Molecularcharacterization of mango (*Mangiferaindica* L.) cultivars using SSR markers

ABSTRACT

Aims: Nowadays, molecularcharacterisationis essential for studying the varietaldiversity of species. In addition, molecular markers are very importants to characterisespecificgenesto a plant. The general objective is to determine the variabilitystructuringlevelin the 18 mangocultivars in Burkina Faso.

Place and Duration of the Study: the studywascarried outsince2021 and 2022 at the molecularbiology unit of the Biosciences laboratory at Joseph KI-ZERBO University (Burkina Faso).

Methodology: To do this, genomic DNA wasextractedfromyoungleaves of 18 mangovarieties.

Results: Analysis of the resultsshowed a polymorphic percentage rangedbetween 85.7% (primer mMiCIR010) to 100% (primer mMiCIR002; mMiCIR003; mMiCIR011; mMiCIR013; mMiCIR025 ; mMiCIR028 ; mMiCIR029 ; mMiCIR030 ; mMiCIR032 ; mMiCIR034 ; and mMiCIR036), with an average of 97.37%. The geneticsimilarityresultingfrom the calculation of genetic distances wasbetween 0.0002 and 1.09. The greatestgenetic distance (1.09) wascalculated for the pairs (VSB, Valencia) and (VSB, Miamilate). On the other hand, the lowestgenetic distance (0.0002) wascalculatedbetweenAlphonso and Francis, Keitt and Sensation, and Mangot vert and Glazier. Examination of the dendrogram shows that the cultivars can beclassifiedintotwo major groups of ninecultivars each. The first group includesMiamilate, Valencia, Lippens, Zill, Keitt, Sensation, Kent, Brooks and Bewerly. The second group includes the cultivars Mangot vert, Glazier, Amélie, Dixon, Springfield, Francis, Alphonso, VSB and Mangot sabre.

Conclusion : Theseresultsshowedcultivars are geneticallyvery diverse. It istherefore essential to preserve and promotethesemangocultivars in order to boost mango production in Burkina Faso.

Keywords : Characterization, Mango, geneticvariability, SSRs markers, Burkina Faso.

1. INTRODUCTION

One of the conditions for the success of cropbreeding programmes depends on the geneticvariabilityavailable in varietal collections. There are around 1,000 varieties of mango in the world according to [12].According to [11], the high rate of outcrossingcontributes to an

increase in mangogeneticdiversity. In Burkina Faso, 22 mangocultivars have been identified, providing a large geneticdiversity to beexploited. In addition, efforts have been done to measure the variability of the mangovariety collection on the basis of agro-morphological traits. This has enabledcultivars to beidentified on the basis of theirfoliarcharacteristics. In terms of fruit, it has enabled a betterunderstanding of the appreciation of cultivars by growers. Moreover, the selection of cultivars resistant to the desiccationpathogenwasused, but this has its limitations [3].

As a result, the use of new molecularapproaches has become essential for identifyingcultivars, studyingtheirdiversity and establishingtheirphylogeneticrelationships. In addition to theirease of use in the laboratory and theireffectiveness in studyingpolymorphism and drawing up geneticmaps, molecular markers are very importants to characterisespecificgenes to a plant, foreigngenes and geneslinked to resistance or susceptibility to diseases. There are currentlyseveral types of molecular marker: RAPD (RandomAmplifiedPolymorphic DNA), AFLP (Amplified Fragment LengthPolymorphism), RFLP (Restriction Fragment LengthPolymorphism) and SSR (Simple SequenceRepeats).Wehave chosen SSR markers becauseits are the mostpopularmolecular markers used to detectmolecularpolymorphism in mango[15]. In addition, SSRsmarkers are known to be codominant, polymorphic and highlyreproducible[10, 8].Few studies have been carried out on the molecularcharacterisation of mangotrees in Burkina Faso. At the end, thisworkwasthereforeundertakenwith the generalaim of determining the level of variabilitystructuring in the 18 mangocultivars.

2. MATERIAL AND METHODS

2.1.PLANT MATERIAL

The plant materialconsisted of 18 mangocultivars 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

N°	Cultivars	Type of Seed	N°	Cultivars	Type of Seed
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

2.2.STUDY SITE

The studywasconducted at the molecularbiology 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).

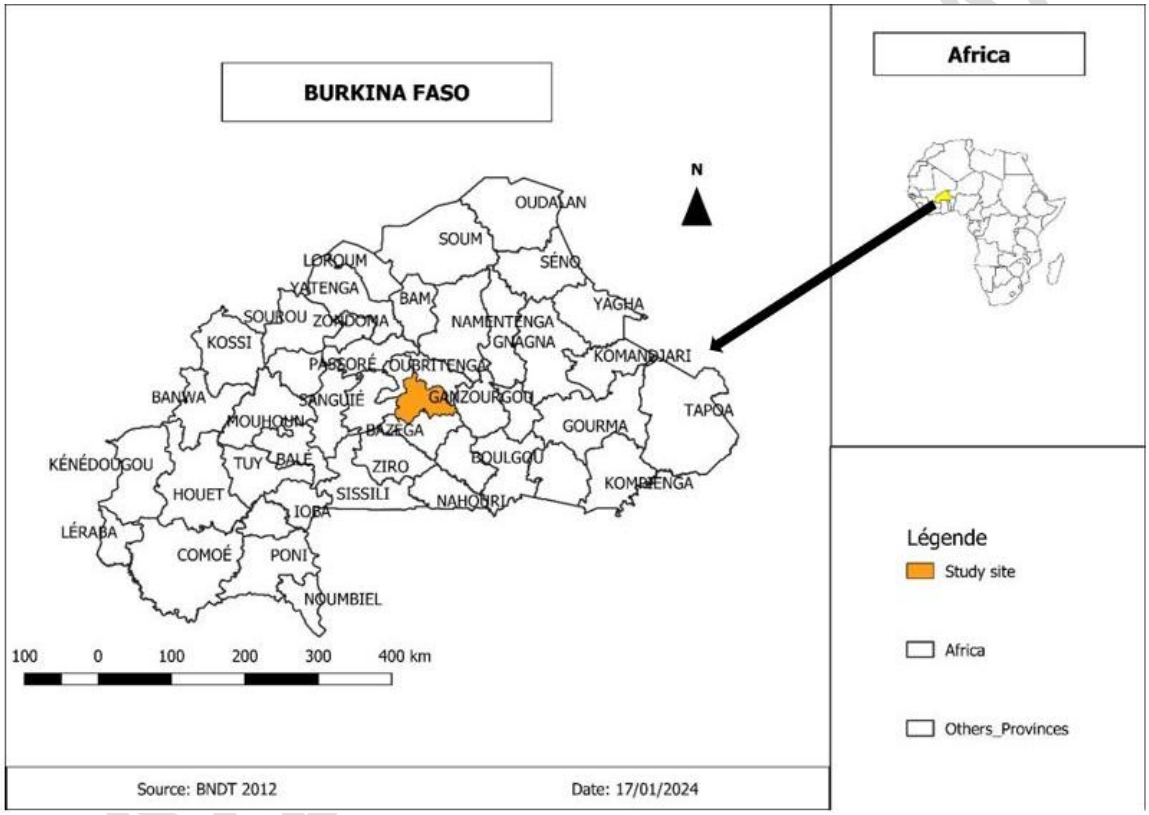

Figure 1:Geographical localisation of the study site

2.3.Extraction of genomic DNA

The extraction of genomic DNA wasextractedsince 2021 and 2022 fromyoungleaves at the molecularbiology unit of the Biosciences laboratory at Joseph KI-ZERBO University (Burkina Faso). It beganwith the preparation of the extraction solution. This preparationwascarried out in accordance with the laboratory's concentration measurement standards. The DNA extraction protocol of [1]wasused to extract the DNA of the 18 cultivars. The extraction process was as shownbelow:

2.3.1. Crushing and celllysis

Eachleafsamplecollected (approximately 200 mg) wasfinelygroundin 2 mL of Tris-EDTA-Sorbitol (TBE) buffer using a mortar and pestle. The crushedmaterialfromeachsamplecollected in an eppendorf tube wascentrifuged at 10,000 RPM for 10 min at 4°C. At the end of centrifugation, the supernatantwasremoved and 750 µL of CTAB (CetylTrimethylAmoniumBromide) buffer pre-warmed to 65°C wasadded to the pellet. The tubes wereshaken to completelyrecover the pellet in lysis buffer. Theywerethenincubated at 65°C in a water bath for 2 h 30 min. The contents of the tubes werehomogenised by invertingthemevery 15 min.

2.3.2. Initial solvent extraction

At the end of incubation, the tubes werecooled for a few minutes at room temperature. A volume of 750 µL of a 24:1 mixture of chloroform and isoamylalcohol (CIAA) wasadded. The tubes werecentrifugedagain at 10,000 RPM for 15 min at 4°C. After centrifugation, the supernatant (approximately 300 µl) wascollected in new 1.5 mLeppendorf tubes.

2.3.3. Initial DNA precipitation

750 µL of veryfresh (-4°C) isopropanol wasadded to each tube to precipitate the DNA. The tubes weregentlyshaken by invertinguntil the DNA pellet wasobserved. Theywerecentrifugedagain at 10,000 RPM at 4°C for 10 min. The supernatantwasdiscarded and the tubes weredried at room temperature.

2.3.4. RNase treatment

The DNA pellet wasthentaken 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 wasadded to each tube and mixed thoroughly. The mixture wasthenincubated at 37°C for at least 30 minutes.

2.3.5. Washing the DNA withethanol

Afterremoval of the supernatant, 200 µL of 70% ethanolwasadded to the DNA pellet and centrifuged at 5000 rpm for 5 minutes.

2.3.6. Final DNA suspension

The DNA pellet obtainedafterremoval of the supernatantwasdried in a concentratorevaporator and thencentrifugedunder vacuum for 1 hour. After drying, the DNA was re-suspendedin 100 µl of TE overnight at room temperature. The DNA wasthenstored at 4°C for immediate use. DNA concentration wasestimatedusing a spectrometer at the regional center of excellence for fruits and vegetables.

2.3.7. Quantification of extracted DNA

The quality and quantity of the extracted DNA werechecked on a 1% agarose gel. The DNA concentrations of eachvarietyweredetermined by comparingthemwiththose of 5 control DNAs (50, 100, 150, 200 and 300 ng/µL). Dilutions werethendone to give a total concentration of 5ng/µL.

2.3.8. Genotyping of DNA extractsusing SSR markers

Fourteen microsatellite markers [15]wereused to study the level of polymorphism in the 18 mangocultivars. Details of the 14 SSR markers used are given in Table 2.

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

Sequencename microsatellite	Repeat motif	Amplification size (bp)	Microsatellite position
mMiCIR002	$(TG)_7$	253	314-327
mMiCIR003	$(TG)_{10}$	319	521-540
mMiCIR008	$(AT)_6G(TG)_{14}(TATG)_6$	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

2.3.9. Amplification of DNA extracts by PCR

For PCR amplification, weused an existingprotocolthat has already been used for similarstudies[1]. The final volume required for the PCR reactionwas set at 13.5 µL. The PCR amplification program usedwastakenfrom the work of [15]. The protocol for this program, called AMSS51, isshown in Table 3.

Table 3: AMSS51 program

Standard program (AMSS51)		
Steps	**Temperature (°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

2.4.Revelation of PCR products by capillaryelectrophoresis

The multiplex PCR productswere mixed in 96 plates to give a total volume of 18.5 µL. The mixture consisted of 10 µL of distilled water, 2.5 µL of master mix, 2 µL of primer and 5 µL of DNA. The plate containing the mixture wascentrifuged and subjected to denaturation at 95°C for 5 minutes. The diluted PCR productswereseparated by capillaryelectrophoresis on an ABI 3700 sequencer (AppliedBiosystems). A set of 14 primer pairs wasused in thisstudy. These primer pairs weresynthesised by BIONEER Corporation, Korea. The molecularweightusedwas Fermentas 100 pbs plus.

2.5.Data analysis

The band profiles generated by the SSR primer pairs wereanalysed and compared to determine the geneticrelationshipbetween the differentmangocultivars. Amplified fragments werescored as present (1) or absent (0). Geneticsimilarity and the similarity matrix wereestimatedusing the Dice coefficient. Dendrogramsshowinggeneticrelationshipswereconstructedusing the Neighbor-Joiningmethodwith DARWIN 6.0.21 software. The 14 SSR primerstestedwereused to calculate the similarity matrices.

3. RESULTS AND DISCUSSION

3.1.RESULTS

3.1.1. Polymorphismdetected by SSR marker analysis

All 14 SSR molecular markers usedwereamplified. Figure 2 shows an extractfrom the results of the polymorphism test, in particular the electrophoretic profile of the microsatellite marker used (mMiCIR011).Allthe markers show bands of different sizes after migration of the PCR products on an agarose gel. Approximate band sizes rangedfrom 156 to 284 bp. The number of bands variedfrom 12 (Figure 2 A) to 6 (Figure 2 B).However, the mMiCIR003 marker onlyrevealed bands from five mango cultivars.PPBrangedbetween85.7% (primer mMiCIR010) to 100% (primer mMiCIR002; mMiCIR003; mMiCIR011; mMiCIR013; mMiCIR025 ; mMiCIR028 ; mMiCIR029 ; mMiCIR030 ; mMiCIR032 ; mMiCIR034 ; and

mMiCIR036), with an average of 97.37%. PIC value variedfrom 0.83 (primer mMiCIR009) to 0.95 (primer mMiCIR003; mMiCIR025 ; mMiCIR034) (Table 4).

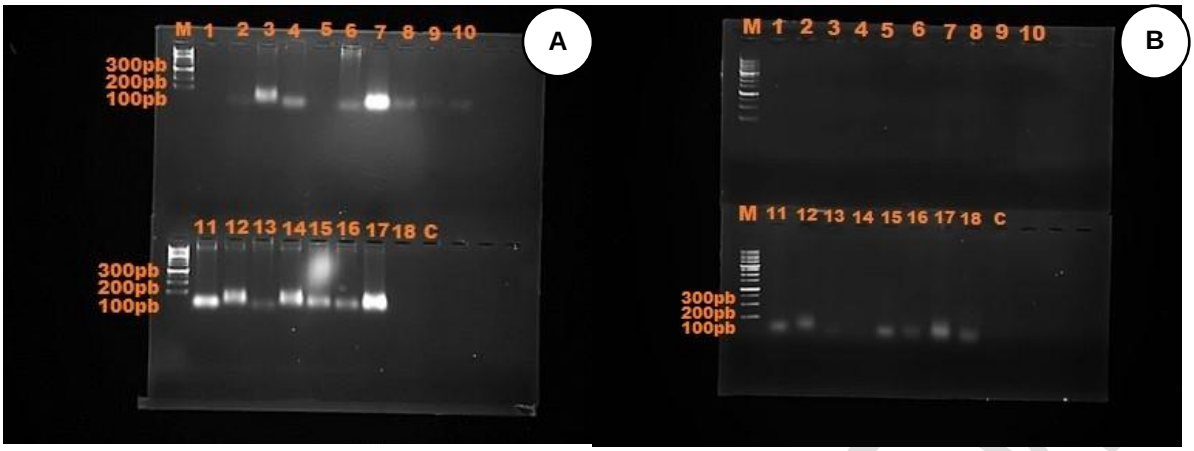

Figure 2: Agarose gel electrophoretic profile of PCR productsfrom DNA extracts of cultivars with microsatellite marker 11 (A) and marker 3 (B).
***Legend:M**: size marker; **1 to 18**: samplessubjected to PCR analysis; **C**: negative control.*

3.1.2. Organisation of geneticdiversity

The matrix resultingfrom the calculation of the genetic distance coefficient revealed the genetic distances of the cultivars in pairs (Table 5). The geneticsimilarityresultingfrom the calculation of genetic distances rangedfrom 0.0002 to 1.09. The greatestgenetic distance (1.09) isbetween VSB and Valencia, VSB and Miamilate. On the other hand, the lowestgenetic distance (0.0002) wasobservedbetweenAlphonso and Francis, Keitt and Sensation, Mangot vert and Glazier. Examination of the dendrogram (Figure 3) enables the cultivars to beclassifiedintotwo major groups. For each of thesegroupings, the distances betweencultivars are relatively large, indicatingsignificantintra-groupvariability. The first group includes the cultivars Miamilate, Valencia, Lippens, Zill, Keitt, Sensation, Kent, Brooks and Bewerly. 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 relativelylow. On the other hand, bootstrap values are relatively high in the second group.

However, when the number of bands amplified for all 14 markers istakenintoaccount, four cultivars werediscardedbecausetheydid not admit enough bands (number of bands lessthan 3 for all markers used). Examination of the dendrogram (Figure 4) enables the cultivars to beclassifiedintothree major groups. The first group comprises seven cultivars, including Dixon, Springfield, Alphonso, Francis, VSB, Bewerly and Kent. The second comprises four cultivars, including Mangot vert, Amélie, Brooks and Sensation. The third comprises two cultivars, including Mangot sabre, Keitt and Glazier.

Table 4:Characteristics of DNA profiles generatedin 18mangocultivars by using14SSRsprimers.

SSRsPrimers	SB	NPB	PPB (%)	PIC
mMiCIR002	11	11	100.0	0.91
mMiCIR003	6	6	100.0	0.95
mMiCIR008	8	7	87.5	0.84
mMiCIR009	10	9	90.0	0.83
mMiCIR010	7	6	85.7	0.85
mMiCIR011	12	12	100.0	0.93
mMiCIR013	9	9	100.0	0.94
mMiCIR025	10	10	100.0	0.95
mMiCIR028	12	12	100.0	0.94
mMiCIR029	11	11	100.0	0.91
mMiCIR030	12	12	100.0	0.94
mMiCIR032	12	12	100.0	0.93
mMiCIR034	12	12	100.0	0.95
mMiCIR036	12	12	100.0	0.90
Sum	144	141	-	-
Average	10.28	10.07	97.37	0.91

SB:Scored bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphism information content.

Table 5:Estimatedgeneticsimilarity (Dice 1945) betweencultivars

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

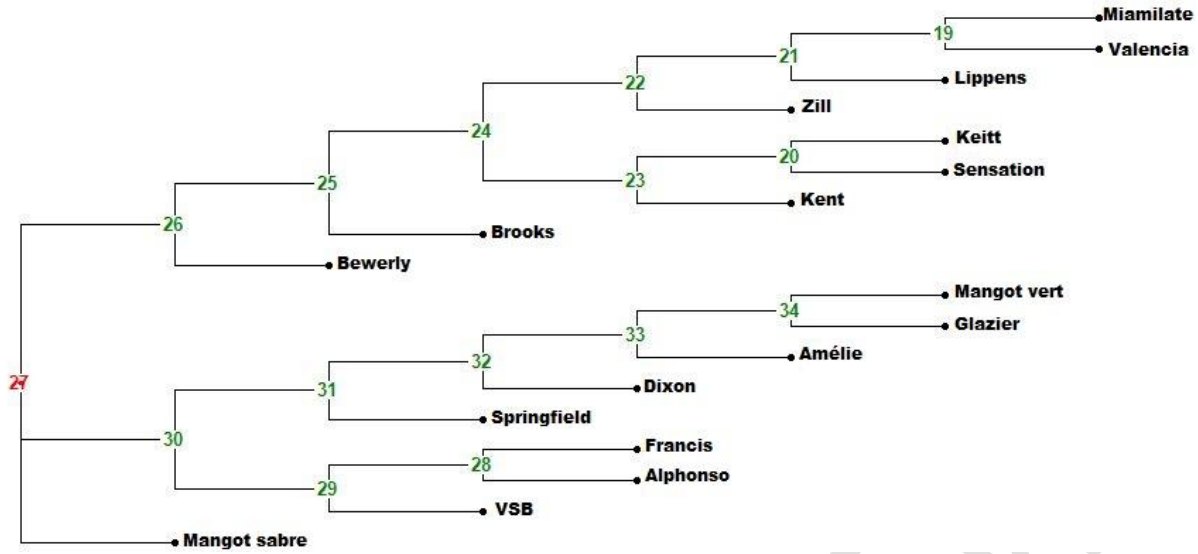

Figure 3:Dendrogramshowing the distribution of mangocultivars using the Neighbour-Joiningmethod.

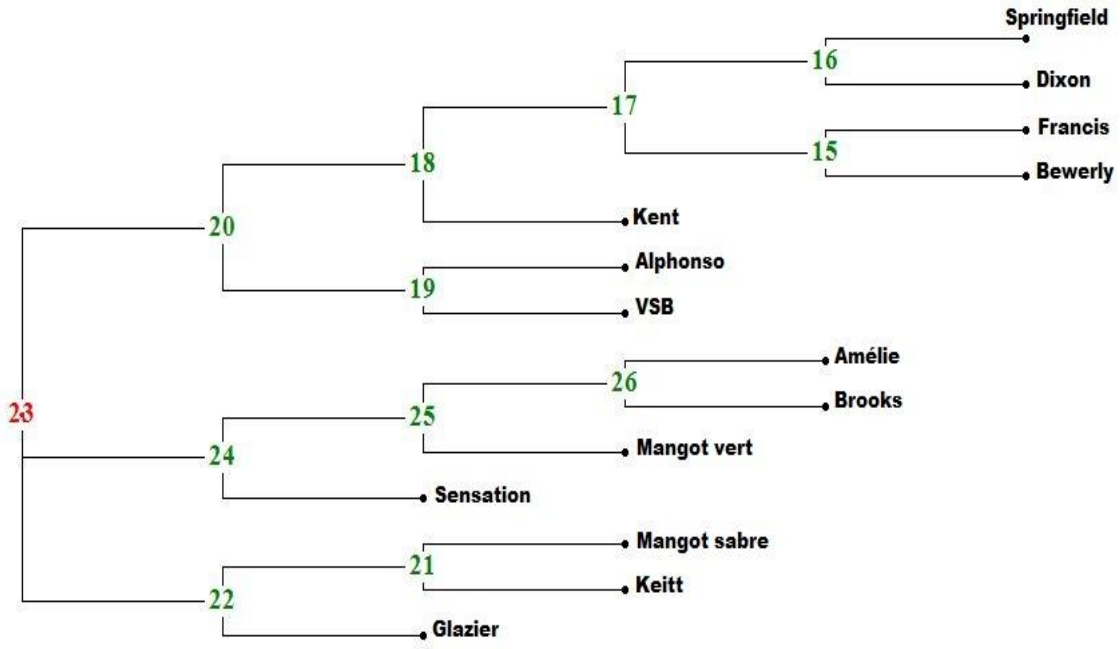

Figure 4:Dendrogramshowing the distribution of 14 mango cultivars thatemitted the most bands using the Neighbour-Joiningmethod.

Principal CoordinateAnalysis (PCoA) based on the similarity matrix confirmsthisgenetic structure within the 18 mangocultivars. Table 6summarises 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: Eigenvalues 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

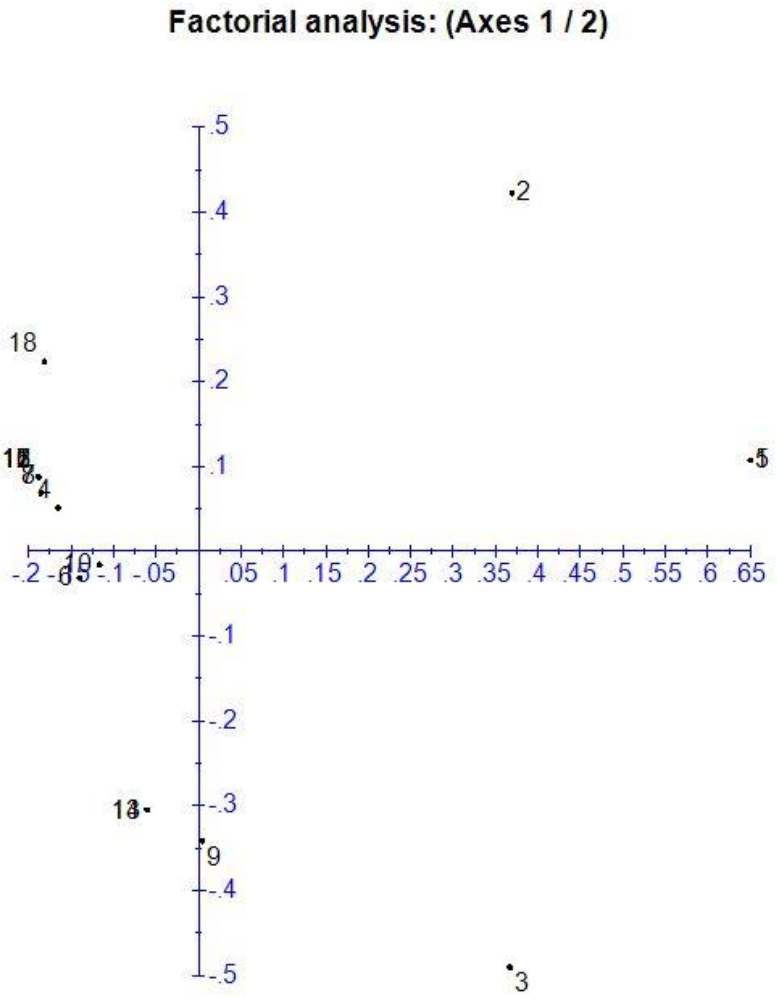

Figure 5: Principal coordinateanalysis of mangocultivars based on SSR molecular data

3.2.DISCUSSION

The 100% polymorphism rate observedindicates a very high level of polymorphism in the SSR markers used. A very high level of polymorphism (100%) wasalsoobtained by [15]using

the same SSR primers. Theseresultsconfirm the effectiveness of SSR primers for discriminatingbetweenindividualswithin a species, evenwith a narrowgenetic base.The high geneticdiversityrevealed by SSR markers between cultivars maybe due to the heterogeneity of their parents [7].Thus, analysis of the genetic distance betweenmangocultivars revealedsufficientdifferences for thesecultivars to begroupedtogether. The greatestgenetic distance (1.09) between VSB and Valencia, VSB and Miamilatereflectsverysignificantgeneticdifferencesbetweenthesetwocultivars. In addition, Valencia and Miamilatemayreactdifferently to mangopathogens and droughtthan VSB. As for the smallestgenetic distance (0.0002) measuredbetweenAlphonso and Francis, Keitt and Sensation, and Mangot vert and Glazier, this shows thatthesecultivars are very close genetically. The large genetic distances betweendifferent cultivars can beexplained by variations in leafcharacteristics and the effect of the environment[16]. This results in the genetic divergence of mango cultivars [13].This shows thatthesecultivars could have the samereaction to pathogenattack and droughtbetweenthem, as indicated by the measurements. Similarly, if the Glaziervariety (monoembryonic) isgenetically close to the Mangot vertvariety, whichispolyembryonic, thiswouldmeanthatthesetwocultivars have the samegene, otherthan the one thatgovernsembryo multiplication. The genetic distances obtained show the greatvariability of mangocultivars in the world. [9]provides ample evidence of this. The twogenetic groups developed by the dendrogram are highly divergent and can beused to advantage in crosses for the geneticbreeding of mango cultivars in Burkina Faso. However, itremains to bedemonstratedthatthesegenetic groups are heterogeneous in the sensethat crosses betweenthem or with local cultivars can give a positive response in terms of important agronomic traits.[2]reported five groups for mangogenotypescollected in Australia. [5]found no significantdifferencewhencomparing cultivars from Myanmar withthosefromIndia and South-East Asia. Molecularanalysis of the cultivars will support the study of theirresistance to Lasiodiplodiatheobromae.[14]alsoreported the existence of gene pools in Banganapalli, Dashehri and Langramangocultivars using SSR markers. The resultsclearlydemonstratedthat PCR-basedtesting of dominant markers is a good tool for geneticanalysis of mangocultivars. This wasdemonstrated by [17]. In addition, marker evaluation has shownthatmostcultivars can beeasilyidentified. Overall, these data expand the knowledge on the application of SSR markers as a molecularcharacterisationtool in mango[6,4].

4. CONCLUSION

This molecularcharacterisation has enabled new knowledge to beacquired at molecularlevel about thesemangocultivars. The general objective was to determined the level of variabilitystructuringin 18 mangocultivars. The fourteenSSRs markers used in thisstudywere all polymorphic. The geneticsimilarityresultingfrom the calculation of genetic distances wasbetween 0.0002 and 1.09. In addition, examination of the dendrogramenabled the cultivars to beclassifiedintotwo major groups of ninecultivars each. This studywillmakeiteasier to conserve the geneticheritage and enhance the adaptation and breeding of mangocultivars in Burkina Faso.

COMPETING INTERESTS

Authors have declaredthat no competinginterestsexist.

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