

Molecular characterization of mango (*Mangifera indica* L.) cultivars using SSR markers

ABSTRACT

Nowadays, molecular characterization is essential for studying the varietal diversity of species. In addition, SSR molecular markers are widely used to identify and distinguish the genetic relationship of mango cultivars. The study aims to determine the variability structure level of 18 mango cultivars in Burkina Faso. Thus, genomic DNA was extracted in 2022 from young leaves at the molecular biology unit of the Biosciences laboratory at Joseph KI-ZERBO University (Burkina Faso). Analysis of the results showed a polymorphic percentage average of 21.49% per marker. Genetic distance showed that the similarity coefficient range is 0.0002 to 1.09. The greatest genetic distance (1.09) was calculated for the pairs (VSB, Valencia) and (VSB, Miami late). On the other hand, the lowest genetic distance (0.0002) was calculated between Alphonso and Francis, Keitt and Sensation, and Mangot vert and Glazier. Examination of the dendrogram shows that the cultivars can be classified into two major groups of nine cultivars each. The first group includes Miami late, Valencia, Lippens, Zill, Keitt, Sensation, Kent, Brooks, and Beverly cultivars. The second group includes the cultivars Mangot vert, Glazier, Amélie, Dixon, Springfield, Francis, Alphonso, VSB and Mangot sabre. These results showed that cultivars are genetically very diverse. Therefore, our findings could be used for genetic diversity analysis and the marker-assisted breeding of mango germplasm.

Keywords : Characterization, Mango, genetic variability, SSR markers, Burkina Faso.

INTRODUCTION

One of the conditions for the success of crop breeding depends on the genetic variability available in varietal collections. According to [1], there are around 1,000 varieties of mango in the world. [2] claim that the high rate of outcrossing justified the increase in mango genetic diversity. In Burkina Faso, 22 mango cultivars have been identified, providing a large genetic diversity to be exploited. In addition, efforts are needed to measure the variability of the mango variety collection based on agro-morphological traits. This has enabled cultivars to be identified based on their foliage characteristics. In terms of fruit, it has enabled a better understanding of the appreciation of cultivars by growers. Moreover, the

selection of cultivars resistant to the desiccation pathogen was used, but this has its limitations [3].

As a result, molecular markers become essential to study plants genetic diversity. In addition to their ease of use in the laboratory and their effectiveness in studying polymorphism and drawing up genetic maps, molecular markers are essential to study foreign genes and genes linked to disease resistance or susceptibility. There are currently several types of molecular markers such as RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism) and SSR (Simple Sequence Repeats). We have chosen SSR markers because it is the most popular molecular markers used to detect molecular polymorphism in mango [4]. In addition, SSRs markers are known to be codominant, polymorphic and highly reproducible ([5] ; [6]). Few studies have been carried out on the molecular characterization of mango trees in Burkina Faso. This is necessary to develop this new approach to studying the genetic diversity of mango trees.

MATERIAL AND METHODS

PLANT MATERIAL

The plant material consisted of 18 mango cultivars seedlings (Table 1).

Table 1 : List of plant material

N°	Cultivars	Type of Seed	N°	Cultivars	Type of Seed
1	Sensation	Mono-embryonic	10	Alphonso	Mono-embryonic
2	Glazier	Mono-embryonic	11	Keitt	Mono-embryonic
3	Brooks	Mono-embryonic	12	Amelie	Mono-embryonic
4	Miami-late	Mono-embryonic	13	Mangot vert	Poly-embryonic
5	Valencia	Mono-embryonic	14	Mangot sabre	Poly-embryonic
6	Zill	Mono-embryonic	15	Dixon	Mono-embryonic
7	VSB	Mono-embryonic	16	Bewerly	Mono-embryonic
8	Springfield	Mono-embryonic	17	Francis	Mono-embryonic
9	Kent	Mono-embryonic	18	Lippens	Mono-embryonic

STUDY SITE

The study was conducted at the molecular biology unit of the Biosciences laboratory at Joseph KI-ZERBO University (Burkina Faso) (longitude 01°31'05" W, latitude 12°21'58" N and altitude 450 m) (Figure 1).

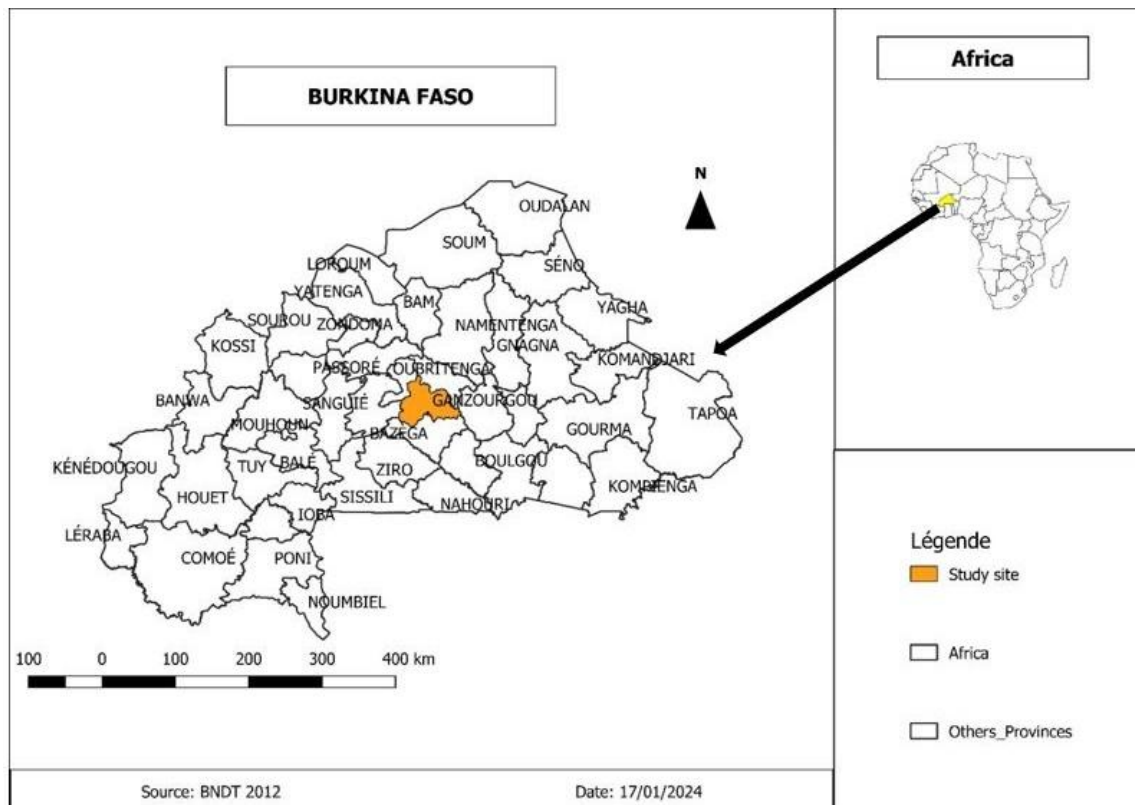


Figure 1: Geographical localisation of the study site

Extraction of genomic DNA

Genomic DNA was extracted in 2022 from young leaves at the molecular biology unit of the Biosciences laboratory at Joseph KI-ZERBO University (Burkina Faso). It began with the preparation of the extraction solution. This preparation was carried out by the laboratory's concentration measurement standards. The DNA extraction protocol of [7] was used to extract the DNA of the 18 cultivars. The extraction process was as shown below :

Crushing and cell lysis

Each leaf sample collected (approximately 200 mg) was finely ground in 2 mL of Tris-EDTA-Sorbitol (TBE) buffer using a mortar and pestle. The crushed material from each sample collected in an Eppendorf tube was centrifuged at 10,000 RPM for 10 min at 4°C. At the end of centrifugation, the supernatant was removed and 750 µL of CTAB (Cetyl-trimethyl-ammonium bromide) buffer pre-warmed to 65°C was added to the pellet. The tubes were agitated to recover the pellet in lysis buffer. They were then incubated at 65°C in a water bath for 2 h 30 min. The contents of the tubes were homogenised by inverting them every 15 min.

Initial solvent extraction

At the end of incubation, the tubes were cooled for a few minutes at room temperature. A volume of 750 µL of a 24 :1 mixture of chloroform and isoamyl alcohol (CIAA) was added.

The tubes were centrifuged again at 10,000 RPM for 15 min at 4°C. After centrifugation, the supernatant (approximately 300 µl) was collected in new 1.5 mL Eppendorf tubes.

Initial DNA precipitation

750 µL of fresh (-4°C) isopropanol was added to each tube to precipitate the DNA. The tubes were gently shaken by inverting until the DNA pellet was observed. They were centrifuged again at 10,000 RPM at 4°C for 10 min. The supernatant was discarded and the tubes were dried at room temperature.

RNase treatment

The DNA pellet was then taken up in Tris-EDTA (TE) buffer and stored in a freezer at -20°C. A volume of 200 µL of TE buffer and 30 mg of RNase was added to each tube and mixed thoroughly. The mixture was then incubated at 37°C for at least 30 minutes.

Washing the DNA with ethanol

After removal of the supernatant, 200 µL of 70% ethanol was added to the DNA pellet and centrifuged at 5000 rpm for 5 minutes.

Final DNA suspension

The DNA pellet obtained after supernatant elimination was dried in a concentrator evaporator and then centrifuged under vacuum for 1 hour. After drying, the DNA was re-suspended in 100 µl of TE overnight at room temperature. The DNA was then stored at 4°C for immediate use. DNA concentration was estimated using a spectrometer.

Quantification of extracted DNA

The quality and quantity of the extracted DNA were checked on a 1% agarose gel. The DNA concentrations of each variety were determined by comparing them with those of 5 control DNAs (50, 100, 150, 200 and 300 ng/µL). Dilutions were then done to give a total concentration of 5 ng/µL.

Genotyping of DNA extracts using SSR markers

Fourteen microsatellite markers [4] were used to study the level of polymorphism in the 18 mango cultivars. Details of the 14 SSR markers used are given in Table 2.

Table 2 : List of SSR markers used to characterise the 18 mango cultivars

Sequencename microsatellite	Repeat motif	Amplification size (bp)	Microsatellite position
mMiCIR002	(TG) ₇	253	314-327
mMiCIR003	(TG) ₁₀	319	521-540
mMiCIR008	(AT) ₆ G(TG) ₁₄ (TATG) ₆	163	78-142
mMiCIR009	(AC) ₁₀	165	278-297
mMiCIR010	(TG) ₁₃	284	196-221
mMiCIR011	(TG) ₁₀	191	119-137

mMiCIR013	(GT) ₁₀	156	147-166
mMiCIR025	(CA) ₆	211	261-272
mMiCIR028	(CA) ₇	187	46-59
mMiCIR029	(AC) ₁₁	176	180-201
mMiCIR030	(TG) ₁₂	193	334-357
mMiCIR032	(TG) ₄	189	442-457
mMiCIR034	(AATA) ₃ (AC) ₈	201	252-277
mMiCIR036	(TG) ₁₁	264	350-363

Amplification of DNA extracts by PCR

A PCR amplification protocol developed by [7] was used as a guide. The final volume required for the PCR reaction was set at 13.5 μ L. PCR amplification programme used was taken from the work of Sitbon [4]. The protocol for this programme, called AMSS51, is shown in Table 3.

Table 3 : AMSS51 program

Standard program (AMSS51)		
Steps	Temperature ($^{\circ}$ C)	Time
1	94	5 mn
2	94	30''
3	51	1 mn
4	72	1 mn
Cycle : 35 times		
5	72	8 mn
6	15	24 h

Revelation of PCR products by capillary electrophoresis

The multiplex PCR products were mixed in 96 plates to give a total volume of 18.5 μ L. The mixture consisted of 10 μ L of distilled water, 2.5 μ L of master mix, 2 μ L of primer and 5 μ L of DNA. The plate containing the mixture was centrifuged and subjected to denaturation at 95 $^{\circ}$ C for 5 minutes. The diluted PCR products were separated by capillary electrophoresis on an ABI 3700 sequencer (Applied Biosystems). A set of 14 primer pairs was used in this study and synthesised by BIONEER Corporation, Korea. The molecular weight used was Fermentas 100 pbs plus.

Data analysis

The band profiles of the SSR primers generated were analysed and compared to determine their polymorphism. Amplified fragments were scored as present (1) or absent (0). Genetic similarity and the similarity matrix were estimated using the Dice coefficient.

Dendrogram showing genetic relationships were constructed using the Neighbor-Joining method with DARWIN 6.0.21 software. The 14 SSR primers tested were used to calculate the similarity matrices.

RESULTS

Polymorphism detected by SSR marker analysis

All 14 SSR molecular markers used were amplified. Figure 2 shows an extract from the results of the polymorphism test, more specifically, the electrophoretic profiles of primers mMiCIR011 and mMiCIR003. Approximate band sizes ranged from 156 to 284 bp. The number of bands varied from 12 (Figure 2 A) to 6 (Figure 2 B). However, the mMiCIR003 marker only revealed bands from five mango cultivars. PPB ranged between 8.33% (primer mMiCIR028 ; mMiCIR032 and mMiCIR036) to 37.5% (primer mMiCIR008), with an average of 21.49% (Table 4).

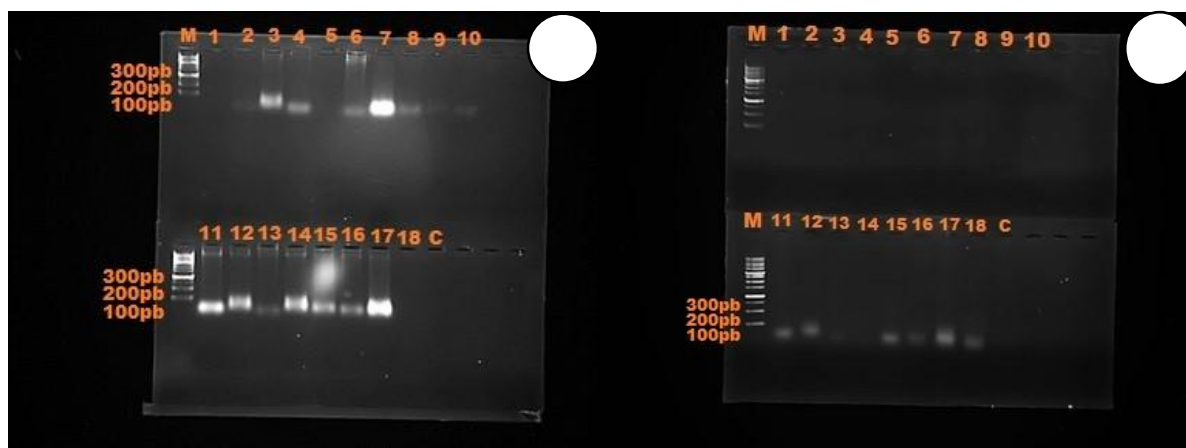


Figure 2 : Agarose gel electrophoretic profile of PCR products from DNA extracts of cultivars with microsatellite marker 11 (A) and marker 3 (B).

Legend: M: size marker; 1 to 18: samples subjected to PCR analysis; C: negative control.

Organisation of genetic diversity

The matrix resulting from the calculation of the genetic distance coefficient revealed the genetic distances of the cultivars in pairs (Table 5). Genetic distance showed that the similarity coefficient range is 0.0002 to 1.09. The greatest genetic distance (1.09) is between VSB and Valencia, VSB and Miamilate. On the other hand, the lowest genetic distance (0.0002) was observed between Alphonso and Francis, Keitt and Sensation, Mangot vert and Glazier. Examination of the dendrogram (Figure 3) enables the cultivars to be classified into two major groups. The first group includes the cultivars Miamilate, Valencia, Lippens, Zill, Keitt, Sensation, Kent, Brooks and Beverly. The second group includes the cultivars Mangot vert,

Glazier, Amélie, Dixon, Springfield, Francis, Alphonso, VSB and Mangot sabre. Within the first group, bootstrap values are relatively low. On the other hand, bootstrap values are relatively high in the second group. However, when the number of bands amplified for all 14 markers is taken into account, four cultivars were discarded because they did not admit enough bands (number of bands less than 3 for all markers used). Examination of the dendrogram (Figure 4) enables the cultivars to be classified into three major groups.

Table 4: Characteristics of DNA profiles generated in 18 mango cultivars by using 14 SSRs primers.

SSRs Primers	SB	NPB	PPB (%)
mMiCIR002	11	2	18.18
mMiCIR003	6	2	33.33
mMiCIR008	8	3	37.5
mMiCIR009	10	1	10.0
mMiCIR010	7	2	10.0
mMiCIR011	12	3	25.0
mMiCIR013	9	3	33.33
mMiCIR025	10	2	20.0
mMiCIR028	12	1	8.33
mMiCIR029	11	2	18.18
mMiCIR030	12	2	16.66
mMiCIR032	12	1	8.33
mMiCIR034	12	2	16.66
mMiCIR036	12	1	8.33
Sum	144	27	-
Average	10.28	2.21	21.49

SB: Scored bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands.

Table 5: Estimated genetic similarity (Dice 1945) between cultivars

N°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Valencia																	
2 Lippens	1																
3 Zill	1.06	0.94															
4 VSB	1.09	0.96	0.92														
5 Miamilate	1	1	1.06	1.09													
6 Bewerly	0.97	0.85	0.80	0.29	0.97												
7 Alphonse	1	0.88	0.83	0.08	1.005	0.21											
8 Francis	1.005	0.88	0.83	0.083	1.005	0.21	0.0002										
9 Kent	0.96	0.84	0.79	0.59	0.96	0.48	0.51	0.51									
10 Brooks	0.91	0.79	0.74	0.34	0.91	0.23	0.26	0.26	0.42								
11 Dixon	0.99	0.87	0.83	0.12	0.99	0.20	0.04	0.04	0.51	0.25							
12 Amélie	0.99	0.87	0.83	0.12	0.99	0.20	0.04	0.04	0.51	0.25	0.0003						
13 Sensation	0.89	0.78	0.73	0.53	0.89	0.42	0.45	0.45	0.33	0.36	0.44	0.44					
14 Keitt	0.89	0.78	0.73	0.53	0.89	0.42	0.45	0.45	0.33	0.36	0.44	0.44	0.0002				
15 Glazier	0.99	0.87	0.83	0.12	0.99	0.20	0.04	0.04	0.51	0.25	0.0004	0.0003	0.44	0.44			
16 Springfield	0.99	0.87	0.83	0.12	0.99	0.20	0.04	0.04	0.51	0.25	0.0003	0.0004	0.44	0.44	0.0005		
17 Mangot vert	0.99	0.87	0.83	0.12	0.99	0.20	0.04	0.04	0.51	0.25	0.0004	0.0003	0.44	0.44	0.0002	0.0005	
18 Mangot sabre	1.1	0.97	0.92	0.28	1.1	0.30	0.19	0.19	0.6	0.35	0.19	0.19	0.54	0.54	0.19	0.19	0.19

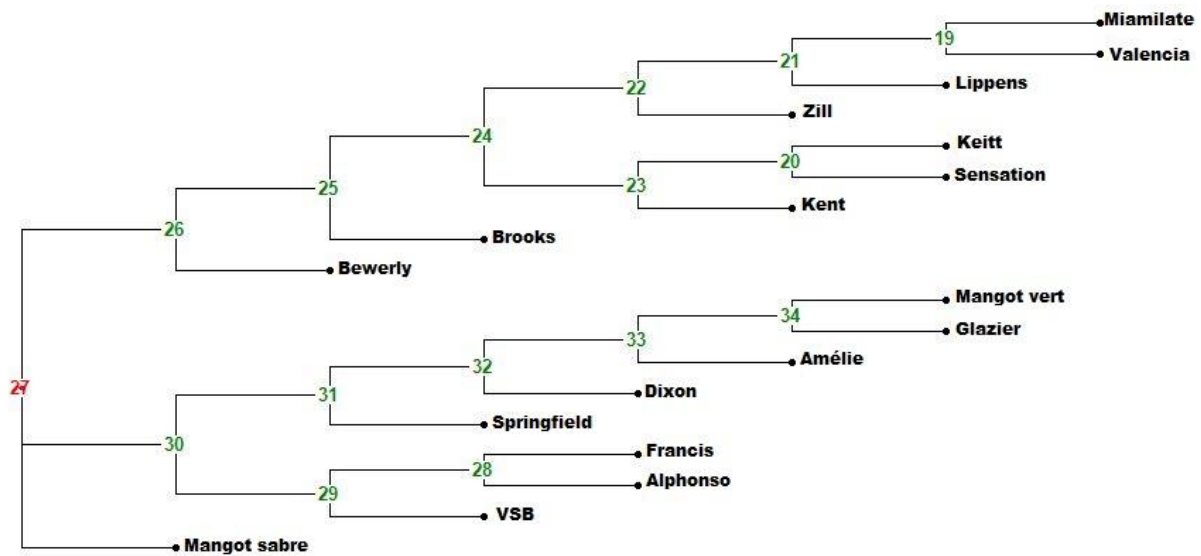


Figure 3: Dendrogram showing the distribution of mango cultivars using the Neighbour-Joining method.

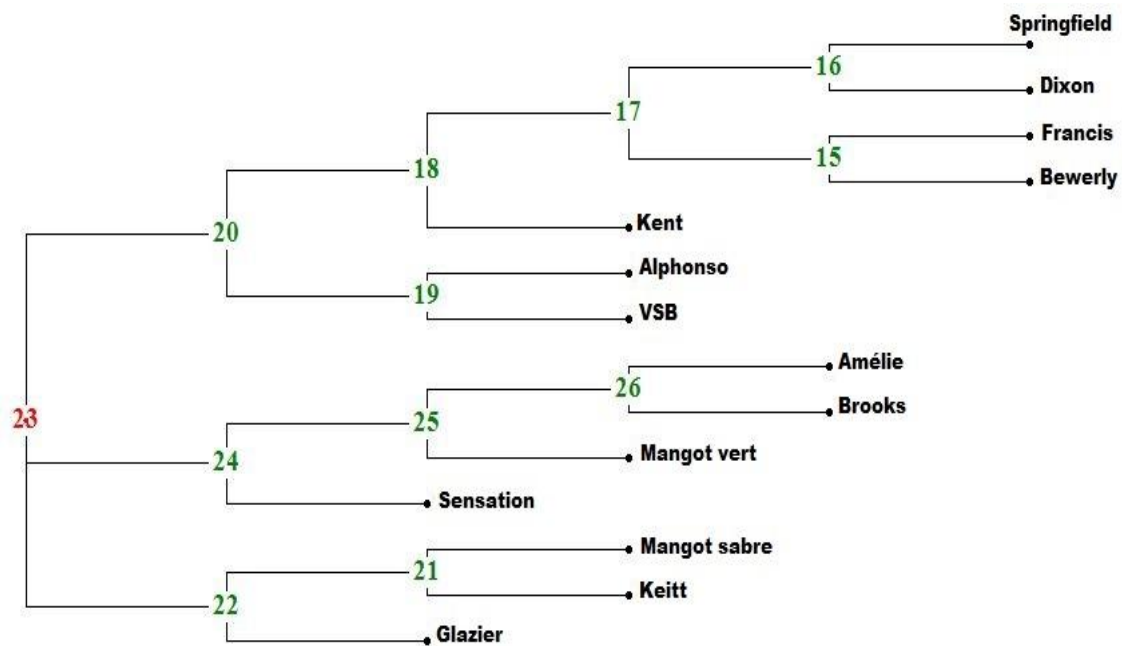


Figure 4: Dendrogram showing the distribution of 14 mango cultivars that emitted the most bands using the Neighbour-Joining method.

Principal Coordinate Analysis (PCoA) based on the similarity matrix confirms this genetic structure within the 18 mango cultivars. Table 6 summarises the contribution of the first five axes to the total inertia. The first principal component explains 38.96% of the total variation, the second 22.52% and the third 13.31%. The first factorial design (1 and 2) explaining

61.48% of the total variance clearly shows the twogenetic groups revealed by the UPGMA method (Figure 5).

Table 6: Eigen values of the first five axes obtained by principal coordinateanalysis of SSR molecular data

Axis no	Eigen value	Total variance (%)	Cumulative variance (%)
1	0,08	38,96	38,96
2	0,05	22,52	61,48
3	0,03	13,31	74,79
4	0,03	12,59	87,38
5	0,01	4,85	92,23

Factorial analysis: (Axes 1 / 2)

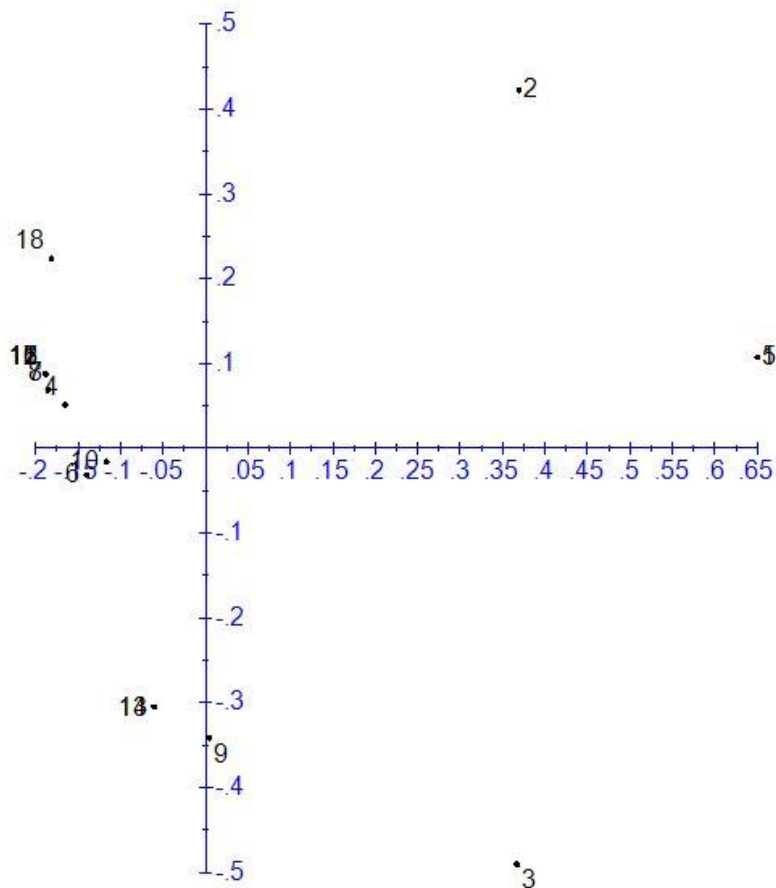


Figure 5 : Principal coordinateanalysis of mango cultivars based on SSR molecular data

DISCUSSION

The 100% polymorphism rate observed indicates a very high level of polymorphism in the SSR markers used. A very high level of polymorphism (100%) was also obtained by [4] using

the same SSR primers. These results confirm the effectiveness of SSR primers for discriminating between individuals within a species, even with a narrow genetic base. The high genetic diversity revealed by SSR markers between cultivars may be due to the heterogeneity of their parents [8]. Thus, analysis of the genetic distance between mango cultivars revealed sufficient differences for these cultivars to be grouped. The highest genetic distance has been obtained between VSB and Valencia, VSB and Miami late and it could be explained by the high variability that exists between these cultivars. In addition, Valencia and Miami late may react differently to mango pathogens and drought than VSB. As for the genetic distance (0.0002) measured between Alphonso and Francis, Keitt and Sensation, and Mangot vert and Glazier, it shows that these cultivars are genetically very similar from the perspective of a character. The large genetic distances between different cultivars can be explained by variations in leaf characteristics and the effect of the environment [9]. This results in the genetic divergence of mango cultivars [10]. This shows that these cultivars could have the same reaction to pathogen attack and drought between them, as indicated by the measurements. Similarly, if the Glazier variety (monoembryonic) is genetically similar to the Mangot vert variety, which is polyembryonic, this would mean that these two cultivars have the same gene, other than the one that governs embryo multiplication. The genetic distances obtained show the great variability of mango cultivars in the world. [11] provides ample evidence of this. The two genetic groups developed by the dendrogram are highly divergent and can be used to advantage in crosses for the genetic breeding of mango cultivars in Burkina Faso. [12] reported five groups for mango genotypes collected in Australia. [13] found no significant difference when comparing cultivars from Myanmar with those from India and South-East Asia. Molecular analysis of the cultivars will support the study of their resistance to *Lasiodiplodia theobromae*. [14] also reported the existence of gene pools in Banganapalli, Dashehri and Langramango cultivars using SSR markers. The results clearly demonstrated that PCR-based testing of dominant markers is a good tool for genetic analysis of mango cultivars. This was demonstrated by [15]. In addition, marker evaluation has shown that most cultivars can be easily identified. Overall, these data expand the knowledge on the application of SSR markers as a molecular characterization tool in mango ([16] ; [17]).

CONCLUSION

The study aimed to determine the degree of variability structuring in 18 mango cultivars in Burkina Faso. The fourteen SSR markers used in this study were all polymorphic. Genetic distance showed that the similarity coefficient range is 0.0002 to 1.09. In addition,

examination of the dendrogram enabled the cultivars to be classified into two major groups of nine cultivars each. This study will make it easier to conserve the genetic heritage and enhance the adaptation and breeding of mango cultivars in Burkina Faso.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ACKNOWLEDGEMENTS

We would like to thank the unit of the Biosciences laboratory at Joseph KI-ZERBO University for enabling us to carry out this study.

AUTHORS' CONTRIBUTIONS

CD and MK designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. ERT, JS and MS supervised the study and all authors read and approved the final manuscript.

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