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

ABSTRACT

Nowadays, molecularcharacterizationis essential for studying the varietaldiversity of species. In addition, SSR molecular markers are widelyused to identify and distinguish the geneticrelationship of mango cultivars. The studyaimis to determine the variabilitystructuringlevel of 18 mango cultivars in Burkina Faso. Thus, genomic DNA wasextractedin 2022 fromyoungleaves at the molecularbiology unit of the Biosciences laboratory at Joseph KI-ZERBO University (Burkina Faso). Analysis of the resultsshowed a polymorphic percentage average of 21.49% per marker. Genetic distance showedthat the similarity coefficient range is 0.0002 to 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 nine cultivars each. The first group includesMiamilate, Valencia, Lippens, Zill, Keitt, Sensation, Kent, Brooks, and Bewerly cultivars. The second group includes the cultivars Mangot vert, Glazier, Amélie, Dixon, Springfield, Francis, Alphonso, VSB and Mangot sabre. Theseresultsshowedthat cultivars are geneticallyvery diverse. Therefore, ourfindingscouldbeused for geneticdiversityanalysis and the marker-assistedbreeding of mangogermplasm.

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

INTRODUCTION

One of the conditions for the success of cropbreedingdepends on the geneticvariabilityavailable in varietal collections. According to [1], there are around 1,000 varieties of mango in the world. [2] claim that the high rate of outcrossingjustified the increase in mangogeneticdiversity. In Burkina Faso, 22 mango cultivars have been identified, providing a large geneticdiversity to beexploited. In addition, efforts are needed to measure the variability of the mangovariety collection on the basis of agro-morphological traits. This has enabled cultivars 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, molecular markers become essential to study plants geneticdiversity. In addition to theirease of use in the laboratory and theireffectiveness in studyingpolymorphism and drawing up geneticmaps, molecular markers are essantial to studyforeigngenes and geneslinked to diseasesresistance or susceptibility. There are currentlyseveral types of molecular markers such as RAPD (RandomAmplifiedPolymorphic DNA), AFLP (Amplified Fragment LengthPolymorphism), RFLP (Restriction Fragment LengthPolymorphism) and SSR (Simple SequenceRepeats). We have chosen SSR markers becauseitis the mostpopularmolecular markers used to detectmolecularpolymorphism in mango [4]. In addition, SSRs markers are known to be codominant, polymorphic and highlyreproducible ([5] ; [6]). Few studies have been carried out on the molecularcharacterization of mangotrees in Burkina Faso. This isnecessary in order to developthis new approach to studygeneticdiversity of mangotrees.

MATERIAL AND METHODS

PLANT MATERIAL

The plant materialconsisted 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 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).

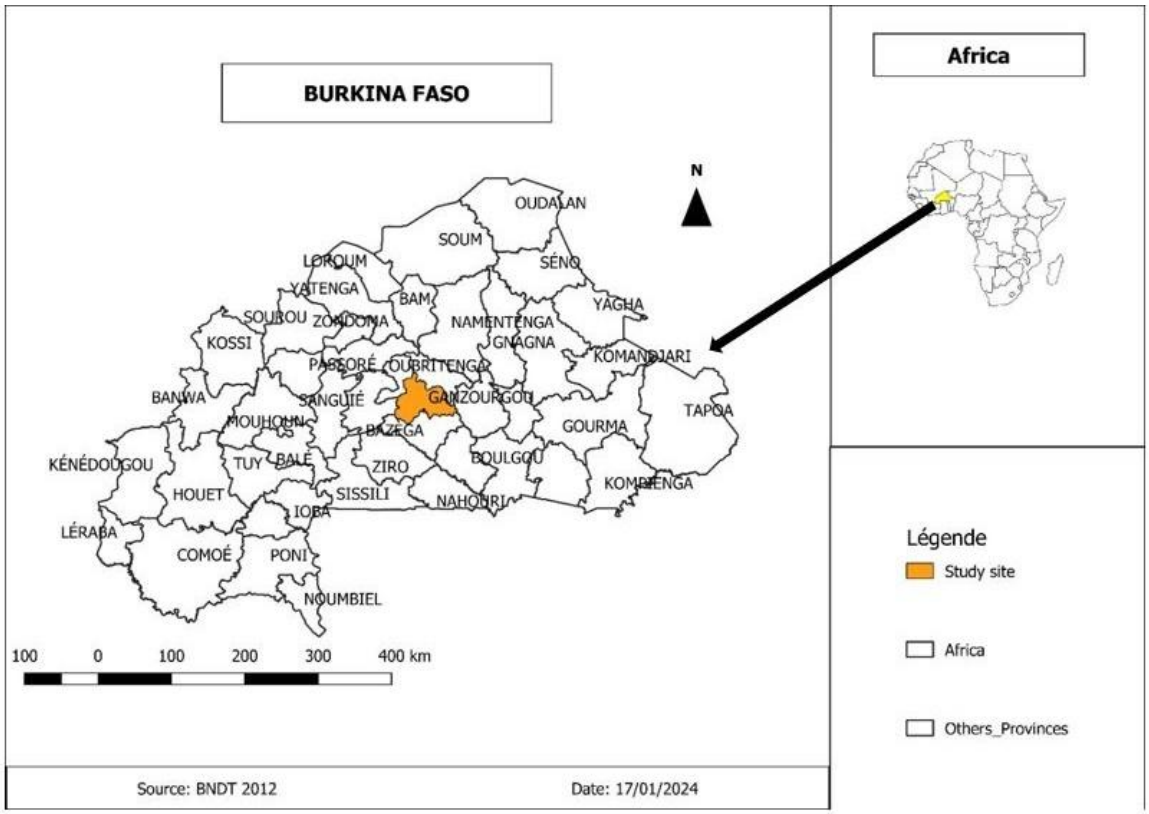

Figure 1:Geographical localisation of the study site

Extraction of genomic DNA

Genomic DNA wasextractedin 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 [7] wasused to extract the DNA of the 18 cultivars. The extraction process was as shownbelow :

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 (Cetyl-trimethyl-ammonium bromide) buffer pre-warmed to 65°C wasadded to the pellet. The tubes wereagitated in order to recover 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.

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.

Initial DNA precipitation

750 µL of fresh (-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.

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 RNasewasadded to each tube and mixed thoroughly. The mixture wasthenincubated at 37°C for at least 30 minutes.

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.

Final DNA suspension

The DNA pellet obtainedaftersupernatantelimination wasdried 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.

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.

Genotyping of DNA extractsusing SSR markers

Fourteen microsatellite markers [4] wereused 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	$(AT)_6G(TG)_{14}(TATG)_6$	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

Amplification of DNA extracts by PCR

A PCR amplification protocoldeveloped by [7] wasused as a guide. The final volume required for the PCR reactionwas set at 13.5 µL. PCR amplification programme usedwastakenfrom the work of Sitbon [4]. The protocol for this programme, 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

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 and synthesised by BIONEER Corporation, Korea. The molecularweightusedwas Fermentas 100 pbs plus.

Data analysis

The band profiles of the SSR primersgeneratedwereanalysed and compared to determinetheirpolymorphism. 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.

RESULTS

Polymorphismdetected by SSR marker analysis

All 14 SSR molecular markers usedwereamplified. Figure 2 shows an extractfrom the results of the polymorphism test, more specifically, the electrophoretic profiles of primers mMiCIR011 and mMiCIR003. 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. PPB rangedbetween 8.33% (primer mMiCIR028 ; mMiCIR032 and mMiCIR036) to 37.5% (primer mMiCIR008), with an average of 21.49%(Table 4).

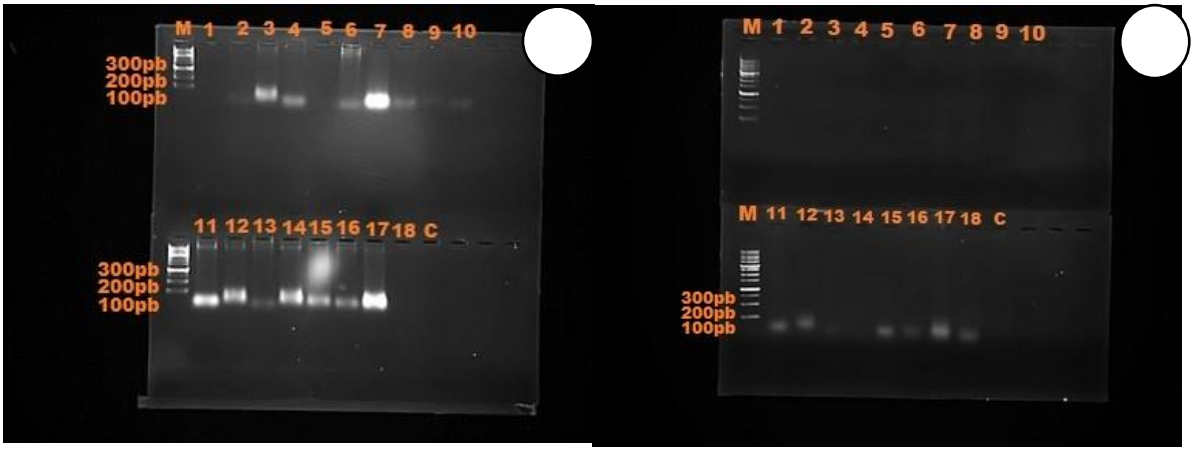

Figure 2 : Agarose gel electrophoretic profile of PCR productsfrom DNA extracts of cultivars with microsatellite marker 11 (A) and marker 3 (B).
Legend:M*: size marker;* ***1 to 18****: samplessubjected to PCR analysis;* ***C****: negative control.*

Organisation of geneticdiversity

The matrix resultingfrom the calculation of the genetic distance coefficient revealed the genetic distances of the cultivars in pairs (Table 5). Genetic distance showedthat the similarity coefficient range is 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. 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.

Table 4:Characteristics of DNA profiles generatedin 18 mango cultivars by using 14 SSRsprimers.

SSRsPrimers	**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:Estimatedgeneticsimilarity (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

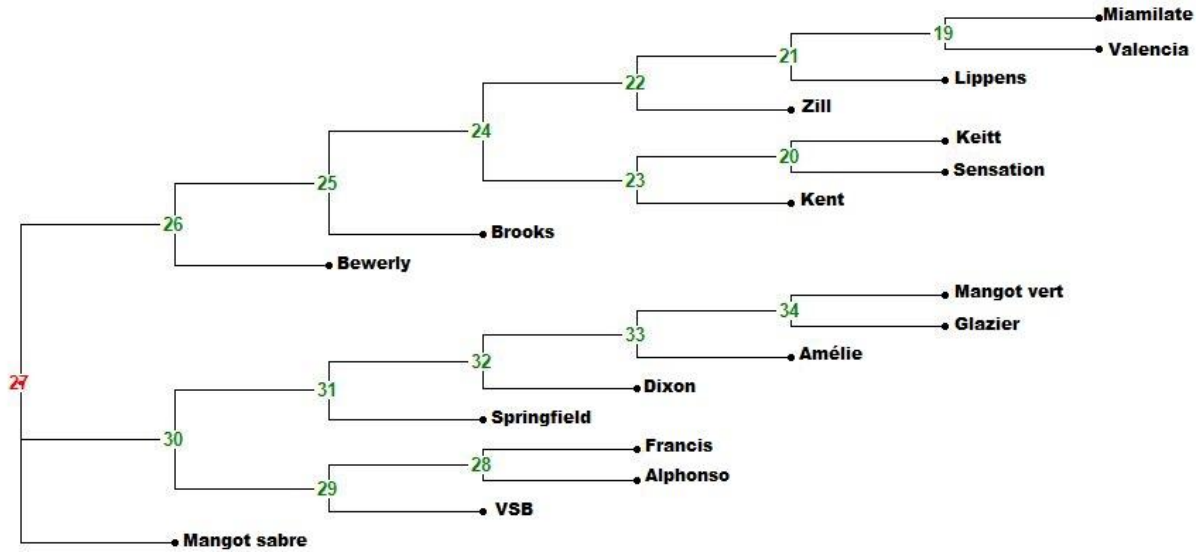

Figure 3:Dendrogramshowing the distribution of mango cultivars using the Neighbour-Joiningmethod.

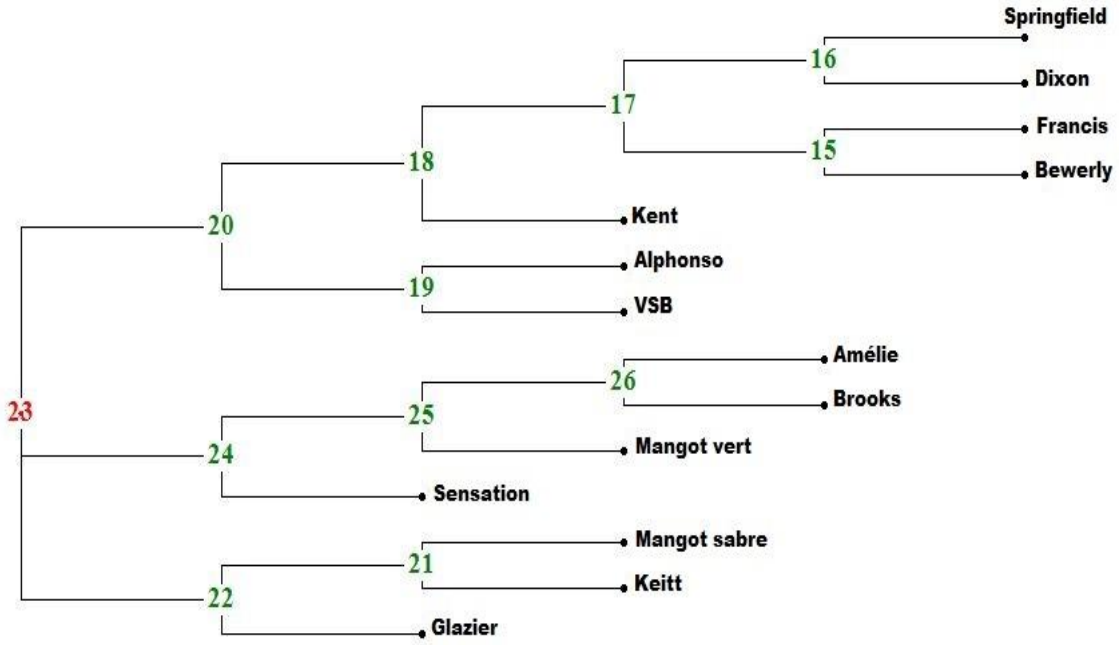

Figure 4:Dendrogramshowing the distribution of 14 mango cultivars thatemitted the most bands using the Neighbour-Joiningmethod.

Principal CoordinateAnalysis (PCoA) based on the similarity matrix confirmsthisgenetic structure within the 18 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

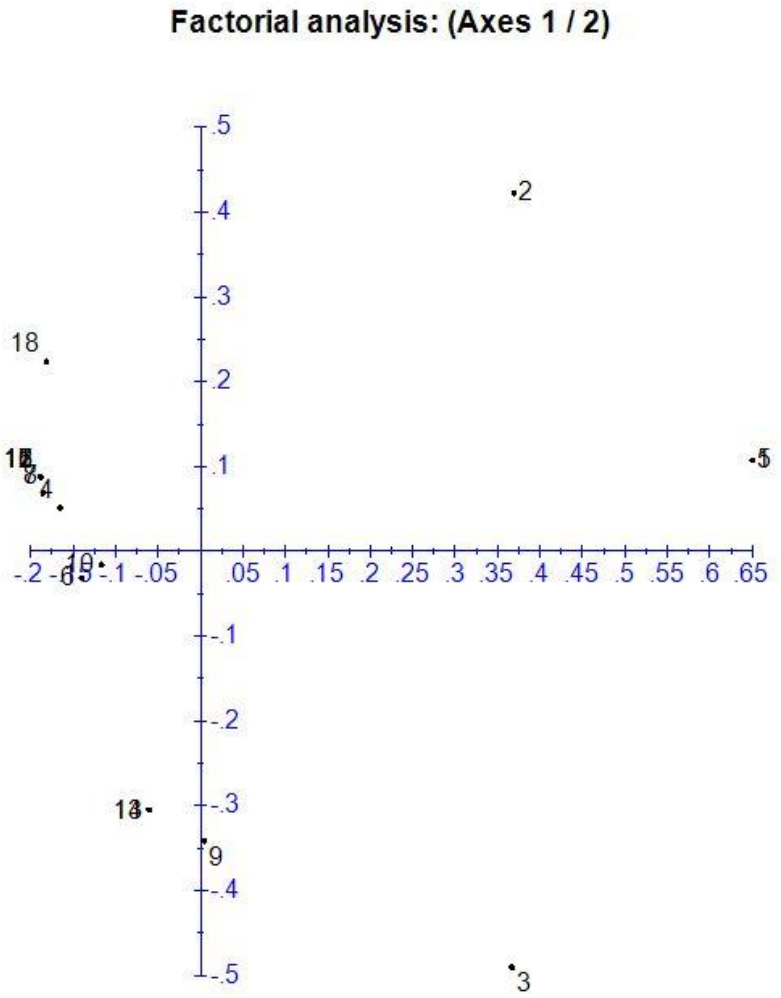

Figure 5 : Principal coordinateanalysis of mango cultivars based on SSR molecular data

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 [4] 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 [8]. Thus, analysis of the genetic distance betweenmango cultivars revealedsufficientdifferences for these cultivars to begroupedtogether. The highestgenetic distance has been obtainedbetween VSB and Valencia, VSB and Miamilate and itcouldbeexplained by the high variabilitythatexistsbetweenthese cultivars. In addition, Valencia and Miamilatemayreactdifferently to mangopathogens and droughtthan VSB. As for the genetic distance (0.0002) measuredbetweenAlphonso and Francis, Keitt and Sensation, and Mangot vert and Glazier, it shows thatthese cultivars are geneticallyverysimilarfrom the perspective of a character. The large genetic distances betweendifferent cultivars can beexplained by variations in leafcharacteristics and the effect of the environment [9]. This results in the genetic divergence of mango cultivars [10]. This shows thatthese cultivars could have the samereaction to pathogenattack and droughtbetweenthem, as indicated by the measurements. Similarly, if the Glaziervariety (monoembryonic) isgeneticallysimilar to the Mangot vert variety, whichispolyembryonic, thiswouldmeanthatthesetwo cultivars have the samegene, otherthan the one thatgovernsembryo multiplication. The genetic distances obtained show the greatvariability of mango cultivars in the world. [11] 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. [12] reported five groups for mangogenotypescollected in Australia. [13] found none 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 Langramango cultivars using SSR markers. The resultsclearlydemonstratedthat PCR-basedtesting of dominant markers is a good tool for geneticanalysis of mango cultivars. This wasdemonstrated by [15]. In addition, marker evaluation has shownthatmost cultivars can beeasilyidentified. Overall, these data expand the knowledge on the application of SSR markers as a molecularcharacterizationtool in mango ([16] ; [17]).

CONCLUSION

The aim of the studywas to determine the degree of variabilitystructuringin 18 mango cultivars in Burkina Faso. The fourteenSSRs markers used in thisstudywere all polymorphic. Genetic distance showedthat the similarity coefficient range is 0.0002 to 1.09. In addition,

examinationof the dendrogramenabled the cultivars to beclassifiedintotwo major groups of nine cultivars each. This studywillmakeiteasier to conserve the geneticheritage and enhance the adaptation and breeding of mango cultivars in Burkina Faso.

CONFLICT OF INTEREST

The authorsdeclarethatthereis no conflict of interest

ACKNOWLEDGEMENTS

Wewould like to thank the unit of the Biosciences laboratory at Joseph KI-ZERBO University for enabling us to carry out thisstudy.

AUTHORS' CONTRIBUTIONS

CD and MK designed the study, performed the statisticalanalysis, wrote the protocol and the first draft of the manuscript. ERT, JS and MS supervised the study and all authorsread and approved the final manuscript.

REFERENCES

1. Rivier M, Méot JM, Ferré T, Briard M. Le Séchage des Mangues. Éditions Centre Technique de Coopération Agricole et Rurale (CTA) : Pays Bas ; 2009.
2. Ravishankar KV, Lalitha A, Dinesh MR, Anand L. Assessment of geneticrelatednessamongmango cultivars of Indiausing RAPD markers. J. Hortic. Sci. Biotechnol. 2000 ; 75(2): 198-201.
3. Drabo C, Dianda ZO, Sanou J, Nikiema Z, Dao A, Sawadogo M. Study of mango (*Mangiferaindica* L.) cultivars reactionsagainst*Lasiodiplodiatheobromae* (Pat.) Griffon &Maubl. in Burkina Faso. Journal of Applied Biosciences. 2022 ; 178: 18644-18657.
4. Sitbon C. Study of the geneticdiversity of West Indianmangotrees (Mangiferaindica L.) using microsatellite markers. Dissertation D.E.S.S. Université Paris 7, France ; 2004.
5. Rajwana IA, Khan IA, Malik AU, Saleem BA, Khan AS, Ziaf K, Amin M. Morphological and biochemical markers for varietalcharacterization and qualityassessment of potentialindigenousmango (*Mangiferaindica* L.) germplasm. Int. J. Agric. Biol. 2011; 13: 151-158.
6. Kumari S, Chakrabarty SK, Bhowmick PK, Singh VJ, Prasad AH. Validation of hybridriceseedvigour traits using SSR marker (*Oryza sativa* L.). Indian J. Genet. Plant Breed. 2020 ; 80(2): 204-208.

7. Agbangla C, Ahanhanzo C, Tostain S, Dansi A, Dainou O. Evaluation of geneticdiversity by RAPD of a sample of Dioscoreaalatafrom a region of Benin, the Savè sub-prefecture. J. Rech. Sci. Uni. 2002 ; 6(1): 197-202.
8. Kumar G, Srivastav M, Sreekanth HS, Kumar C, Prakash J, Singh SK, Vinod. SSR assisted identification of mango (*Mangiferaindica*L.) hybrids and development of DNA barcodes. Indian J. Genet. Plant Breed. 2023 ; 83(3): 437-445.
9. Syed RI, Kumari K, Abha K, Abhay M, Feza A. VarietalCharacterization and QualityAssessment of Mango Hybrid and their Parents throughMorphological and Biochemical Markers. Int. J. Curr. Microbiol. App. Sci . 2019 ; 8(4): 697-706.
10. Rymbai H, Laxman RH, Dinesh MR, Johnsunoj VS, Ravishankar KV, Jha AK. Diversity in leafmorphology and physiologicalcharacteristicsamongmango (*Mangiferaindica*L.) cultivars popular in different agro-climatic regions of India. Sci. Hort. 2014 ;176: 189-193.
11. Mir JI, Karmakar PG, Chattopadhyay S, Chaudhury SK, Ghosh SK, Roy A. SSR and RAPD Profile BasedGrouping of Selected Jute Germplasmwith Respect to Fibre Fineness Trait. J. Plant Biochemistry&Biotechnology. 2008 ; 17(1) : 29-35.
12. Dillona NL, Ballya ISE., Wrighta CL, Hucksa L, Innesb DJ, Dietzgen RG. Geneticdiversity of the Australian National Mango Genebank. Sci. Hortic. 2013 ;150: 213-226.
13. Hirano R, HtunOoT, Watanabe KN. Myanmar mangolandracesrevealgeneticuniqueness over common cultivars from Florida, India, and South-east Asia. Genome. 2010 ; 53(4): 321-330.
14. Singh S, Gaikwad AB, Karihaloo JL. Morphological and molecularanalysis of intra cultivarvariation in Indianmango (*Mangiferaindica* L.) cultivars. Acta Horti. 2009 ; 829 : 205-212.
15. Ukoskit K. Development of microsatellite markers in mango (*Mangiferaindica* L.) using 5' anchored PCR. Thammasat Int. J. Sci. Technol. 2007 ; 12 : 1-7.
16. Krauss SL. Accurategenediversityestimatesfromamplified fragment lengthpolymorphism (AFLP) markers. MolecularEcol. 2000 ; 9 : 1241-1245.
17. González A, Coulson M, Brettell R. Development of DNA markers (ISSRs) in Mango. Acta Horticulturae. 2002 ; 575 : 139-143.