African Journal of Biotechnology

Full Length Research Paper

The use of microsatellites in the characterization of three plantain cultivars from the Democratic Republic of Congo

Mave Dhed'asi Gracia[1]*, Dhed'a Djailo Benoit[1], Kateregga John N.[2] and Nanyiti Sarah[3]

[1]Department of Biotechnology, University of Kisangani, P. O. Box 2012 Kisangani, Democratic Republic of Congo.
[2]College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, P. O. Box 7062 Kampala, Uganda.
[3]National Crop Resources Research Institute, Namulonge Uganda, P. O. Box 7084 Kampala, Uganda.

Knowledge of the genetic variability of plantain cultivars in the Democratic Republic of the Congo (DRC) is limited. This study assessed genetic diversity and relationships in three plantain accessions differing in pseudostem color at seven microsatellite loci using SSR markers. DNA was extracted from banana leaves, and PCR was performed with unlabeled and fluorescent-labeled SSR primers and eventual capillary sequencing. Different population genetic analysis software was used to interpret the fragment analysis data. There was a high allelic power with a mean polymorphic information content of 0.83. A high average genetic diversity was indicated by Nei's Gene Diversity Index (h: 1.38) and the Shannon Information Index (I: 2.22). Pairwise genetic similarity between plantain genotypes averaged 0.82. A dendrogram grouped the 60 genotypes into three clusters. AMOVA showed high genetic variation within the population (98%) compared to the variation between populations, with no significant difference between the three cultivars regarding the color of the pseudostem. In contrast, these cultivars were closely related to each other. No correlation between the morphological and molecular characterization of cultivars was observed. We recommend a follow-up study with other plantain cultivars from other regions and large sample size for more representative data.

Key words: Characterization, microsatellites, genetic diversity, plantain cultivars.

INTRODUCTION

Bananas and plantains are of great economic, cultural, and nutritional importance worldwide, especially in developing countries, including the Democratic Republic of the Congo (DRC). They occupy the second position as a food crop, which also contributes greatly to the food security of the population of this mostly poor country, especially in the Oriental province (Onautshu, 2013). In 2019, banana production in the DRC was estimated at 64110596 tons, including 4856474 tons of plantains coming mainly from the lowland forest region (National Institute of Statistics (NIS), 2020). In addition, it is also an important source of household income, along with

cassava, rice, maize, and palm oil (Kasaka et al., 2021).

Most cultivated bananas are triploid and belong to the Eumusa section of the Musaceae family (Tenkouano et al., 2007). These cultivars are derived from intra- and interspecific crosses of two diploid ancestral species, *Musa acuminata* Colla (genome AA) and *Musa balbisiana* Colla (genome BB), originating from Malaysia and India, respectively (Lusty et al., 2006). Plantains that were characterized in this study belong to the AAB group (Perrier et al., 2011). The DRC has rich genetic diversity in bananas, especially plantains. The estimated plantain diversity in the Democratic Republic of the Congo is more than 100 cultivars. Most cultivars followed the traditional banana cultivar classification and identification system, which is mainly based on morphological and quantitative characteristics such as bunch type, crop size, bunch orientation, pseudostem color, and fruit peel color (Adheka, 2016).

However, it is challenging to classify accessions from the same variety using morphology since this method has some limitations when it comes to accurately identifying them. For example, there are only a limited number of traits available for characterization, and these traits can be strongly influenced by environmental factors for quantitative traits (Blazakis et al., 2017). Additionally, some heritable traits may show insignificant variations, and there can be difficulties in distinguishing physical traits due to the morphological similarity of the accessions (Rana, 2018). Color variation usually affects the pseudostem more than any other part of the plant. In the case of False Horn plantain accessions, for instance, the three cultivars used in this study share identical traits except for the color of their pseudostems. The pseudostem of Libanga Likale is green, that of Libanga Lifombo is red, and that of Libanga black is black (Adheka et al., 2018).

Some molecular characterization has been done, but not all plantain cultivars have been genetically characterized. Hence, the extent of molecular diversity among plantain varieties in the DRC is not very well understood. Various types of genetic markers are utilized to measure variation among genotypes. Microsatellites, also known as Simple Sequence Repeats (SSRs), are the most commonly used because they are dependable and can be used across different laboratories. These markers are codominant and can identify a large number of variable bands in a single sample rather than having high levels of variation at each genetic locus (Kitavi, 2015). SSRs are valuable because they are widespread, present throughout the genome, have multiple alleles, are highly reproducible, and can work with small quantities of material or degraded or ancient DNA (Senan et al., 2014). However, the mutation rates of SSRs are excessively high, leading to alterations in conserved regions between species and homoplasy (alleles that are identical in the state but not origin) (Barkley et al., 2009). There is currently a group of 22 SSR markers utilized for the molecular characterization of bananas that can distinguish subgroups and subspecies (Christelová et al., 2011; Hippolyte et al., 2012). This study was thus aimed at determining genetic diversity within and among the Congo basin plantain populations using microsatellite markers to determine if there is a correlation between morphological diversity and genetic diversity among the three plantain cultivars.

MATERIALS AND METHODS

Study location

Samples were obtained from two plantain collections in the Congo basin at the University of Kisangani, located in the North-Eastern region of the DRC. The first collection is located at the Faculty of Sciences, shown as the faculty collection on the map, and the second collection is the intendance collection (Figure 1).

Plant

Three False Horn plantain cultivars, which differ only in their pseudostem color (Figure 2), were used in this study. The samples (20 per cultivar) were collected by punching banana leaf discs directly into 2 mL microcentrifuge tubes (Changadeya et al., 2012). The leaf samples were put in the ice box containing ice packs and were authorized by the University of Kisangani and the Institute of Agriculture for transportation and analysis at NaCCRI, Uganda. The samples were kept in the deep freezer at -80°C for better preservation and to prevent denaturation of DNA.

DNA isolation

The extraction of DNA was done using Cetyltrimethylammonium Bromide (CTAB) as described by Changadeya et al. (2012) with a few modifications. Leaf discs of about 100 mg each were ground using a mortar and pestle and then transferred to the 2.0 mL microcentrifuge tube. 400 µL of extraction buffer (1.5% CTAB), 100 mM Tris-HCl, 20 mM EDTA, 1.4 mM NaCl, and 0.2% ß-mercaptoethanol were added to the sample and then incubated at 65°C for 30 min, shaking after 10 min. An equal volume of Chloroform: Isoamyl alcohol (24:1) was added, and the homogenate was hand-mixed for 10 min at room temperature. The mixture was centrifuged at 12,000 rpm for 10 min. 300 µL of supernatant was transferred to a 2.0 mL microcentrifuge tube, and 210 µL of ice-cold isopropanol was added and incubated at -20°C for 1 h to precipitate DNA. The precipitated DNA was separated from the suspension by centrifugation at 13,000 rpm for 10 min; the supernatant was decanted, and the resultant pellet was washed in 400 µL of 70% cold ethanol to remove salt from the pellet and centrifuged for 10 min. The ethanol was decanted, and the DNA pellet was air dried for 10 to 15 min before dissolution in 100 µL nuclease-free water. The quantity and quality of the extracted DNA were determined using the Thermo Scientific Nanodrop 1000; DNA of good quality was defined as having an A260/A280 nm ratio between 1.8 and 2.0.

DNA amplification

To amplify the housekeeping actin gene in the extracted DNA, PCR was used as a check on the quality of the DNA extraction before using it in the upstream molecular analysis, including PCR
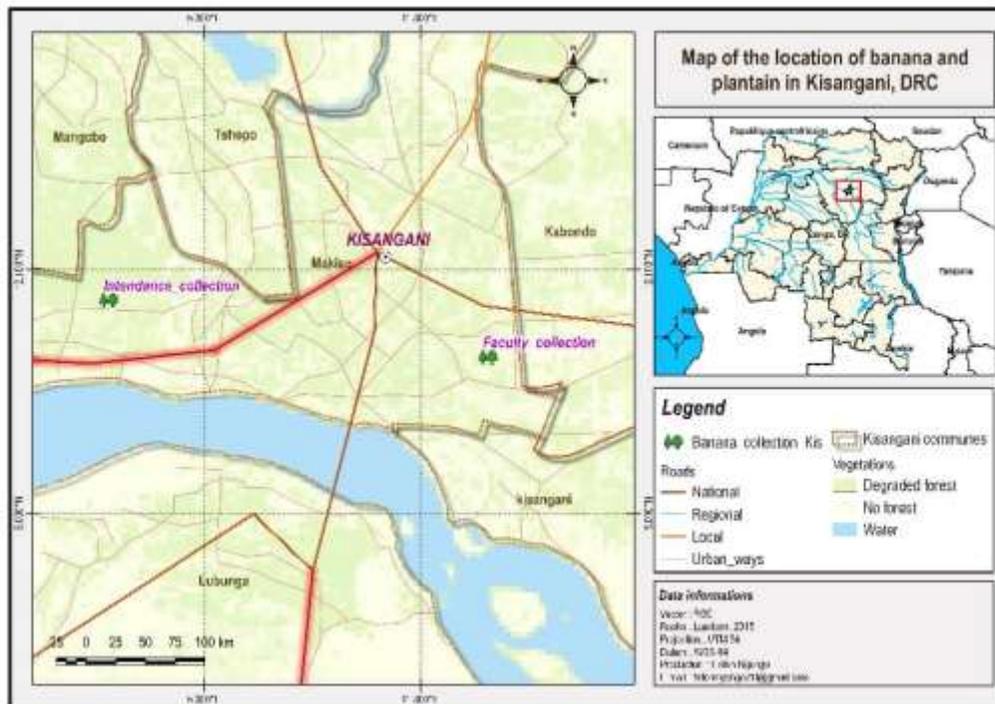
Figure 1. Map of the study area showing the banana collection sites including the Intendance collection and the Faculty of Sciences collection.
Sources: Fiston Ngongo Muselemu.

Libanga Likale (Green pseudostem)   Libanga Lifombo (Red pseudostem)   Libanga Black (Black pseudostem)
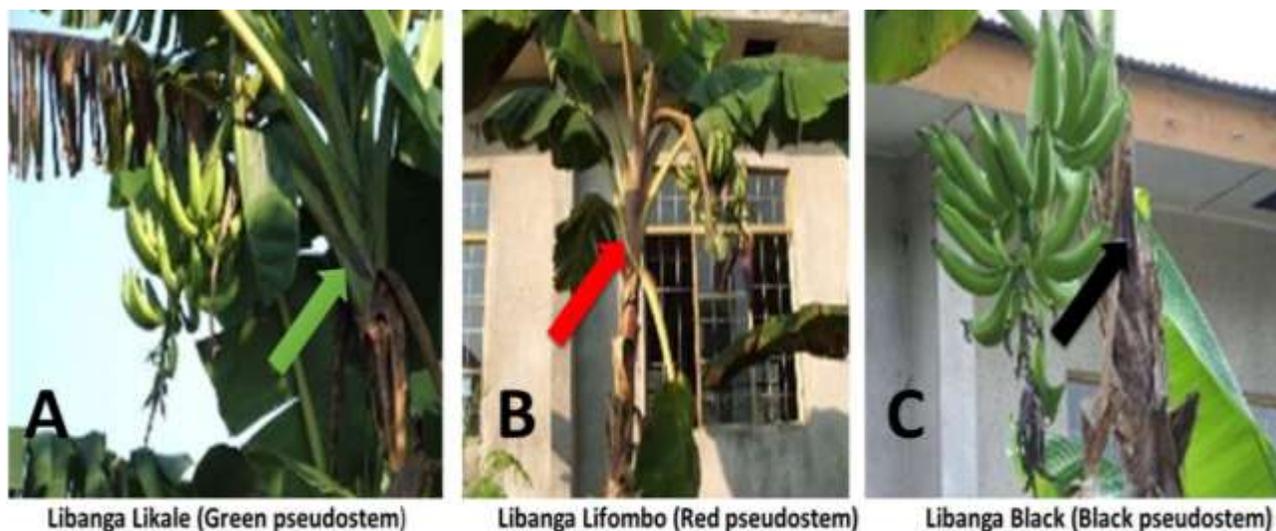
Figure 2. Plantain cultivars with different pseudostem colors used as biological material. (A) is the Libanga Likale with the green arrow pointing to the green pseudostem on the banana plant. (B) is the Libanga Lifombo with the red arrow pointing to the red pseudostem on the banana plant. (C) is the Libanga Black with the black arrow pointing to the black pseudostem on the banana plant. The three plantain cultivars are the most cultivated in the DRC.
Sources: Authors

amplification with SSR primers and capillary sequence analysis. Banana-specific actin gene primers forward, 5'ACCGAAGCCCCTCTTAACCC3' and reverse, 3'GTATGGCTGACACCATCACC5' reported in Tripathi et al. (2010) were used. The primers were manufactured by Macrogen (The Netherlands). The expected product size with the actin primers was

Table 1. Microsatellite primers used in the study.

Loci	Primes sequences	Expected product size	Ta (°C)
STMS1F/R	TGAGGCGGGGAATCGGTA GGCGGGAGACAGATGGAGTT	110-154	58
STMS7F/R	AAGAAGGCACGAGGGTAG CGAACCAAGTGAAATAGCG	234-252	54
STMS10F/R	ATGATCATGAGAGGAATATCT TCGCTCTAATCGGATTATCTC	116	53
AGMI93/94F/R	AACAACTAGGATGGTAATGTGTGGAA GATCTGAGGATGGTTCTGTTGGAGTG	124-158	57.5
Ma_1/16F/R	TTTGCCTGGTTGGGCTGA CCCCCCTTTCCTCTTTTGC	140-166	58.5
Ma_1/17F/R	AGGCGGGGAATCGGTAGA GGCGGGAGACAGATGGAGT	100-156	57.7
Ma_1/18F/R	TTTGCCTGGTTGGGCTGA CCCCCCTTTCCTCTTTTGC	138-182	57.5
Ma_1/24F/R	GAGCCCATTAAGCTGAACA CCGACAGTCAACATACAATACA	154-168	54
Ma_1/27F/R	TGAATCCCAAGTTTGGTCAAG CAAACACATGTCCCCATCTC	114-132	54
Ma_3/2F/R	GGAACAGGTGATCAAAGTGTGA TTGATCATGTGCCGCTACTG	204-238	56.2
Ma_3/90F/R	GCACGAAGAGGCATCAC GGCCAAATTTGATGGACT	128-162	53.2
Ma_3/103F/R	TCGCCTCTCTTTAGCTCTG TGTTGGAGGATCTGAGATTG	138-160	54

F-Forward; R- reverse; Ta- annealing temperature.
Sources: Authors

about 250 bp. A positive PCR with actin primers indicated good-quality DNA extraction and hence the DNA was subsequently used for molecular analysis using SSR primers. Polymerase Chain Reaction was carried out in the Thermocycler (BioRAD) in a 20 μL of final reaction volume that contained 10 μL One Taq Quick Load (New England Biolabs), 6 μL of Nuclease free water, 2 μL of genomic DNA and 1 μL of forward (10 μM) and reverse (10 μM) of banana actin primers. For each PCR, appropriate positive and negative controls were used. During the amplification process, denaturation at 94°C for 5 min was followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 1 min, and then 72°C for 1 min, followed by 7 min of extension at 72°C.

Twenty DNA samples chosen at random before labeling with fluorescent dyes were amplified using 12 different primer sets (Table 1) used by Changadeya et al. (2012) and manufactured by

Macrogen (Netherlands). Each SSR marker was amplified separately using the Thermocycler (Bio-Rad) by conducting PCR reactions. The total reaction mixture volume of 20 μL comprised 10 μL of One Taq Quick Load, 1 μL of forward (10 μM) and reverse (10 μM) SSR primers, 6 μL of Nuclease free water, and 2 μL of genomic DNA. The PCR reaction was carried out with the following conditions: denaturation at 94°C for 30 s, followed by 35 amplification cycles consisting of denaturing at 94°C for 30 s, annealing at an optimal temperature (which varied based on the melting temperature (Tm) of the primers used) for a specific primer pair for 15 s, and elongation at 72°C for 30 s (the elongation time varied depending on the expected product size of the PCR product). The final extension was performed at 65°C for 20 min, followed by a 4°C soaking temperature. The markers such as Ma_3/90, STMS1, STMS10, and Ma_1/18, and Ma_3/2 were not

Table 1. Dyes and primers selected for capillary sequencing.

Panel	Marker	Annealing temp. (°C)	Allele size range (bp)	Dye color
1	Ma_3/103FP	54	138-160	FAM
	STMSTFP	54	234-252	FAM
	AGM193/94FP	57.5	124-158	HEX
2	Ma_1/24FP	54	154-168	FAM
	Ma_1/27FP	54	114-132	HEX
3	Ma_1/17FP	57.7	100-156	FAM
	Ma_1/16FP	58.5	140-166	HEX

Sources: Authors

labeled since they did not display the expected level of variation.

Seven of the twelve microsatellite primer pairs were chosen because of their reported level of variation in the Musa cultivar (Changadeya et al., 2012). For the remaining polymorphic markers, the forward primer was labeled at the 5' end with either FAM (blue color) or HEX (green color) as indicated in Table 2. To avoid allele overlap, primers with similar product sizes were dyed in various colors (Masi et al., 2003). The labeled primers were then used in PCR. The total reaction volume of 20 µL PCR mix contained 3 µL DNA, 1 µL each of the labeled forward primer (10 µM) and reverse primer (10 µM), 5 µL of Nuclease Free Water, and 10 µL One Taq Quick-Load (New England Biolabs). The amplification consisted of an initial denaturation at 94°C for 35 s, followed by 35 cycles of denaturation at 89°C for 30 s, annealing for 30 s with temperature variations according to the primers used, and extension at 72°C for 30 s. A final extension phase was carried out at 72°C for 5 min, and the PCR products labeled with dye and expected to have the same fragment size were combined in the same PCR mixture to create a single panel, which was then separated by capillary electrophoresis.

The samples were then placed on 96 microplate wells and sent to Macrogen Europe (Netherlands) for capillary sequencing of the amplicons. Genemarker v2.6.3 Software was used to analyze and identify alleles of the resulting data. The process of allele sizing and identification followed the instructions in the user manual, and the alleles were manually scored as fragment sizes in base pairs (Christelová et al., 2011).

Statistical analysis

Analysis of genetic variation

Polymorphism Information Content (PIC) was calculated as described by Botstein et al. (1980) using the formula:

$$PIC = 1 - \sum_{i=1}^{n} Pi^2$$

Pi represents the frequency of the ith allele out of the total number of alleles at the Simple Sequence Repeats (SSR) locus, while n indicates the total number of different alleles present at that locus. Each SSR allele was treated as a separate marker, and the presence or absence of each PCR amplification product was recorded as either "1" or "0," respectively, resulting in a binary data matrix because determining the number of copies of individual alleles in polyploid species is difficult (Lian et al., 2003; Mengoni et al., 2000). The genetic diversity parameters within populations and between banana cultivar collections were estimated using GenAlex Version 6.503 Software (Peakall and Smouse, 2012). The diversity

was analyzed using these parameters: the number of alleles (Na), the number of effective alleles (Ne), gene diversity (h), Shannon's information index (I), and gene differentiation (Gst).

Analysis of cultivar similarity

The binary data matrix was generated and scored as either present (1) or absent (0) based on Nei's genetic distance to identify the genetic similarity among banana cultivars (Nei and Li, 1979). The Unweighted Pair Group Method Using Arithmetic Mean (UPGMA) cluster analysis was used to process similarity data using the R-3.6.1 Software Agnes package. A Principal Component Analysis (PCA) based on the SSR frequency was carried out to further evaluate the genetic linkages of plantain accessions as individual plants.

Structure analysis

The genetic structure of three cultivars was performed using the Bayesian model-based classification implemented in the STRUCTURE v2.3.4 software (Hilal et al., 2014). Using the mixing model, the value of K ranged from 1 to 10, with 100,000 MCMC, twenty iterations, and a break-in period of 200,000 (Workneh et al., 2022). The best probable K value was determined using the web-based structure harvester version 0.6.92 (Earl and Von Holdt, 2012).

Genetic differentiation and Analysis of Molecular Variance (AMOVA)

AMOVA was performed to compare differences within and between populations. The statistical significance of the AMOVA was assessed using a non-parametric permutation approach with 99 permutations. The degree of genetic differentiation among sub-populations was evaluated based on Gst values. Additionally, unbiased genetic distance (Nei, 1978) was used to calculate pairwise genetic distances.

RESULTS

Gene diversity and polymorphism

The genetic diversity of plantains was assessed using seven SSR markers, all of which showed variation, with a

Table 3. Number of alleles observed in the different populations.

Marker	Ma_1/103		STMS7		AGM93/94		Ma_1/24		Ma_1/27		Ma_1/16		Ma_1/17		Total
Population	TNA	ASR	TNA	ASR	TNA	ASR	TNA	ASR	TNA	ASR	TNA	ASR	TNA	ASR	
Libanga Likale	3	147-159	6	234-249	7	137-151	3	160-167	7	116-122	5	154-164	8	117-134	39
Libanga Noir	6	147-157	7	234-248	9	137-152	6	160-168	8	116-123	5	154-165	7	117-128	48
Libanga Lifombo	3	147-152	6	234-244	9	137-153	4	161-167	6	116-124	6	154-166	8	117-124	42
Total	12	-	19	-	25	-	13	-	21	-	16	-	23	-	129
PIC	-	0.67	-	0.97	-	0.85	-	0.85	-	0.95	-	0.7	-	0.72	-

TNA:Total number of alleles; ASR: allele size range; PIC: polymorphism information content.
Sources: Authors

total of 129 alleles detected across the loci, averaging 18.4 alleles per locus. The polymorphism information content (PIC) of the microsatellite primers ranged from 0.68 to 0.97, averaging 0.82 (Table 3.) The number of alleles observed ranged from 6.66 to 16.66, with an average of 11.61, while the number of valid alleles ranged from 6.31 to 14.64, with an average of 10.74. The average gene diversity (h) from Nei (1973) ranged from 0.28 to 2.72, with an average of 1.38 for the loci that produced polymorphic bands in 60 psyllium. This finding was further supported by Shannon's information index, with locus AGMI93/94 having the highest value of 4.11, while the lowest value of 0.57 was observed at locus Ma_1/103 (Table 4).

Similarity coefficient among the different plantain cultivars

Jaccard similarity coefficients based on seven SSR markers were used to assess the genetic similarities of all plantain genotypes under study. Among the sixty plantain genotypes, the similarity coefficient ranged from 0.35 to 0.94, with an average of 0.82. This finding suggests that the

plantain accessions cultivated in the Congo basin have a high level of genetic diversity.

Genetic relationships among the plantain genotypes

A dendrogram was created based on the genetic similarities of the genotypes, and it showed that the three populations formed three distinct clusters (Figure 3). Observations in Cluster 1 are closer to those in Cluster 2 than those in Cluster 3. Sample 1 to sample 16 in population 1 is more similar to each other. PCA did not reveal any significant clustering among the 60 populations. The first component comprised 7.76% and the second principal component 10.15% of the total variation (Figure 4).

Genetic differentiation and AMOVA

AMOVA indicated that almost all of the genetic diversity was attributable to variation within individual populations (98%), while only a small proportion (2%) of the genetic variation was found among the three populations (green, black, and

red) (Table 5). The statistical analysis (PhiPT= 0.019, P = 0.060) revealed no significant genetic differentiation between the plantain populations. The statistics on population structure and heterozygosity showed that the Gst values were estimated to be 43% of the total population differentiation. Using the model-based Bayesian algorithm, it was possible to identify six distinct clusters (k = 6). The Nei genetic distance pairwise population matrix revealed that the three accessions were closely related to each other, with values of genetic distance ranging from 0.014 to 0.016. The value of total heterozygosity (Ht) and gene diversity of individual relatives to their population (Hs) was high in all populations (1.43 and 1.40), respectively.

DISCUSSION

The results obtained in this work are similar to those of other work done in this direction. However, it should be noted that the discriminatory power of SSRs of the cultivars studied was not effective on Agarose compared to a study done by Dowiya et al. (2018) and on polyacrylamide gel

Table 4. The average genetic variability for plantain populations at 7 microsatellite loci.

Locus	Na	Ne	I	h
Ma_1/103	6.66	6.31	0.57	0.28
STMS7	12	12.1	2.23	1.36
AGMI93/94	16.66	14.64	4.11	2.72
Ma_1/24	7	8.02	1.15	0.69
Ma_1/27	14	12.03	3	1.9
Ma_1/16	10	9.73	1.85	1.15
Ma_1/17	15	12.39	2.65	1.6
Mean	11.61	10.74	2.22	1.38
SD	3.89	2.87	1.18	0.80

Sources: Authors
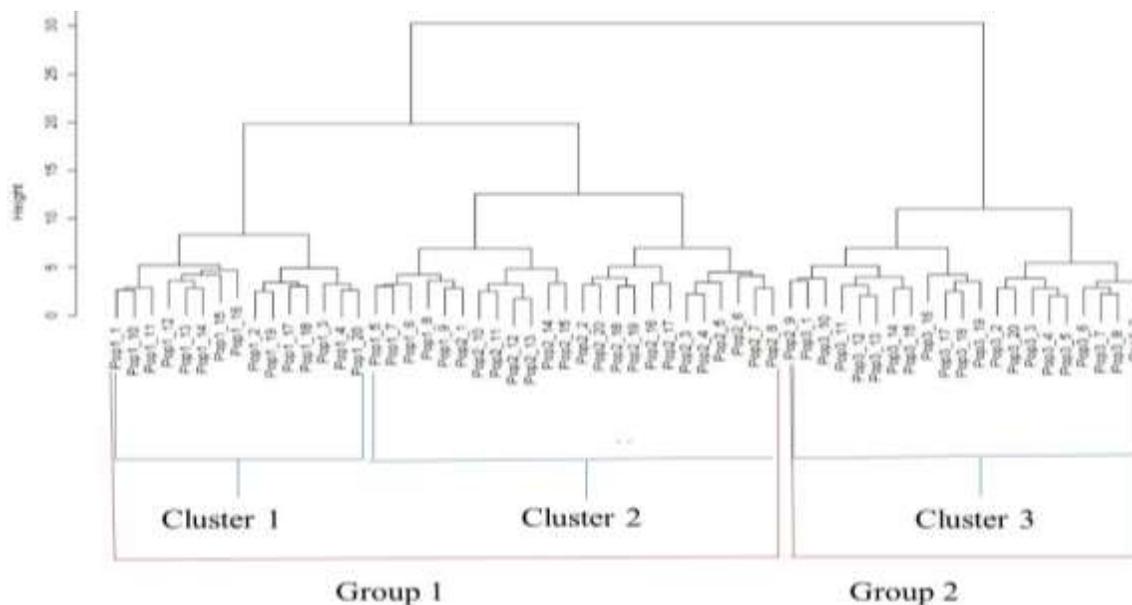
Figure 3. The dendrogram of genetic relationship among the three plantains cultivars generated by UPGMA. Cluster 1 represents a population of Libanga Likale with green pseudostem. Cluster 2: Population of Libanga Black with black pseudostem and Cluster 3: Population of Libanga Lifombo with red pseudostem. Samples 5, 6, 7, 8, and 9 of Population 1 crossed to Population 2, and sample 9 of Population 2 crossed to Population 3.
Sources: Authors

electrophoresis compared to a study done by Changadeya et al. (2012). This is due to the smaller difference in base pairs observed among the amplified fragments. Capillary electrophoresis is so far the best technique of fragment analysis with smaller base pair differences. Among the three accessions, Libanga Black provided a high number of alleles with high polymorphic information compared to Libanga Likale and Libanga Lifombo.

This study agrees with the findings of earlier research on Musa germplasm that employed SSrs, indicating a significant level of polymorphism. Christelová et al.

(2011) analyzed 38 triploid accessions of plantains from the International Transit Center (ITC) in Leuven (Belgium) using 19 microsatellite primers and obtained 267 alleles in total with an average PIC of 0.85. Changadeya et al. (2012) tested 12 SSR primers to assess genetic diversity and relationship in 141 banana cultivars growing in Malawi and obtained a high allelic diversity (174 alleles) and the discriminatory power of the SSR primer used was high with a mean PIC of 0.74. Workneh et al. (2022) in Ethiopia characterized 96 banana genotypes and obtained PIC averaged 0.82. The dissimilarities in the number and nature of polymorphic markers utilized, the
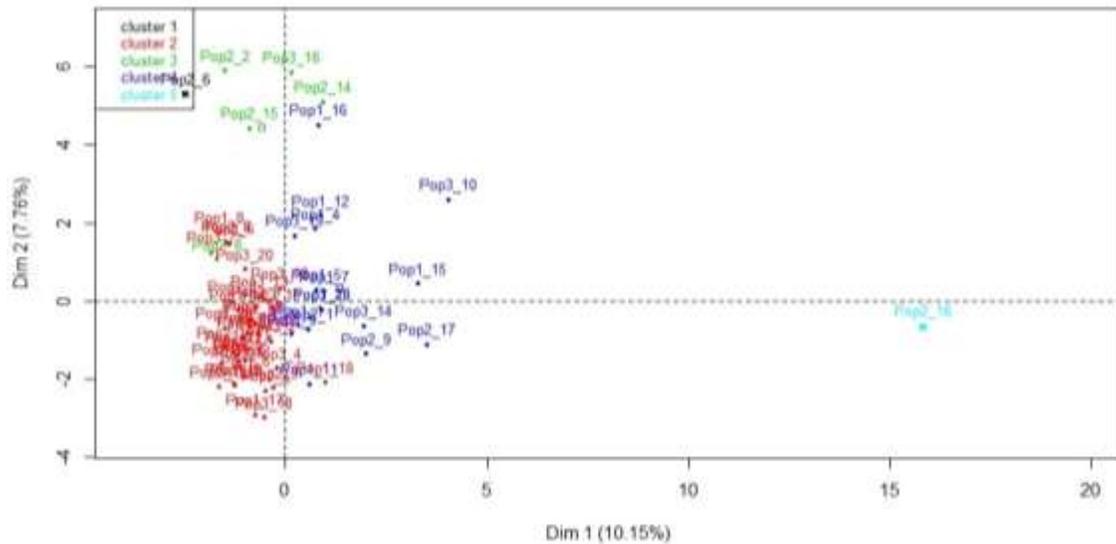
Figure 4. Principal component analysis (PCA) of populations of plantain cultivars.
Sources: Authors

Table 5. Genetic variance among and within the population of plantains.

Source	df	SS	MS	Est. Var.	%	Stat	Value	P-value
Among populations	2	14.417	7.208	0.102	2	PhiPT	0.019	0.060
Within populations	57	294.750	5.171	5.171	98			
Total	59	309.167	-	5.273	100			

Df= Degree of freedom, SS=sum of squares, MS=mean square.
Sources: Authors

sample sizes, the collection locations, and the geographical sources of the genotypes are plausible reasons for the substantial differences in the alleles found in this study versus those in previous studies on Musa germplasm (Gyang et al., 2017). The greater degree of polymorphism detected in this study relative to the research conducted by Changadeya et al. (2012) could be a consequence of the improved comprehensiveness of the plantain cultivar variation present in the Congo Basin and a higher resolution provided by capillary electrophoresis. The seven SSR markers in this research made it possible to differentiate between distinct genotypes. Studies have shown that SSR loci can cause large segregation between closely related individuals, even when only a few loci are used (Powell et al., 1996). According to Al-Badeiry et al. (2014), Polymorphic Information Content (PIC) is indicative of the strength of SSR loci and their ability to identify genetic distinctions between breeds. Hayden et al. (2010) classified PIC values into three categories: low information content (PIC < 0.25), moderate information content (0.25 ≤ PIC ≤ 0.5), and high information content (PIC > 0.5).

Shannon information index (I) and the Nei genetic diversity (h) values obtained in this study showed a significant degree of genetic diversity. This is consistent with findings from Quain et al. (2018), who also reported high genetic diversity in 40 Ghanaian plantain accessions (with a Nei genetic diversity of 0.61 and a Shannon information index of 0.41). By analyzing the genetic variation and structure of plantain (*Musa sapientum* L.) landraces from Côte d'Ivoire using Microsatellite markers, Kouamé et al. (2019) obtained a high genetic diversity (I = 0.726 0.023 and He = 0.506 0.008). The significant genetic diversity present in the collection is expected to enhance the effectiveness of selecting accessions for DRC plantain management and breeding initiatives.

The study found that the genetic variation, as determined by AMOVA, did not show statistical significance in differentiating strains based on the color of their pseudostems. Only 2% of the total genetic variation was attributed to differences between populations. Therefore, the primary source of genetic variation observed in the study was within the population itself. Nei's genetic identity analysis confirmed that the three populations had a close relationship. This finding is consistent with previous reports of a close relationship

among diploid banana cultivars in the *M. acuminata* group, including those by Changadeya et al. (2012), Kitavi (2015) for East African highland banana cultivars, and Quain et al. (2018) for forty plantain genotypes collected in Ghana. These results support the hypothesis proposed by several authors (De Langhe et al., 2005; Noyer et al., 2005) of a common origin of the plantain landraces.

Noyer et al. (2005), showed that all plantain cultivars originated from a single genotype, which underwent epigenetic mutations during successive vegetative propagations in the domestication process. The present study revealed high genetic variation within the plantain genotypes used but little differentiation among populations. This differentiation value was higher than the average for crop species (Gst = 0.34) (Oriero et al., 2006) and the Brazilian diploid and triploid banana accessions using SSR data (Rst=0.105) (Creste et al., 2004). The average differentiation value found by Changadeya et al. (2012) for *Musa* L cultivars was lower (0.14) than in this study. However, the differentiation value was lower than in studies of wild *Musa balbisiana* Colla using cpDNA PCR-RFLP (Gst = 0.77) (Ge et al., 2005). Domestication reduced genetic diversity in banana species due to human interventions and the artificial selection of preferred cultivars, leading to the disappearance of genes over time (Changadeya et al., 2012). Banana is a polyploid. The three selected plantain cultivars used in this study are triploid, but the molecular data was converted to binary and analyzed as haploid. This led to a loss of some information. Molecular analytical platforms for polyploidy species are still under development and currently unavailable. That was the limitation of the study.

Conclusion

There is significant genetic variability in plantain genotypes grown in the Congo basin, the variation of which remains to be confirmed in a larger number of samples considering different regions when collecting samples. The continuation of this study by analyzing other cultivars collected in distant regions and a follow-up study with tools such as RFLP, SNPs, and AFLP be used to verify the findings are recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Al-Badeiry NAH, Al-Saadi AH, Merza TK (2014). Analysis of genetic diversity in maize (*Zea mays* L.) varieties using simple sequence repeat (SSR) markers. Journal of University of Babylon, Pure and Applied Sciences 22(6):1768-1774.

Adheka J (2016). Contribution to the characterization and classification of the Congo basin African plantains (Musa AAB) in the Democratic Republic of Congo. Ph.D. thesis, University of Kisangani.

Adheka J, Dhed'a DB, Karamura D, Blomme G, Swennen R, De Langhe E (2018). The morphological diversity of plantain in the Democratic Republic of Congo. Scientia Horticulturae 234:126-133. 10.1016/j.scienta.2018.02.034.

Barkley NA, Krueger RR, Frederici CT, Roose ML (2009). What phylogeny and gene genealogy analyses reveal about homoplasy in citrus microsatellite alleles. Plant Systematics and Evolution 282:71-86. DOI 10.1007/s00606-009-0208-2.

Blazakis KN, Kosma M, Kostelenos G, Baldoni L, Bufacchi M, Kalaitzis P (2017). Description of olive morphological parameters by using open access software. Plant Methods 13(1):111.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32(3):314-331.

Changadeya W, Kaunda E, Ambali, AJD (2012). Molecular characterization of Musa L. cultivars cultivated in Malawi using microsatellite markers. African Journal of Biotechnology 11(18):4140-4157. https://doi.org/10.5897/AJB10.1738.

Creste S, Tulmann NA, Vencovsky R, de Oliveira SS, Figueira A (2004). Genetic diversity of Musa diploid and triploid accessions from the Brazilian banana breeding program estimated by microsatellite markers. Genetic Resources and Crop Evolution 51:723-733.

Christelová P, Valárik M, Hřibová E, Van den Houwe I, Channelière S, Roux N, Doležel J (2011). A platform for efficient genotyping in Musa using microsatellite markers. AoB Plants 2011:plr024. doi: 10.1093/aobpla/plr024.

De Langhe E, Pillay M, Tenkouano A, Swennen R (2005). Integrating morphological and molecular taxonomy in Musa: The African plantains (Musa spp. AAB group). Plant Systematics and Evolution 255:225-236. 10.1007/s00606-005-0346-0.

Earl DA, von Holdt BM (2012). Structure Harvester: A website and program for visualizing structure output and implementing the Evanno method. Conservation Genetics Resources 4(2):359-361.

Earl DA, von Holdt BM (2012). Structure Harvester: A website and program for visualizing structure output and implementing the Evanno method. Conservation Genetics Resources 4(2):359-361.

Ge XJ, Liu MH, Wang WK, Schaal BA, Chiang TY (2005). Population structure of wild bananas, Musa balbisiana, in China determined by SSR fingerprinting and cpDNA PCR-RFLP. Molecular Ecology 14(4):933-944. https://doi.org/10.1111/j.1365-294X.2005.02467.x

Gyang PJ, Nyaboga E, Muge EK (2017). Molecular Characterization of Common Bean (*Phaseolus vulgaris* L.) Genotypes Using Microsatellite Markers. Journal of Advances in Biology and Biotechnology 13(2):1-15. https://doi.org/10.9734/jabb/2017/33519

Hayden MJ, Tabone TL, Nguyen TM, Coventry S, Keiper FJ, Fox RL, Chalmers KJ, Mather DE, Eglinton JK (2010). An informative set of SNP markers for molecular characterization of Australian barley germplasm. Crop and Pasture Science 61(1):70-83. https://doi.org/10.1071/CP0914

Hilal AQ, Pinnamaneni SR, Akanksha K, Penna S, Sujata B (2014). Alterations in stem sugar content and metabolism in sorghum genotypes subjected to drought stress. Functional Plant Biology 41(9):954-962 https://doi.org/10.1071/FP13299

Hippolyte I, Jenny C, Gardes L, Bakry F, Rivallan R, Pomies V, Cubry P, Tomekpe K, Risterucci AM, Roux N, Rouard M, Arnaud E, Kolesnikova-Allen M, Perrier X (2012). Foundation characteristics of edible Musa triploids revealed from allelic distribution of SSR

markers. Annals of Botany 109(5):937-951. https://doi.org/10.1093/aob/mcs010.

Kasaka DL, Onautshu O, Muliwambene K, Lebisabo BC, Baert G, Swennen R, Haesaert G, Dhed'a DB (2021). Importance and diversity of Mycorrhizae under plantain cultivation in the slash-and-burn and non-burn cropping systems in the forest region of Kisangani, Tshopo Province, D.R. Congo. African Journal of Agricultural Research 17:809-821. 10.5897/AJAR2020.15411.

Kitavi M (2015). Genetic diversity, evolutionary history and epigenetic analysis of East African Highland Bananas. PhD Thesis. National University of Ireland Galway.

Kouamé K, Cyrille G, Désiré PN, Sylvère SR, Deless T, Fulgence E (2019). Genetic diversity and structure of plantain (*Musa sapientum* L.) landraces from Côte d'Ivoire using SSR markers Genetic diversity and structure of plantain (*Musa sapientum* L.) landraces from Côte d'Ivoire using SSR markers. European Journal of Biotechnology and Bioscience 7(1):36-43.

Lian C, Oishi R, Miyashita N, Nara K, Nakaya H, Wu B, Zhou Z, Hogetsu T (2003). Genetic structure and reproduction dynamics of Salix rein during primary succession on Mount Fuji, as revealed by nuclear and chloroplast microsatellite analysis. Molecular Ecology 12(3):609-618. doi:10.1046/j.1365-294X.2003.01756.x

Lusty C, Akyeampong E, Dvey MW, Newilah NG, Markham R (2006). A staple food with nutritious appeal. InfoMusa 15(1/2):39-43.

Masi P, Spagnoletti Zeuli PL, Donini P (2003). Development and analysis of multiplex microsatellite markers sets in common bean (*Phaseolus vulgaris* L.). Molecular Breeding 11(4):303-313. https://doi.org/10.1023/A:1023443109985.

Mengoni A, Gori A, Bazzicalupo M (2000). Use of RAPD and microsatellite (SSR) variation to assess genetic relationship among populations of tetraploid alfafa, *Medicago sativa*. Plant Breeding 119(4):311-317. https://doi.org/10.1046/j.1439-0523.2000.00501.x

National Institute of Statistics (NIS) (2020). DRC Statistical Yearbook. Ministry of Planning, DRC P 398.

Nei M (1973). The Theory and Estimation of Genetic Distance. In N. E. Morton (Ed.), Genetic Structure of Populations (pp. 45-54). Honolulu, HI: University of Hawaii Press.

Nei M (1978). Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics 89(3):583-590.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76(10):5269-5273. doi:10.1073/pnas.76.10.5269.

Noyer JL, Causse S, Tomekpe K, Bouet A, Baurens FC (2005). A new image of plantain diversity assessed by SSR, AFLP and MSAP markers. Genetica 124(1):61-9. doi: 10.1007/s10709-004-7319-z. PMID: 16011003.

Onautshu O (2013). Characterization of Mycosphaerella fijiensis populations and epidemiology of black Sigatoka of banana Kisangani (DRC). PhD thesis, The University of Toulouse.

Oriero CE, Odunola OA, Lokko Y, Ingelbrecht I (2006). Analysis of B-genome derived simple sequences repeat (SSR) markers in Musa spp. African Journal of Biotechnology 5(2):126-128.

Peakall R, Smouse PE (2012). GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research- An Update. Bioinformatics 28:2537-2539. http://dx.doi.org/10.1093/bioinformatics/bts460

Perrier X, De Langhe E, Donohue M, Lentfer C, Vrydaghs L, Bakry F, Carreel F, Hippolyte I, Horry JP, Jenny C, Lebot V, Risterucci AM, Tomekpe K, Doutrelepont H, Ball T, Manwaring J, de Maret P, Denham T (2011). Multidisciplinary perspectives on banana (Musa spp.) domestication. Proceedings of the National Academy of Sciences USA 108(28):11311-11318. doi: 10.1073/pnas.1102001108.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2(3):225-238. https://doi.org/10.1007/BF00564200

Quain MD, Adelaide A, Dzomoku BM (2018). Assessment of plantain (*Musa sapientum* L.) accessions genotypic groups relatedness using simple sequence repeats markers. African Journal of Biotechnology 17(16):541-551.https://doi.org/10.5897/ajb2017.16363.

Rana MK (2018). Molecular Characterization of Plant Genetic Resource. Division of Genomic Resources, ICAR- NBPGR, New Delhi.

Senan S, Kizhakayil D, Sasikumar B Sheeja TE (2014). Methods for Development of Microsatellite Markers: An Overview. Notulae Scientia Biologicae 6(1):1-13.

Tenkouano A, Vuylsteke D, Swennen R (2007). Sink Competition and Desuckering Effects on Field Performance of Triploid and Tetraploid Plantain Genotypes. Journal of Crop Improvement 20(1-2):31-51.

Tripathi L, Mwaka H, Tripathi JN, Tushemereirwe WK (2010). Expression of sweet pepper Hrap gene in banana enhances resistance to Xanthomonas campestris pv. musacearum. Molecular Plant Pathology 11(6):721-731. https://doi.org/10.1111/j.1364-3703.2010.00639.x

Workneh S, Alemu S, Olani G, Debebe A, Berhanu B, Dagnew MA, Wagayehu A (2022). Molecular characterization of banana genotypes by SSR markers. African Journal of Plant Science 16(9):258-269. 10.5897/AJPS2022.2268.