

Molecular Characterization of Local Algerian Olive Cultivars

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Article history: Received: August 15th 2025 ; Revised: November 15th 2025 ; Accepted: December 11th 2025

Abstract

Algeria hosts a multitude of olive varieties, with 36 officially recognized and conserved at the ITAFv station. Nonetheless, prior research, predominantly centered on these 36 varieties, may undervalue the actual genetic diversity of Algerian olive trees. Ancient and indigenous varieties, frequently neglected and endangered by climate and socio-economic influences, require particular consideration. This study employed microsatellite markers (SSR) to investigate 23 varieties from several locations in Algeria, specifically Bejaia, Skikda, Guelma, Khenchela, and Biskra, to enhance the understanding of this diversity. The extraction and analysis of DNA identified 134 distinct alleles. The mean number of alleles per locus was 11.17, with a range from 6 to 16. Genetic diversity was evaluated using several parameters, including observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphic information content (PIC). The findings indicate considerable variability among the examined varieties, with heterozygosity values ranging from 0.33 to 1.00 and an average of 0.82. The mean PIC was 0.87, signifying considerable variability among the varieties. The differentiation indices (F_{st}) indicate significant differentiation among the varieties. These results emphasize the importance of characterizing lesser-known and unregistered olive cultivars to safeguard Algeria's olive germplasm and to inform future strategies for conservation and sustainable management and conservation efforts.

Keywords: olive, local varieties, genetic diversity, SSR markers, Algeria.

المخلص

تحتضن الجزائر مجموعة متنوعة من أصناف الزيتون، حيث تم الاعتراف رسمياً بـ 36 صنفاً منها وحفظها في محطة المعهد التقني للأشجار المثمرة والكروم (ITAFv). ومع ذلك، فإن الأبحاث السابقة التي ركزت بشكل رئيسي على هذه الأصناف الـ 36 قد تقلل من القيمة الحقيقية للتنوع الوراثي لأشجار الزيتون الجزائرية. فالأصناف القديمة والمحلية، التي غالباً مايتجاهلها والمهددة بالتغيرات المناخية والضغط الاجتماعي والاقتصادية، تتطلب اهتماماً خاصاً. اعتمدت هذه الدراسة على مؤشرات الميكروساتلايت (SSR) لدراسة 23 صنفاً من مناطق مختلفة في الجزائر، وهي: بجاية، سكيكدة، قالمة، خنشلة و بسكرة، بهدف تعزيز فهم هذا التنوع. أبتعملية استخراج وتحليل الحمض لن و و يالبتحديد 134 أليلاً مميزاً. وكان المتوسط العام لعدد الأليلات تلك لموقع وراثي 11.17، تراوح بين 6 و 16. تمقيم التنوع الوراثي باستخدام عدة مؤشرات، منها التباين الوراثي الملحوظ، (H_o) والتباين الوراثي المتوقع، (H_e) ومحتوى المعلومات متعددة الأشكال (PIC). أشارت النتائج إلى وجود تباين كبير بين الأصناف المدروسة، حيث تراوح تقيم التباين الوراثي من 0.33 إلى 1.00 بمتوسط قدره 0.82. و بلغ متوسط قيمة PIC حوالي 0.87، مما يدل على وجود تنوع كبير بين الأصناف. كم أظهرت مؤشرات التمايز (F_{st}) وجود تمايز ملحوظ بين الأصناف. تؤكد هذه الدراسة على ضرورة توصيف الأصناف غير المعروفة وغير المدرجة من أشجار الزيتون، من أجل حماية التنوع الوراثي في الجزائر وإبراز أهميتها في جهود الإدارة والحفاظ المستقبلية.

الكلمات المفتاحية: الزيتون، الأصناف، التنوع الوراثي، الميكروساتلايت، الجزائر.

Introduction

Algeria, like other Mediterranean countries, has a long history of olive cultivation. The area dedicated to its cultivation occupies more than a third of the land allocated to fruit tree farming. With its highly diverse bioclimatic zones, Algeria represents a significant reservoir of olive resources. While the Western region is dominated by the Sigoise variety and a few introduced varieties, the central and eastern areas exhibit a very high varietal richness.

Algeria officially has 36 identified and preserved varieties in an experimental station (ITAFv). The latter is the result of identification following the work carried out by Mendil and Sebai (2006) in collaboration with the International Olive Council. In fact, Hauvill, in his research on the prospecting and identification of Algerian olive resources conducted in 1953, noted the existence of 150 varieties, more

or less abundant. Historical events that Algeria experienced throughout the ages (conquests and migrations of populations) allowed the olive tree to settle in various regions, even far from the Mediterranean basin, under extreme climatic conditions. Thus, we know today that in several traditional olive-growing areas in central and eastern Algeria, a considerable number of denominations have unique characteristics. (e.g, Khenchela, Biskra, Tizi-ouzou, Bejaia, Setif, Skikda, Guelma, Batna et El Taref).

Over the past ten years, several studies on genetic characterization using molecular markers have been conducted. The majority of studies have focused on the 36 varieties previously mentioned, which leads to an underestimation of the level of diversity that the olive tree could have in Algeria. However, the number of varieties studied is very far from the actual number of existing varieties in Algeria. Thus, several other ancient Indigenous varieties exist in different regions that remain uncharacterized. These varieties are underutilized and threatened with extinction due to various technical, climatic, and socio-economic constraints. The significant lack of information about the diversity of this culture and the accelerated climate changes that Algeria is experiencing, compounded by issues of poor management (inappropriate practices, lack of resources and modern expertise, promoting the cultivation of certain Algerian varieties like Chemlal or introduced ones at the expense of Indigenous varieties, absence of an effective national program for the preservation of phytogenetic resources, etc.) threatens genetic richness due to the risk of extinction of old varieties with limited distribution. These varieties can exhibit very interesting characteristics in terms of production, quality, resistance, and adaptation. The problem lies in the permanent loss of a portion of olive resources that remain poorly understood and whose consequences are not currently assessed adequately.

In several olive-growing regions of central and eastern Algeria (El Taref, Bouira, Setif, Tizi-Ouzou, Bejaia, Batna, Constantine, Skikda, Guelma, etc.), some varieties are very limited in distribution and very little known, unregistered, and traditionally maintained by local populations. It is also encountered in some remote areas, with an arid to Saharan climate, numerous small olive-growing regions rich in unrecorded resources (Alloum, 1974). This is the case for the regions of Khirene and Zaouia (Khenchela province) and Ain Zaatout (the village of AthFerrah, Biskra province), where this culture has been known since at least Roman times, as evidenced by the presence of ancient oil mills and trees that are several hundred to thousands of years old. (Camps-Fabrer, 1953). These varieties are of great importance due to their adaptation to the region's climate, their agronomic characteristics, and their socio-economic and ecological significance.

In this work, a portion of these varieties with very limited use is analyzed using microsatellite markers and molecularly compared to widely distributed varieties to see if genetic specificities exist between them. This information will help inform decisions during the management and preservation plan for olive resources.

Materials and Methods

A total of 23 local olive (*Olea europaea* L.) varieties underwent molecular analysis using (SSR) markers. These varieties are sampled in the following areas of eastern Algeria: Bejaia (ITAFv of Sidi Aiche), Skikda, Guelma, Setif, Khenchela (Khirene and Zaouia), and Biskra. (AineZaatout).

DNA extraction

100 mg of the young leaves of each of the 23 previously sampled varieties, stored at -80°C, were ground in liquid nitrogen. The extraction of genomic DNA was carried out according to the method described by Sá et al. (2011).

The quality of the DNA was verified by electrophoresis in a 0.8% agarose gel, and the concentration was estimated using a Nanodrop microvolume spectrophotometer, mySPECTwin. (VWR, Germany).

SSR markers used

Twelve (12) SSR markers (Table01), previously published and tested as useful for characterizing olive varieties, were used to study the genetic diversity of the 23 varieties.

PCR amplification

The PCR was performed for a final volume of 20 µL containing 20 ng of DNA, 2 µL of colorless 5X GoTaq® reaction buffer (Promega), 2.5 mM of MgCl₂, 200 µM of each dNTP, 0.25 µM of each primer (the forward primer was labeled with a fluorescent dye, either 6-FAM or HEX), and 0.03 units of

GoTaq® DNA Polymerase. (Promega). The PCRs were performed in a MyCycler thermocycler (Bio-Rad) with an initial denaturation at 94 °C for 4 minutes, followed by 35 cycles of 92 °C for 30 seconds, 50-55 °C for 30 seconds, and 72 °C for 1 minute, then a final extension at 72 °C for 7 minutes. Before moving on to the next step (Capillary Electrophoresis), a check for the presence of bands of the amplified segments is performed on a 2% agarose gel.

Table 01: SSR Markers Used

SSR Marker	Primer Sequences (5' – 3')	Authors
DCA03	F CCC AAG CGG AGG TGT ATA TTG TTA C	Sefc et al., 2000
	R TGC TTT TGT CGT GTT TGA GAT GTT G	
DCA05	F AACAAATCCCATACGAACTGCC	
	R CGTGTGCTGTGAAGAAAATCG	
DCA09	F AAT CAA AGT CTT CCT TCT CAT TTC G	
	R GAT CCT TCC AAA AGT ATA ACC TCT C	
DCA14	F AATTTTAAATGCACTATAATTTAC	
	R TTGAGGTCTCTATATCTCCAGGGG	
DCA18	F AAG AAA GAA AAA GGC AGA ATT AAG C	
	R GTT TTC GTC TCT CTA CAT AAG TGA C	
GAPU-59	F CCCTGCTTTGGTCTTGCTAA	
	R CAAAGGTGCACTTTCTCTCG	
GAPU-101	F CATGAAAGGAGGGGGACATA	Carriero et al., 2002
	R GGCACCTTGTGTGCAGATTG	
GAPU-71B	F FAM-GATCAAAGGAAGAAGGGGATAAA	
	R ACAACAAATCCGTACGCTTG	
UDO99-024	F GGATTTATTTAAAGCAAAACATACAAA	Cipriani et al., 2002
	R CAATAACAAATGAGCATGATAAGACA	
UDO99-043	F TCGGCTTTACAACCCATTTT	
	R TGCCAATTATGGGGCTAACT	
UDO99-039	F AAT TAC CAT GGG CAG AGG AG	
	R CCC CAA AAG CTC CAT TAT TGT	
EM090	F CATCCGGATTCTTGCTTTT	de la Rosa et al., 2002
	R AGCGAATGTAGCTTTGCATGT	

Capillary electrophoresis

The PCR products were separated by capillary electrophoresis, and the genotype profiles were visualized and assessed using Peak Scanner V 1.0 software.

The sizes of the amplified products were determined based on an internal standard included with each sample.

Studied parameters

The following parameters are determined using various bioinformatics software:

- The number of detected alleles (Na), the number of effective alleles (Ne), the observed heterozygosity (Ho), the expected heterozygosity (He), the number of private alleles, and the percentage of polymorphic alleles are calculated using the software Popgene 1.32.
- The polymorphic information content (PIC) for each locus was determined with GenePop version 4.2.2
- The allele frequency, differentiation index (Fst), and PCoA are performed using the software GenAlEx 6.51b2.
- Genetic relationships among the 23 olive varieties were illustrated using a dendrogram created using the software Popgene 1.32.

Results and discussion

Table 02 presents the different parameters analyzed for the 12 SSRs used in this study. They identify rank, the number of detected alleles, the effective number of alleles, polymorphic information content, observed heterozygosity, expected heterozygosity, differentiation index, and the number of private alleles.

Table 2. Genetic parameters of each locus in all studied varieties.

Locus	Allelic range (bp)	Na*	Ne*	PIC	Ho	He	Fst	Private Alleles
EM090	183-195	6	4,11	0,81	1,00	0,77	0,34	0
GAPU-71B	116-160	11	6,68	0,87	0,78	0,87	0,75	4
DCA09	161-195	16	11,11	0,92	0,93	0,93	0,48	4
UDO99-024	177-203	7	3,68	0,83	0,58	0,74	0,70	2
UDO99-043	158-216	9	5,62	0,85	0,86	0,84	0,66	3
GAPU-101	173-253	13	8,91	0,93	0,33	0,91	0,85	6
UDO99-039	104-182	9	3,84	0,81	0,67	0,75	0,54	3
DCA03	231-255	11	5,86	0,85	0,97	0,84	0,41	3
DCA14	147-193	15	9,23	0,90	1,00	0,91	0,43	3
DCA5	176-214	14	4,39	0,84	0,89	0,79	0,51	5
DCA18	153-207	14	9,19	0,93	0,86	0,91	0,67	7
GAPU-59	204-228	9	4,13	0,84	0,93	0,77	0,42	3
Mean		11,17	6,40	0,87	0,82	0,84	0,56	3,58

A total of 134 different alleles were detected, with sizes ranging from 104 bp (marker UDO99-039 for the following varieties: Aeleh, Ahia Ousbah, Akenane, Azeradjde AZ, Azizawth, Alslith, and Mekki) to 255 bp (marker DCA03 for the variety Azizawth). This result is higher than that obtained by Dominguez-Garcia et al. (2012), who detected 77 alleles by analyzing 19 varieties, including 16 from Algeria, using a set of 10 SSR markers. Other studies on Algerian olive resources report detected allele numbers comparable to our results (122 to 210 detected alleles). (Haouane et al., 2011 ; Abdessemed et al., 2015 ; Boucheffa et al., 2016 ; di Rienzo et al., 2018 ; El Bakkali et al., 2020 ; Haddad et al., 2020). Mansouri and Benbouza (2014), in a study conducted on 7 Algerian varieties using 7 SSR markers, report the presence of only 48 alleles.

The average number of alleles detected per locus is 11.17 (ranging from 6 for EM090 to 16 for DCA09). This number is very close to those obtained by Atrouz et al. (2021) and Abdessemed et al. (2015) (10,6 et 11,7, respectively). Other authors (Haddad et al., 2020; Dominguez-Garcia et al., 2012) obtained lower average numbers of detected alleles, while others reported much higher average numbers (ranging from 16.27 to 18.5) (Haouane et al., 2011; El Bakkali et al., 2020; di Rienzo et al., 2018). The DCA09 marker records the highest number of detected alleles (16 alleles), followed by the DCA05, DCA14, and DCA18 markers, which recorded a number of 14 to 15 detected alleles. On the other hand, the EM090 and UDO99-024 markers recorded the lowest numbers, with 6 to 7 alleles detected.

Among the 134 amplified alleles, 43 were private, with an average of 3.58 alleles per marker. The markers DCA18 and GAPU-101 expressed the most private alleles (6 to 7 alleles), while the marker EM090 did not yield any private alleles. The other remaining markers yielded a number of private alleles ranging from 2 to 5 alleles.

The average number of effective alleles was 6.40 (ranging from 3.68 for UDO99-024 to 11.11 for DCA09). This result is close to that obtained by Boucheffa et al. (2016), who recorded an average of 5.87. Haddad et al. (2020), in a study involving 34 varieties, recorded an average of less than 3.6. Di Rienzo et al. (2018), in a study on 128 varieties, including 25 Algerian ones, found a higher average number (7,4).

The analysis and combination of the 134 alleles amplified by the 12 SSRs allowed us to distinguish the 23 varieties studied clearly. However, none of the SSRs used were able to differentiate all the varieties on their own. The locus that allowed for the distinction of the greatest number of varieties is DCA14 (10 varieties), while the markers EM090 and GAPU-59 only allowed for the distinction of 3 varieties.

The observed heterozygosity (Ho) ranged from 0.33 for the locus GAPU-101 to 1.00 for EM090, DCA14. The average value of the observed heterozygosity appears to be very high. (0,82). Different values are obtained by various authors on Algerian varieties (between 0.488 and 0.851). (Haouane et al., 2011 ; Mansouri et Benbouza, 2014 ; Abdessemed et al., 2015 ; Boucheffa et al., 2016 ; di Rienzo

et al., 2018). In a study conducted in Tunisia on a group of individuals resulting from inter-varietal crossings, Dridi et al. (2018) recorded a very high average value of H_o at 0.987, which is higher than ours. The observed heterozygosity was greater than 0.50 for most loci, indicating significant and high diversity among the analyzed varieties.

The expected heterozygosity (H_e) ranged from 0.74 for UDO99-024 to 0.93 for DCA09, with an average of 0.83. The average value of the expected heterozygosity obtained is identical to that obtained by Di Rienzo et al. (2018) in a study on Algerian varieties. Lower average values have been recorded in other studies on Algerian varieties (between 0.699 and 0.776) (Boucheffa et al., 2016; El Bakkali et al., 2020; Haddad et al., 2020), while in other studies (Mansouri & Benbouza, 2014; Abdessemed et al., 2015), much lower values have been recorded (0.334 and 0.312, respectively).

The polymorphic information content (PIC) showed values ranging from 0.81 for the markers EM090 and UDO99-039 to 0.93 for GAPU-101 and DCA18, with an average of 0.87. This result is close to that recorded by di Rienzo et al. (2018) but higher than the averages obtained by other authors (Dominguez-Garcia et al., 2012; El Bakkali et al., 2020; Haddad et al., 2020) who found averages around 0.66. PIC values greater than 0.8 indicate that the studied varieties exhibit a high level of diversity and that the SSRs used are effective for molecular characterization.

The differentiation index (F_{st}) shows all positive values ranging from 0.34 to 0.85, with an average of 0.56, indicating a fairly high degree of differentiation among the varieties.

The Aeheh and Blanquette de gastu varieties exhibited the highest number of private alleles (4) at the UDO99-043, UDO99-039, DCA5, DCA18 markers for the Aeheh variety, and at the GAPU-71B, GAPU-71B, GAPU-101, DCA14 markers for the Blanquette de gastu variety. The varieties Abeskri, Balbal (petit), Derdi, Mekki, and Reyabeach exhibited 3 private alleles. The other varieties exhibited private allele counts ranging from 1 to 2 (Table 03).

Table 3. Private Alleles by Variety

Cultivars	Markers				Total
Abeskri	GAPU-71B (123)	DCA18	GAPU-59		3
Aeheh	UDO99-043	UDO99-039 (123)	DCA5	DCA18	4
Ahia ousbaa	DCA18				1
Akenane	DCA5				1
Azerradj AZ	DCA5				1
Balbal (gros)	DCA5				1
Balbal (petit)	GAPU-101	DCA03	DCA18		3
Blanquette de gastu	GAPU-71B	GAPU-71B	GAPU-101 (123)	DCA14 (123)	4
Blanquette de Guelma	UDO99-039				1
Boukaila	DCA09	UDO99-024			2
Bouricha	UDO99-039				1
Derdi	GAPU-101	GAPU-101	GAPU-59		3
Grosse de Hamma	DCA14				1
Hamraya	DCA14				1
Hebraya	UDO99-043				1
Kahlaya	GAPU-101	GAPU-59			2
Khadraya	GAPU-71B				1
Mekki	DCA09	DCA03	DCA03		3
Nebjmel	DCA09	DCA18			2
Oukhelfa	DCA09				1
Reyab	GAPU-101	DCA18	DCA18		3
Rougette fejourj	UDO99-043				1
Souidi	UDO99-024	DCA5			2
Total					43

The allele frequencies (Table 04) vary from a minimum of 0.02 at several loci and for several alleles to a maximum of 0.44 for allele (185) at the UDO99-024 locus and allele (204) at the DCA5 locus.

Principal Component Analysis PCoA

The analysis represented in Figure 01 expressed 20.76% of the total information and divided our varieties into several groups that sometimes aligned with their geographical origins (distance and/or climatic zone), as is the case, for example, with the varieties of Abeskri KH and Ferkani, Reyab and Hamraya, and Souidi and Bouguenfou. At other times, they are grouped according to their commercial purposes (table olive or oil), as seen with Balbal and Abeskri AZ, which are table olives, and Blanquette de Guelma, Hebraya, Mekki, and Boukaila; which are generally intended for oil production.

Table 4. Allelic frequencies of the SSR markers used.

Locus															
EM090	183	185	187	189	191	195									
	0,15	0,35	0,08	0,10	0,28	0,03									
GAPU-71B	116	118	120	122	124	126	136	140	160						
	0,08	0,03	0,25	0,19	0,06	0,06	0,06	0,22	0,06						
DCA09	161	167	169	171	173	175	177	179	181	183	185	187	189	191	
	0,15	0,02	0,02	0,08	0,02	0,08	0,02	0,05	0,12	0,07	0,13	0,03	0,05	0,08	
	193	195													
	0,03	0,05													
UDO99-024	177	179	183	185	189	191	203								
	0,04	0,23	0,08	0,44	0,04	0,13	0,04								
UDO99-043	158	174	176	180	186	194	196	214	216						
	0,02	0,26	0,21	0,12	0,05	0,02	0,02	0,19	0,10						
GAPU-101	173	179	181	183	185	187	189	193	199	231	239	249	253		
	0,02	0,05	0,07	0,05	0,14	0,12	0,07	0,02	0,02	0,17	0,17	0,07	0,02		
UDO99-039	104	106	144	170	174	176	178	180	182						
	0,25	0,42	0,02	0,10	0,02	0,10	0,03	0,02	0,05						
DCA03	231	233	235	239	241	243	245	249	251	253	255				
	0,25	0,02	0,02	0,25	0,03	0,12	0,03	0,13	0,02	0,10	0,03				
DCA14	147	153	155	157	161	167	169	171	173	175	177	179	181	187	
	0,02	0,05	0,10	0,02	0,07	0,03	0,10	0,07	0,03	0,03	0,18	0,03	0,07	0,18	
	193														
DCA5	176	178	188	192	196	198	200	202	204	206	208	210	212	214	
	0,09	0,02	0,04	0,06	0,02	0,02	0,04	0,06	0,44	0,07	0,04	0,06	0,04	0,02	
DCA18	153	159	165	167	169	171	173	175	177	181	183	197	203	207	
	0,02	0,05	0,05	0,14	0,12	0,12	0,14	0,17	0,02	0,02	0,05	0,05	0,02	0,02	
GAPU-59	204	208	210	212	214	218	222	224	228						
	0,02	0,19	0,12	0,41	0,09	0,03	0,10	0,02	0,02						

It is worth noting that the 20.76% information percentage expressed in this analysis is very low, indicating a significant loss of information.

Dendrogram

Figure 02, which represents the dendrogram results, does not truly reflect the PCoA results, as there was a significant loss of information at this level. That said, it noted that grouping by region and commercial purpose is more explicit here and divides the varieties studied into 6 or 7 distinct groups.

For certain varieties, the grouping is done according to economic purpose because farmers have pushed selection in such a way that there is a closeness between varieties, such as in the case of Derdi and Reyab (oil), Bouricha and Nebjmel (oil), Chemlal and Melissi (oil), Abeskri and Balbal (table olives), Boukaila and Hebraya (oil). In other cases, the selective pressure from the farmer is not significant, and therefore, the grouping is based on agro-ecological belonging, as is the case with Bouguenfou and Souidi (Zaouia, Khenchela), Hamraya and Khadraya. (Khirene, Khenchela).

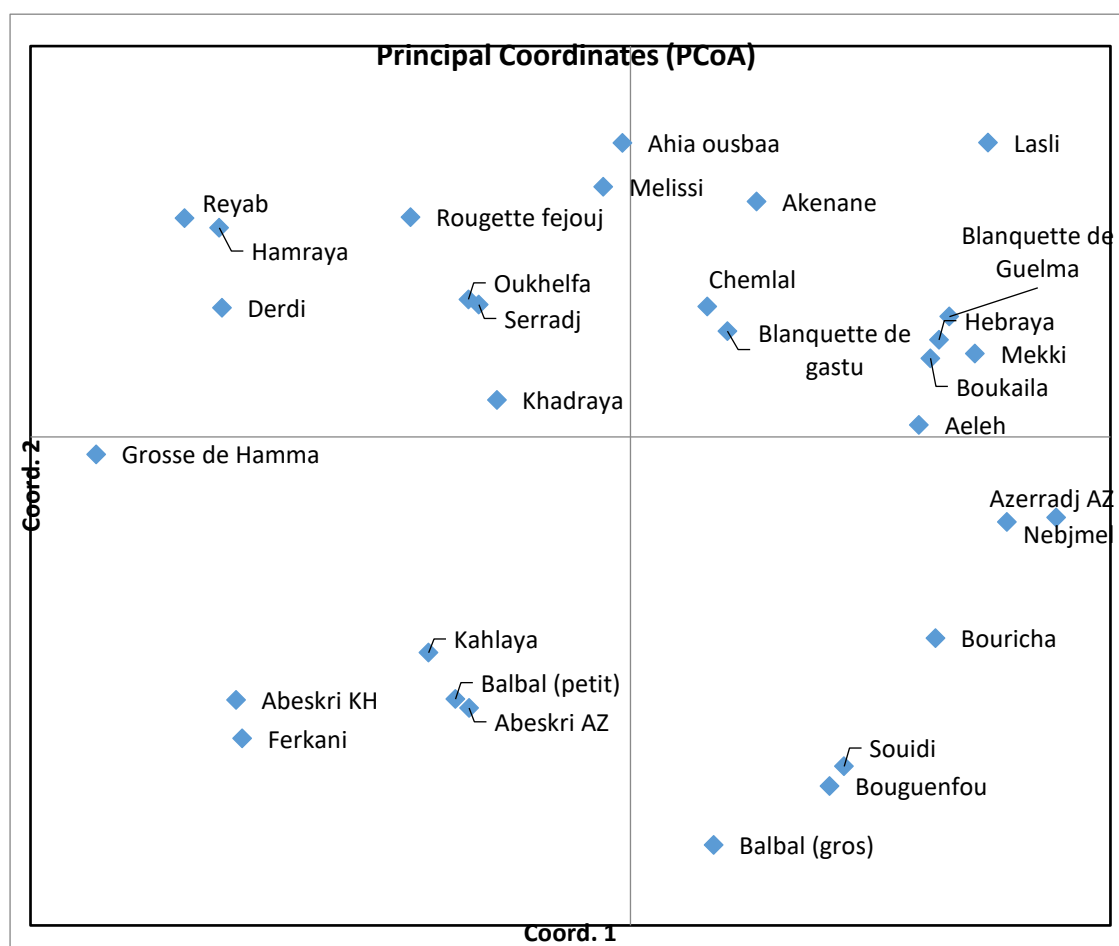


Figure 1. Principal component analysis PCoA

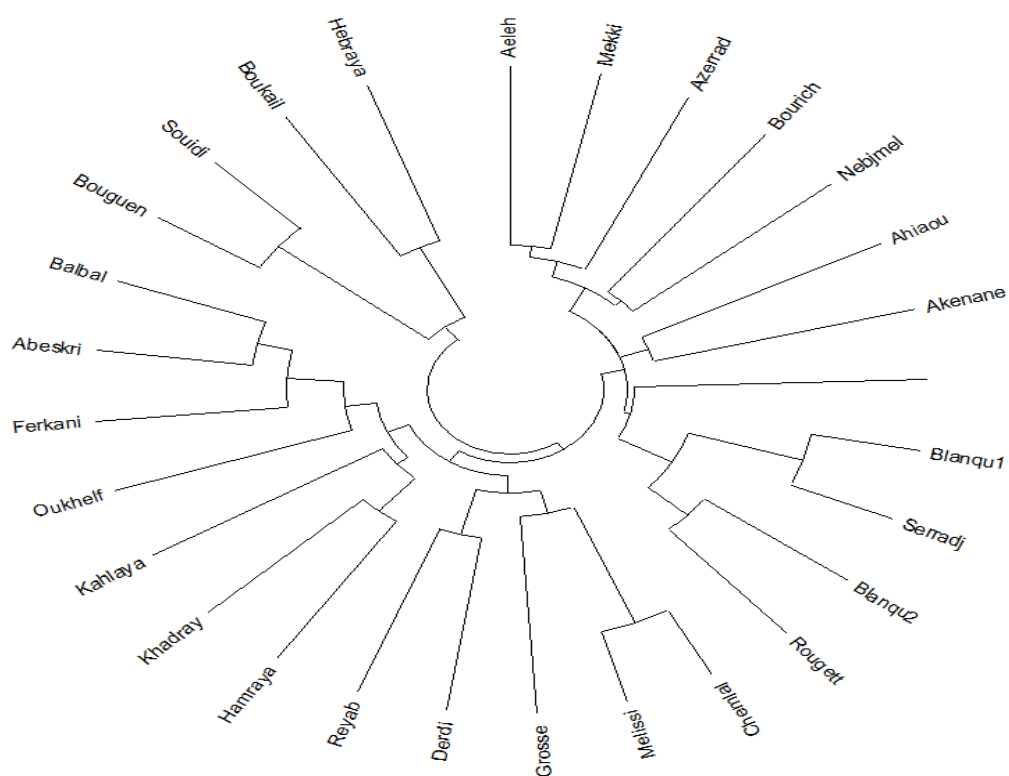


Figure 2. Dendrogram of 23 Algerian olive accessions obtained with 12 SSR markers, based on the UPGMA methodology.

Conclusion

The genetic diversity of the olive tree in Algeria is both vast and complex, as demonstrated by this study based on the analysis of microsatellite markers (SSR) from 23 olive varieties originating from different regions of eastern Algeria. The findings indicate significant genetic diversity, with 134 alleles identified and a comparatively elevated average of effective alleles per site. This diversity is marked by high polymorphic content (PIC) and considerable observed heterozygosity, indicating the richness and genetic variability of the examined varieties.

The analyzed varieties also exhibit a considerable number of private alleles, highlighting the existence of unique traits that could be potentially valuable for future breeding and conservation programs. The differentiation between the varieties is notable, with differentiation indices (F_{st}) indicating a good genetic separation between the groups.

Numerous obstacles threaten this diversity, including climate change, management difficulties, and socio-economic limitations. The lack of a robust national initiative for the conservation of plant genetic resources, coupled with a preference for certain introduced varieties over indigenous ones, endangers these invaluable genetic resources.

To ensure the preservation of this wealth, it is crucial to implement appropriate management and conservation strategies. This includes the recognition and protection of ancient and lesser-known varieties, as well as the development of research programs to better understand and harness their agronomic, ecological, and socio-economic potential. The pursuit of genetic research and the execution of programs for conservation are vital to preserving Algeria's olive diversity for future generations. Further studies involving a larger number of genotypes and additional molecular markers could help clarify the evolutionary relationships among Algerian olive cultivars.

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