groups.

FST values for the pairwise population matrix demonstrated low degrees of divergence among the populations. Also, the pairwise population matrix based on Nei genetic distance revealed both intimate and broad genetic links among the studied plantain accessions. As a result, the findings are corroborated

by a report on the linkages between diploid *Musa acuminata* cultivars (Changadeya et al., 2012).

Based on the Nei genetic distance matrix, the cluster analysis utilizing PCoA did not reveal a distinct cut among the three plantain populations. As a consequence, the unweighted neighbor-joining approach was used to explain the differences between plantain populations. The dissimilarity matrix was used to produce a dendrogram, which validated the linkages and dissimilarities of plantain accessions across populations. This analysis led to three cluster groups. Therefore, the report by Cyrille et al. (2019) backs up these findings.

Conclusion

The present study is one of the earliest to use SSR markers to investigate the genetic diversity of plantain genotypes in Nigeria. The use of these SSR markers revealed significant genetic variation and connections among 20 plantain accessions. The adoption of these primers was predicated on the informativeness of their high PIC values, which have been previously reported in the literature. They duplicated the same high scores on average, resulting in careful genetic variation among the 20 plantain genotypes. The main allele frequency was at its peak. The key factors seen on the genetic differentiation index scores were Shannon's information index and the inbreeding coefficient component. All of these findings correlate with the gene flow rate (Nm), which refers to the number of cross-over genes every generation. This might be thought of as the essence of the entire diversity. The results on Nm are supported by the pairwise population matrix and the AMOVA. Surprisingly, the PCoA and dendrogram generated corroborated the linkages and differences among the 20 plantain accessions. They were regrouped (rearranged inside the same initial three (3) cluster groups, but with clear mixes among the individuals throughout the three populations: Cluster 1, Cluster 2, and Cluster 3). As a result, plant breeders can choose from any of the accessions in distinct clusters for exceptional crop development schemes for sustainable agriculture. More research on the effects of co-dominant markers on genetic diversity, the assembly of desired traits, and plant improvement will be much appreciated as a precaution against any unexpected hybrid breakdown.

Supplementary Materials

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_719401.html.

Supplementary Table 1. Responses of SSR markers to genetic diversity indexes across entire populations.

Supplementary Table 2. AMOVA based on SSR markers for 20 accessions of *Musa* spp.

Supplementary Table 3. Pairwise population matrix FST values (below the diagonal) and Nei genetic distance (above the diagonal).

Author Contributions

Conceptualization, D. O. and O. N.; methodology, F. O.; software, D. O. and F. O.; validation, C. O., O. U. and H. O.; formal analysis, C. O. and F. O.; investigation, D. O.; resources, D. O.; data curation, O. N. and F. O.; writing—original draft preparation, D. O. and F. O.; writing—review and editing, O. U. and H. O.; visualization, H. O. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest Statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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مقاله پژوهشی

بررسی تنوع ژنتیکی چنار (*Musa* spp.) در منطقه حفاظت‌شده آفریقای غربی با استفاده از نشانگرهای توالی‌های تکراری ساده

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چکیده: ارزیابی تنوع ژنتیکی ژرم‌پلاسم برای مدیریت صحیح ژرم‌پلاسم گیاهی و استفاده در برنامه‌های اصلاحی ضروری است. این مطالعه با هدف برآورد تنوع ژنتیکی در میان توده‌های چنار و برقراری ارتباط بین ژنوتیپ‌ها با استفاده از نشانگرهای توالی تکراری ساده (SSR) انجام شد. با استفاده از نشانگرهای SSR، ۲۱ آلل با فراوانی ۳/۵ آلل در هر مکان ژنی و با فراوانی آلل اصلی ۰/۸۰ ± ۰/۳۴ (SD ± میانگین) در ۲۰ توده چنار شناسایی گردید. میزان تغییرات محتوای اطلاعات چندشکل (PIC) و شاخص تنوع شانون به ترتیب ۰/۰۵۴ الی ۰/۹۱۹ و صفر الی ۱/۸۶۴ متغیر بود. تحلیل واریانس مولکولی نشان داد که ۸۸ درصد از تنوع ژنتیکی شناسایی شده، تنوع درون جمعیتی بوده در حالیکه فاصله ژنتیکی کمتری بین جمعیت‌های مورد بررسی مشاهده شد. بنظر می‌رسد این موضوع سبب اندک شدن برآورد فاصله ژنتیکی Nei و مقادیر FST در هنگام تفکیک جمعیت‌ها گردید. نرخ جریان ژنی به‌طور قاطع کارایی استفاده از نشانگرهای همبارز را نشان داد، همان‌طور که از طریق تجزیه به مولفه‌های اصلی (PCoA) و دندروگرام به اثبات رسید. این تحقیق بیانگر وجود تنوع ژنتیکی بین ژنوتیپ‌های جمعیت‌های چنار بوده و اطلاعات حاصله از گروه‌بندی ژنوتیپ‌های را می‌توان در برنامه‌های آتی اصلاحی مورد استفاده قرار داد.

کلمات کلیدی: نشانگرهای SSR، Musa spp، تنوع ژنتیکی، فاصله ژنتیکی، شدت جریان ژنی، هتروزیگوسیتی.

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