European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 07-11-2018; Accepted: 09-12-2018
www.biosciencejournals.com
Volume 7; Issue 1; January 2019; Page No. 36-43

Genetic diversity and structure of plantain (*Musa sapientum* L.) landraces from Côte d'Ivoire using SSR markers

Koffi Kouamé Germain Cyrille[1], Pokou N'da Désiré[2], Sie Raoul Sylvère[3], Thiémélé Deless Edmond Fulgence[4]
[1, 2, 4] Laboratoire Central de Biotechnologies (LCB), Centre National de Recherche Agronomique (CNRA), Abidjan, Côte-d'Ivoire.
[3] UFR Science de la nature, Université Nangui Abrogoua (UNA), Laboratoire de Biologie et Amélioration des Productions Végétales, Abidjan, Côte d'Ivoire

Abstract
Plantains are a key crop for food security in Africa, particularly in the humid regions of Central and West Africa, where they are a major staple food. Plantain collection of the Centre National de Recherche Agronomique (CNRA) in Côte d'Ivoire contains about 42 accessions, who was little genetically characterized. The objective of this work was therefore to perform molecular characterization of this collection and assess relationship among known genotypic groups (populations) using Simple Sequence Repeat (SSR) loci. The microsatellite profiles of 42 plantain genotypes, were detected a total of 14 alleles, with an average of 2.33 alleles per locus. A high level of genetic variation was obtained within accessions, with the Shannon diversity index (*I*) and the Nei genetic diversity index (*He*) respectively of $I = 0.726 \pm 0.023$ and $He = 0.506 \pm 0.008$. The mean values of observed heterozygosity ($Ho = 0.897 \pm 0.025$) were higher than the expected mean heterozygosity values ($He = 0.506 \pm 0.008$), which confirms plantain's highly heterozygous nature. The study also revealed that the SSR loci used, were not able to split the plantains into their known distinct morpho groups based on inflorescence characteristics (French plantain, True Horn and False Horn). The result of this work will contribute to the conservation strategies.

Keywords: genetic diversity, germplasm, genotype, microsatellite (SSR), plantain

1. Introduction
Banana (*Musa* spp.) is one of the most important fruit crops grown in the tropical and subtropical regions of the world. The major edible species are in the section *Eumusa* of the genus *Musa*, and originated from inter and intra-specific hybridizations between two wild diploid species, *Musa accuminata* Colla (donor of A genome) and *Musa balbisiana* (donor of B genome) (Simmonds & Shepherd, 1955) [1]. The most economically important cultivated types used by farmers worldwide are triploids (2n = 3x = 33 chromosomes) and divided into three major groups: AAA (Cavendish or dessert bananas), ABB (cooking bananas) and AAB (plantain). The plantain subgroup (AAB) is mostly grown by small farmers for local consumption and is of considerable importance to the agriculture of tropical humid forest regions in Africa, Central and South America, and Asia (Noyer *et al.*, 2005) [2]. It is indigenous to South East Asia but are so predominant in the humid lowlands of west and central Africa so that this region is now considered as the secondary centre of diversification (Simmonds & Sheppard, 1955) [1]. These two regions produces more than 70% of African production (14.5 million tonnes) (FAO, 2017) [3]. Plantain is very appreciated for organoleptic, cooking and agronomic qualities. It is also a major source of income for many people and actors in the supply chain in the rural and urban sectors (Jacobsen *et al.*, 2004) [4].
In Côte d'Ivoire, plantain has an important role in both the diet of populations and cropping systems, where it serves as shading for the establishment of perennial crops such as cocoa and coffee. Plantain is the third food crop after yam and cassava, with an annual production estimated at 1.6 million tonnes (FAO, 2017) [3]. This production is mainly use to cover domestic demand. However, the production is affected by an array of diseases among which, the black sigatoka disease. Attempts to deal with these problems have led to the development of new hybrid varieties through breeding programs to creat line with resistance or tolerance. These hybrids have been introduced into some of the West and Central African countries (Quain *et al.*, 2018) [5]. Some of the improved high-yielding and Sigatoka-tolerant varieties such as PITA 3 and FHIA 21 are being released in Côte d'Ivoire from 2005 onward (Traore *et al.*, 2008) [6]. However, they are still marginal because of the cost of plant material, taste and sensory quality that which is not so much appreciate by consumers (Perrin, 2015) [7]. Thus, the best local genotypes could be selected in breeding programs to develop hybrids adapted to local ecological conditions and consumer preference. In this context, the plantain collection of the Centre National de Recherche Agronomique (CNRA) in Côte d'Ivoire has been enlarged by introduction of several plantain cultivars collected on farm in plantain production areas in Côte d'Ivoire. An insight of genetic make-up may contribute to information, vital for both breeders and consumers. Several methods have been used to assess the genetic variability in *Musa* germplasm (Ortiz, 1997; Noyer *et al.*, 2005; Resmi *et al.*, 2011; Thiémélé *et al.*, 2017) [8, 2, 9, 10]. However, the technologies based on molecular markers provide the best tools that are able to reveal polymorphism at the DNA sequence level, which are adequate to detect genetic variability between individuals and within the populations (Kresovich *et al.*, 1995) [11].
Recently, several molecular markers have been used for plantain germplasm management (Ude *et al.*, 2003; De Langhe *et al.*, 2005; Noyer *et al.*, 2005; Thiémélé *et al.*, 2017)

[12, 13, 2, 10]. Thus, using SSR and AFLP markers, Noyer *et al.* (2005) [2] studied the genetic diversity of 30 plantains constituting a representative sample of the phenotypic diversity from the CARBAP collection. They found a very narrow genetic base of this cultivars group. Out of 9 loci SSR used, only one polymorphic locus was observed. With the AFLP marker, on 633 bands provided by 8 primer pairs, only one methylation insensitive polymorphism level appears (0.2%) and all plantains are grouped into two distinct clusters. On the contrary, Quain *et al.* (2018) [5] revealed a high genetic diversity between 40 plantain accessions collected in Ghana using 17 SSR loci are well distributed within the *Musa* genome. However, this study could not discriminate the plantain populations (French plantain, True Horn and False Horn) constituted by considering morphological characteristics of inflorescence.

Microsatellites or simple sequence repeats (SSRs) are among the most used molecular markers to characterize and evaluate the genetic variability of the *Musa* genus. They have specific advantages, including ease of use once the primer giving birth were defined, their high polymorphism; their high reproducibility, co-dominance, multi-allelic, easy to interpret and polymerase chain reaction (PCR) amplification (Crouch *et al.*, 1999) [14].

The objectives of this study was (i) to estimate the genetic variation within and among populations of Côte d'Ivoire plantain collection using SSR markers and (ii) to establish the genetic relationships.

2. Material and methods

2.1 Plant material

Forty two (42) plantain landraces maintained at the germplasm collection of the Centre National de Recherche Agronomique (CNRA) in Azaguié, Côte d'Ivoire, representing the phenotypic diversity was used in this study (Table 1). The accessions are composed of three inflorescence types, including 17 French, seven (7) True Horn, 18 False Horn and one (1) diploid cultivars. The plantain "True Horn" is characterized by the absence of male flowers at maturity and the presence of very large horn-like fruits. While 'French' cultivar present a persistence of male flowers at maturity and smaller fruit size and the "False Horn" cultivar characterized by a restricted number of neutral flowers but without any male bud at maturity (Figure 1).

2.2 Genomic DNA isolation

Genomic DNA of each genotype was isolated from the cigar leaf (youngest unfurled leaf) using the ZR Plant/Seed DNA Mini Prep (Zymo Reseach, USA) according to the manufacturer's instructions. DNA was quantified and assessed for purity using a NanoDrop 2000 spectrophotometer (Thermo Scientifc, USA). DNA concentration was determined by absorbance at 260 nm and DNA purity was estimated by the 260:280 ratio and the 260:230 ratio.

2.3 Microsatellite analysis

Twenty eight Expressed Sequence Tag–Simple Sequence Repeat (EST-SSR) markers developed by Mbandjo *et al.* (2012) [15], were used to screen the 42 plantain genotypes. The SSR patterns of each individual were analyzed following the protocol of Quain *et al.* (2018) [5], as applied with the automated infrared fluorescence technology of a sequencer LICOR 4300 (LICOR, USA). The 28 SSR loci were amplified using specific primers that were adjusted with an extension M13 sequence at 5'-end (5' CACGACGTTGTAAAACGAC-3') of the forward primer (Christelova *et al.*, 2011; Quain *et al.*, 2018) [16, 5].. The PCR amplification was performed in a GeneAmp System 9700 thermocycler (Applied Biosystem, Foster City, California, USA) in a total volume of 20 µl consisting 10 ng of genomic DNA, 5 µl of master Mix ''One Taq Quick Load 2X Master Mix with standart buffer'' (New England BioLabs, USA), 0.1 mM of the M13-labelled primer, 0.1 mM of the other primer, 5% v/v DMSO and 0.1 µM of M13 primer-fluorescent dye IR700 or IR800 (Biosynthesis, Lewisville, Texas, USA). The amplifications were carried out with the following PCR conditions: denaturation step at 94°C for 3 min, followed by 35 amplification cycles of denaturing at 94°C for 45 s, annealing at 58 °C for 1 min and elongation at 68°C for 1 min. The final extension was at 68°C for 20 min followed by a soaking temperature of 4°C. PCR products were separate by electrophoresis in 6.5 % Long Ranger polymer gels (Lonza, Houston, Texas, USA) with a Licor 4300 DNA Analyser System.

2.4 Data analysis

The following parameters were computed to analyse the diversity within and between plantain cultivar accessions: number of alleles (*Na*), mean number of alleles per locus (*A*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Shannon's information index (*I*) and inbreeding coefficient (F_{IS}) across loci per populations. The software GenAlEx 6.5 (Peakall & Smouse, 2012) [17] was employed to compute those measurement. Then, Polymorphic Information Content (PIC) was calculated to evaluate the informativeness of each marker using Power Marker (version 3.25) (Liu & Muse, 2005) [18].

To analyse the genetic structure of different accessions, the Analysis of Molecular Variance (AMOVA) was performed to compare within and between population variations. Significance of AMOVA was tested using a non-parametric permutation approach with 999 permutations. Genetic differentiation were determine by Fst values among sub-populations. Pairwise genetic distances were computed using unbiased genetic distance (Nei, 1978) [19]. In order to describe the structuring, Principal Coordinates Analysis (PCoA), based on the pairwise distance matrix. The software GenAlEx 6.5 were used to perfume these parameters. To refine the genetic structure, a dendrogram was produced using the unweighted neighbor-joining method based on genetic dissimilarity among the 42 plantain accessions by Darwin software, version 6.0.12 (Perrier & Jacquemoud-Collet, 2006) [20].

3. Results

3.1. SSR and genetic diversity analyses

Among the 28 SSRs markers used in this study, only 6 representing 21.45% presented polymorphic profils that can be used for subsequent analysis to assess diversity of 42 genotypes of plantain (Table 2). A total of 14 alleles were identified at the six (6) loci, with an average of 2.33 alleles per locus (Table 3). The highest polymorphism value of, with three (3) alleles found, was observed at loci *DN239472* and *Ma513026332*. Among the six (6) SSR markers considered, only *DN239472* locus produced one (1) unique or rare alleles belonging to in the French populations (Table 3). The discriminatory power of each locus was evaluated by

estimating the Polymorphic Information Content (PIC). Its average value was relatively low (0,468 ± 0.071), with a maximum of 0.593 for the *Ma513026332* locus and a minimum of 0.375 observed with *DN240063* locus.

Genetic diversity parameters are presented in Table 4. Observed heterozygosity (*Ho*) values ranged from 0.647 to 1.000 with an average of 0.897 ± 0.025, whereas the average expected heterozygosity (*He*) (genetic diversity) was 0.506 ± 0.008 ranging from 0.459 (Ma513036168) to 0.612 (Ma513026332) in the True Horn population. The Shannon information index (*I*) showed a high diversity across the accessions ranging from 0.652 to 1.000 in True Horn cultivars, with a mean of 0.726± 0.023. The inbreeding coefficient (F_{IS}) value was negative for the 42 genotypes analysed (-0.776 ± 0.050), indicating an excess of heterozygosity.

3.2. Genetic differentiation

The analysis of the molecular variance (AMOVA) showed no genetic differentiation between the three populations of plantain (PhiPT = 0.011, P = 0.267), only 1% is due to variation among populations, whereas 99 % of the observed variation is due to genetic variation among individuals within each population (Table 5 and Figure 2). The pairwise population of F_{ST} values showed that there was a low degree of differentiation among populations (Table 6). The pairwise population matrix of Nei genetic distance indicated that accessions of the three populations are closely related to each other cultivar, with distance values of 0.006; 0.009 and 0.009 (Table 7).

3.3. Genetic structure analysis

The genetic relationships among the 42 plantain accessions are presented in the principal coordinates analysis plot (Figure 2). The first three most informative principal components explained 54.59 % of the total variation. Graphic representation of PCoA showed a weak differentiation between the three plantain populations based on the phenotypic characteristics of the inflorescence. None of the first two axes clearly separates plantain cultivars. Individuals from true horn cultivars tended to have higher scores high for coordinate 2. However, all three populations overlapped for both coordinates. Within each population, certain individuals appeared highly distinct from certain others. Thus, *Glowa, N'zélou-ka, Bidini, Aboisso-1* and *afoto-2* accessions formed a distinct cluster and were separated from rest of the cultivars (Figure 2). The Neighbor Joining dendrogram based on dissimilarity matrix also confirmed this pattern of structure of plantain accessions (Figure 4). Bootstrapping values of all the nodes were depicted as numbers at the forks of consensus tree diagram. Similarly, no geographical patterns of clustering were observed.

4. Discussion

Knowledge of the extent of genetic diversity in crop germplasm is a prerequisite to develop a strategy for rational management of germplasm and its use in breeding. In Côte d'Ivoire, an important morphological variation was observed in the plantain collection by Thiémélé *et al.* (2017) [10]. However, many phenotype characteristics are highly influenced by the environment with continuous variation (Creste *et al.*, 2004) [21]. Thus, the aim of this study was to characterize the accessions of plantain of this collection and to describe the genetic structure by molecular markers SSRs.

A small fraction of the primers tested (6 out of 28, or 21 %) generated scorable amplicons in the 42 genotypes of plantain analysed. All the others were monomorphic. Creste *et al.* (2004) [21] tested 33 primers SSR on 58 diploid and triploid *Musa* accessions from the Brazilian banana germplasm, and only 15 primers (45 %) yielded amplification products in the predicted size. The limited number of polymorphic primers could be due to the sufficient difference the anchor point of the primers, sequences flanking of the microsatellite loci. Since microsatellite markers were mainly obtained from various diploid genotypes (Calcutta-4 and FHIA diploid) and East Africa Highland Banana cultivars (Mbanjo *et al.*, 2013) [15], all highly genetic diverse. Thus, more universal microsatellite markers are needed for genetic analysis of *Musa*.

To evaluate the informativeness and efficiency of SSR loci in analysis of genetic diversity of plantains, the total number of alleles, mean number alleles per locus and PIC value for each SSR locus were estimated. The six (6) primers amplified a total of 14 alleles, with an average of 2.33 alleles per locus from the 42 plantains genotypes. The low levels of SSR marker polymorphism observed in our study was similar to the levels of polymorphism reported by several authors which assessed genetic diversity of the species to asexual reproduction using SSR markers developed from an EST database. For instance, 75 EST-SSRs used to assess 12 sweet-potato genotypes showed low polymorphism with a mean three (3) alleles per locus and ranging from two (2) to six (6) (Hu *et al.*, 2004) [22]. Likewise, none of the 150 EST-SSRs which revealed to be polymorphic and used of evaluate eight (8) sweet-potato cultivars, does showed more than two (2) alleles in any of this diploid accessions and up to six alleles in hexaploid sweet-potato (Schafleitner *et al*., 2010) [23]. Raji *et al.* (2009) [24] also revealed a weak polymorphis in 29 cassava (*Manihot esculenta* Crantz) cultivars, with a number of alleles per locus ranged from 2 to 10 and an average of 4.55 per locus. In addition, Mbanjo *et al.* (2013) [15], author of EST-SSRs markers used in present study, observed that the majority of this microsatellites produced a mean two (2) alleles per locus, and only 12 primer pairs on 410 showed three (3) to five (5) alleles in 43 *Musa* genotypes. These results suggest that EST-SSR do not be useful as an effective type of molecular markers for identification and estimation of genetic diversity in plantain germplasm because they are less polymorphic. PIC values revealed by SSR markers in this study ranged from 0.375 to 0.593 with an mean of 0,468 ± 0,017, which is lower than that reported by Resmi *et al.* (2011) [9] (0.53) evaluating 38 banana cultivars using 15 SSR markers. The PIC value can be classified into three classes: slightly informative (PIC < 0.25), reasonably informative (0.5 > PIC > 0.25), and highly informative (PIC > 0.5) (Hayden *et al.*, 2010) [25]. Based on this classification, loci (*DN240063* and *Ma513026332*) showed a PIC value greater than 0.5 suggesting that this SSR markers can be ideal to conduct assessments aimed at understanding of genetic diversity of these accessions.

A high level of genetic variation was obtained in this study, with the Shannon diversity index (*I*) and the Nei genetic diversity index (*He*) respectively of *I* = 0.726 ± 0.023 and He = 0.506 ± 0.008. Similar results was reported by Quain *et al.* (2018) [5] in 40 plantain accessions collected from Ghana (*He*= 0.61 and *I* = 0.41). This two countries located in West Africa, in the heart of the secondary center of diversification of plantain in the world (De Langhe *et al.*, 2005) [13], could

contained several plantain genotypes. The high level of genetic variation in the collection will increase the efficiency of the selection of accessions in management and breeding programs of plantain in Côte d'Ivoire.

In this study, the average expected heterozygosity (*He* = 0.506 ± 0.008) was lower than the observed heterozygosity (*Ho* = 0.897 ± 0.025) at all loci and in all populations tested. Quain *et al.* (2018) [5] found similar result (*Ho* = 0.75 and *He* = 0.41) on 40 samples of plantain obtained directly from landraces cultivar, suggesting that plantain is at an excess in heterozygosity. The negative F_{IS} value (0.776 ± 0.050) confirms the highly heterozygous nature of plantain resulting from interspecific hybridizations between two wild diploid species, *Musa accuminata* Colla (Genome A donor) and *Musa balbisiana* (Genome B donor) (Simmonds & Shepherd, 1955) [1].

In the present study, genetic differentiation was measured by AMOVA, F_{ST} values and Nei genetic identity. Analysis of molecular variance (AMOVA) revealed that the differentiation of accessions based on morphological characteristics of inflorescence was insignificant (P = 0.267), and only 1% of total genetic variation assigned to the difference among populations. This implies that the main component of genetic variation observed in the present study was found within populations. Comparison of F_{ST} values by population pairs revealed low genetic differences among the populations (table 6). The Nei genetic identity confirmed the close relatedness between of the three populations (table 7). Close relationships between diploid banana cultivars of *Musa acuminata* group has also been reported by (Changadeya *et al.*, 2012) [26], in East African highland bananas cultivars (Kitavi, 2013) [27] and in 40 genotypes of plantain collected in Ghana by Quain *et al.* (2018) [5]. These results confirm the hypothesis of an unique origin of plantain landraces reported by several authors (Noyer *et al.*, 2005, De Langhe *et al.*, 2005) [2, 13]. According to Noyer *et al.* (2005) [2], all plantain cultivars come from a same initial genotype, whose the differentiation was carried out by epigenetic mutations follow successive vegetative propagations throughout the process of domestication. The results from the present study clearly showed a high genetic variation within genotypes of plantain, but very low differentiation among population based on characteristics of inflorescence. Similarly, (Flajoulot *et al.*, 2005) [28] reported that less than 1% of the total genetic variation differentiated populations of alfalfa, although genetic diversity is high within alfalfa accessions. This information can be applied in selecting a sampling strategy for genetic conservation. The AMOVA results obtained here suggest that future germplasm expeditions should collect more individuals within a limited number of populations.

PCoA analysis were not able to clearly differentiate the plantain landraces into their distinct morphogroups based on inflorescence characteristics, although *Glowa*, *N'zélou-ka*, *Bidini*, *Aboisso-1* and *afoto-2* accessions were separated from rest of the cultivars (Figure 3). This pattern of structure of plantain accessions was also confirmed the Neighbor Joining phylogram, whose bootstrap values of the nodes were all less than 95%. In fact, according to (Felsenstein, 1985)[29] had suggested that only those groups with bootstrap values of 95% or greater could be considered significant. This indicates that no robust groupings plantain accessions were observed. This result is in agreement with the previous studies on plantain using SSR markers (Noyer *et al.*, 2005 and Quain *et al.*, 2018) [2, 5], which showed that this markers was unable to cluster the plantain cultivars in a meaningful morphological order. These observations confirm the hypothesis that morphological diversity in the plantains apparently arose from somatic mutations in an hypervariable region of the genome from a very limited number of botanically different clonal sources (Vuylsteke *et al.*, 1991; De Langhe *et al.*, 2005)[30, 13]. The clustering analysis also did not show any geographical distribution pattern. Similar observations were obtained with the earlier studies of genetic diversity on plantain germplasms (Ude *et al.*, 2003, Noyer *et al.*, 2005 and Quain *et al.*, 2018) [12, 2, 5]. This observation could be the result of frequent seed exchanges (rejection) among different regions. Indeed, farmers traditionally use plantain to create the shade necessary for the proper development of young cocoa and coffee trees. The spatio-temporal dynamics of plantain production are related to those of the coffee / cocoa pair. Thus, the communities attached to coffee and cocoa cultivation have contributed to the dissemination of plantain genetic resources across different production areas.

Table 1: List of plantain accessions and their sampling location

Accession Name	Varietal group	Location	Genome type
Agnirin	French	Gagnoa, Côte d'Ivoire	AAB
Banablé_(Corne_4)	French	Abengourou, Côte d'Ivoire	AAB
Eniaba_(N'Zélouka)	French	Abengourou, Côte d'Ivoire	AAB
Foua	French	Bouaflé, Côte d'Ivoire	AAB
French_2	French	Côte d'Ivoire	AAB
Glowa	French	Côte d'Ivoire	AAB
N'zelou-ka	French	Congo	AAB
Bindi-Mossenjo	French	Congo	AAB
French_clair	French	Akoupé, Côte d'Ivoire	AAB
Ghana_Agnirin	French	Abengourou, Côte d'Ivoire	AAB
Laknao	French	Côte d'Ivoire	AAB
Ngletia_1	French	Côte d'Ivoire	AAB
Ninglinnin_1	French	Bouaflé, Côte d'Ivoire	AAB
Zakoi	French	Côte d'Ivoire	AAB
French_sombre	French	Cameroon	AAB
Bindi	French	Côte d'Ivoire	AAB
Monthan	French	Vietnam	AAB
Saci_1	False horn	Akoupé, Côte d'Ivoire	AAB
Kokoida	False horn	Aboisso, Côte d'Ivoire	AAB
Banablé_Corne_4	False horn	Abengourou, Côte d'Ivoire	AAB

Bana_Boi_1	False horn	Abengourou, Côte d'Ivoire	AAB
Afoto_2	False horn	Gnagnoa, Côté d'Ivoire	AAB
18_Rouge_french	False horn	Congo	AAB
3_vert	False horn	Congo	AAB
Bandiè_2	False horn	Côte d'Ivoire	AAB
Corne_1	False horn	Akoupé, Côte d'Ivoire	AAB
Corne_4	False horn	Côte d'Ivoire	AAB
Corne_5	False horn	Côte d'Ivoire	AAB
Corne_bout_rond	False horn	Côte d'Ivoire	AAB
Corne_tacheté	False horn	Abengourou, Côte d'Ivoire	AAB
Ehô	False horn	Côte d'Ivoire	AAB
Lorougnon	False horn	Abidjan, Côte d'Ivoire	AAB
Lorougnon_2	False horn	Aboisso, Côte d'Ivoire	AAB
Big_Ebanga	False horn	Cameroun	AAB
Orishele	False horn	Abidjan, Côte d'Ivoire	AAB
Red_Ebanga	False horn	Congo	AAB
Aboisso_1	True horn	Côte d'Ivoire	AAB
Ndoria	True horn	Abidjan, Côte d'Ivoire	AAB
Oleakotoi	True horn	Akoupé, Côte d'Ivoire	AAB
M'bomo	True horn	Congo	AAB
Iba	True horn	Nigeria	AAB
Assouba	True horn	Côte d'Ivoire	AAB
kpatrè_gnon	True horn	Gagnoa, Côte d'Ivoire	AAB

Table 2: Microsatellite (SSR) primers sequence used for analysis

Locus	Primers sequences (5'-3')	Allele size range observed	Tann (°C)
DN240063	F : CACGACGTTGTAAAACGACCGCGGATGATTCATCGTGGACCG	170-174	58
	R : ACAGCAGCAACAATCTCGTCGT		
DN239472	F : CACGACGTTGTAAAACGACCCGTGTTACAGATTGTATTCCCTTGT	105-126	58
	R : CGGCTGCGTCAACAAGCC		
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, Tann (°C), annealing temperature

Table 3: Allelic polymorphism of SSR loci used in this study

Locus	Number of allele	frequency of major allele	PIC
DN240063	2	0.50	0.375
Ma513026332	3	0.50	0.593
Ma513019043	2	0.58	0.460
DN239472	3	0.49	0.470
Ma513035997	2	0.52	0.510
Ma513036168	2	0.58	0.404
Total	14		
Mean	2.33	0.53	0.468
Standard deviation	0.55	0.04	0.071

PIC = Polymorphism Information Content

Table 4: Information index (I), Observed heterozygosity (Ho), Expected Heterozygosity (He) and inbreeding coefficient of the 6 SSR loci on 42 plantain accessions

Pop	Locus	*Ne*	*I*	*Ho*	*He*	F_{IS}
French	DN63	2.000	0.693	1.000	0.500	-1.000
	Ma32	2.261	0.889	0.867	0.558	-0.554
	Ma43	1.992	0.691	0.813	0.498	-0.631
	DN72	2.273	0.892	0.933	0.560	-0.667
	Ma97	1.988	0.690	0.923	0.497	-0.857
	Ma68	1.992	0.691	0.938	0.498	-0.882
False horn	DN63	2.000	0.693	1.000	0.500	-1.000
	Ma32	1.954	0.681	0.846	0.488	-0.733

	Ma43	1.895	0.665	0.647	0.472	-0.370
	DN72	2.000	0.693	1.000	0.500	-1.000
	Ma97	1.994	0.692	0.944	0.498	-0.895
	Ma68	1.946	0.679	0.833	0.486	-0.714
True horn	DN63	2.000	0.693	1.000	0.500	-1.000
	Ma32	2.579	1.004	0.857	0.612	-0.400
	Ma43	1.946	0.679	0.833	0.486	-0.714
	DN72	2.000	0.693	1.000	0.500	-1.000
	Ma97	2.000	0.693	1.000	0.500	-1.000
	Ma68	1.849	0.652	0.714	0.459	-0.556
Moyenne		2.037	0.726	0.897	0.506	-0.776
Standard deviation		0.040	0.023	0.025	0.008	0.050

I, Information index; HO, Observation heterozygosity; He, Expected heterozygosity and FIS, Inbreeding coefficients

Table 5: AMOVA of three populations based on 6 SSR markers

Source	df	Sum of squares	Mean squares	Estimate variation	Percentage	Statistics	Value	P value
Among populations	2	4.148	2.074	0.021	1%	PhiPT	0.011	0.267
Intra-population	39	70.423	1.806	1.806	99%			
Total	41	74.571		1.826	100 %			

Df: Degrees of freedom; PhiPT: Analogue of FST fixation index.

Table 6: Pairwise Population Fst Values

French	False horn	True horn	
0.000			French
0.003	0.000		False horn
0.004	0.004	0.000	True horn

Table 7: Pairwise Population Matrix of Nei Genetic Distance

French	False horn	True horn	
0.000			French
0.006	0.000		False horn
0.009	0.009	0.000	True horn

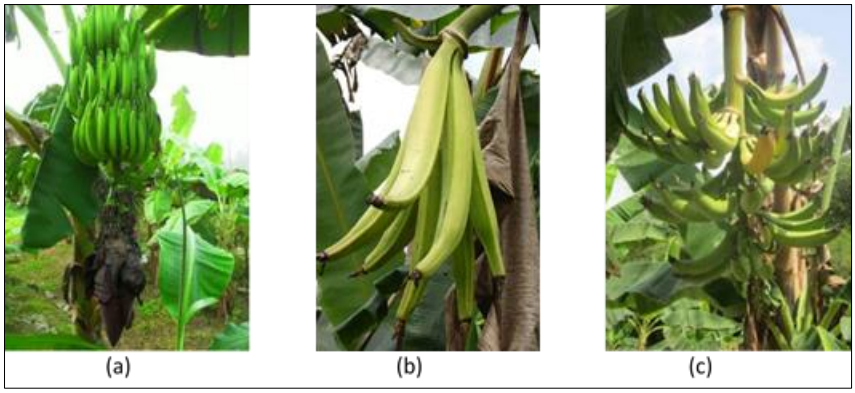

Fig 1: Different types of plantain cultivars: (a) French; (b) True horn; (c) False horn

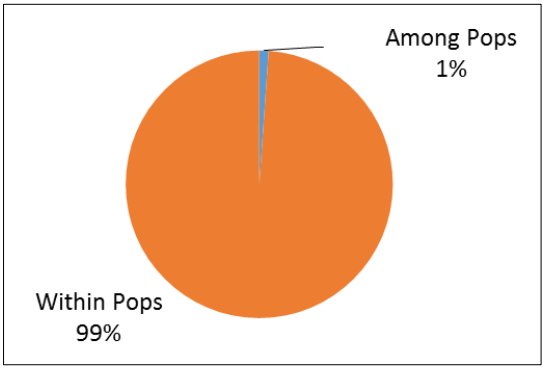

Fig 2: AMOVA of three populations based on 6 SSR markers

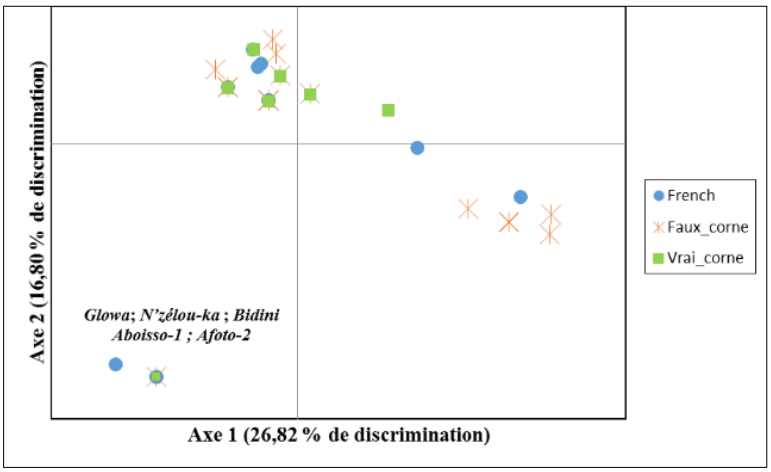

Fig 3: Principal Coordinates Analysis (PCoA) scatter plot of 42 plantain accessions and 1 diploid cultivars. The plane of the first second main PCO axes accounted for 43.62 % of total variation. First axis =26.82% of total information, the second = 16.80 %

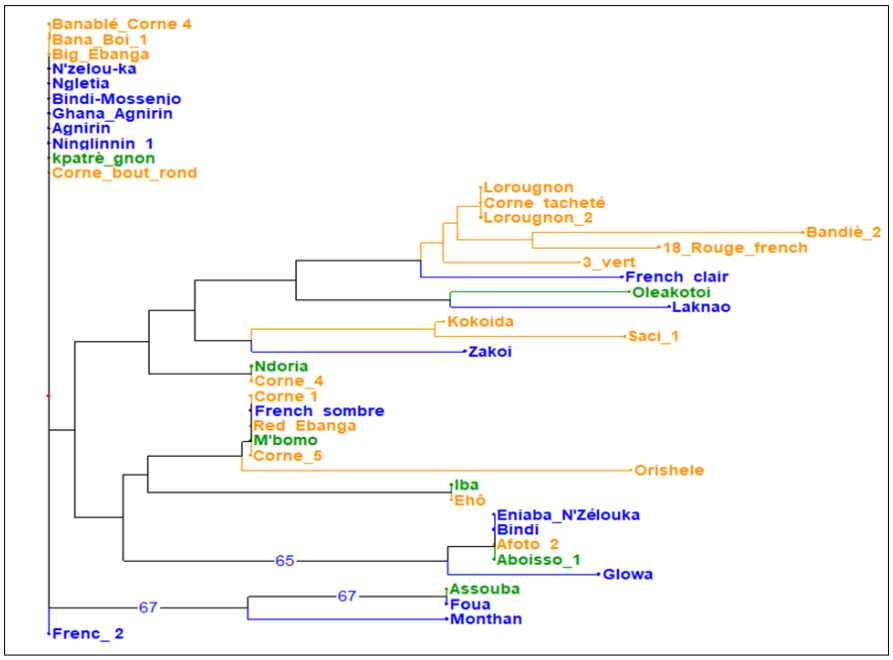

Fig 4: Microsatellite phylogram of plantain germplasms. The dendrogram was produced using the unweighted NeighborJoining method based on genetic dissimilarity among the 42 plantain accessions. The colors of branches indicate accessions corresponding to three populations (French, false horn, true horn). Node labels represent bootstrap support (>60%) out of 1000 bootstrap replicates.

6. Conclusion

This study, to the best of our knowledge, is one of the first ever reports on application of SSR markers to study molecular diversity in genotypes of plantain in Côte d'Ivoire. Although, The SSR markers used in this study revealed a wide genetic diversity in this set of 42 accessions plantain, they were unable to adequately group the accessions into their morphologic groups. There is thus the need to develop markers that will detect variations among genotypic groups of plantain. Genetic diversity found in cultivar of plantain can be used in designing conservation strategies, maximizing its use in breeding programs.

7. Acknowledgements

We thank Yao Amha Lucie and Affian Kakou Natanael for their excellent technical assistance. This work was supported financially by the Fonds Interprofessionnel pour la Recherche et le Conseil Agricoles of Côte d'Ivoire (FIRCA), on behalf of the World Bank and the Government of Côte d'Ivoire, in the framework of the West Agricultural Productivity Program (WAAPP) of the ECOWAS.

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