JJBS

Volume 18, Number 1,March 2025
ISSN 1995-6673
Pages 155 – 161
https://doi.org/10.54319/jjbs/180117

Jordan Journal of Biological Sciences

Genetic Diversity of South Libyan Elite Date Palm Using SSR Markers

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Received: January 21, 2024; Revised: December 18, 2024; Accepted: January 7, 2025

Abstract

Date palms (*Phoenix dactylifera*) reproduce through offshoots, a vegetative propagation method that results in offspring genetically similar to the parent plant. However, the genetic diversity of date palms, particularly in the Southern Libya Region (SLR), remains insufficiently understood at the molecular level. Previous studies relied heavily on phenotypic traits, fruit, leaf, and chemical analyses, which are susceptible to environmental influences and developmental variations. In this study, genetic diversity was assessed using microsatellite markers across 27 palm samples from three cultivars (Taghyat, Tafsert, and Thales) grown in three regions of SLR (Sabha, Ubari, and Murzuq). Twenty microsatellite primers were employed, producing polymorphic results across the samples. A total of 84 alleles were identified, with an average of 4.2 alleles per locus. Allelic distribution varied, with loci ranging from two alleles to seven. Genetic diversity analysis yielded an average diversity index of 0.61, with heterozygosity values ranging from 0% to 100%. The Polymorphism Information Content (PIC) values averaged 0.55, indicating moderate marker efficiency. Cluster analysis revealed two main genetic groups: one consisting of Taghyat and Thales and the other of Tafsert. The AMOVA results indicated that 24% of the genetic variance was attributed to differences between cultivars, while 76% was within cultivars. These findings provide valuable insights into the genetic structure and diversity of date palms in the SLR, highlighting potential for future breeding and conservation efforts.

1. Introduction

The date palm (*Phoenix dactylifera L.*) is a vital crop in arid and semi-arid regions, particularly in the Middle East, North Africa, and South Asia. It serves as a key source of food, income, and shelter for local communities. Beyond its fruit, every part of the date palm—from the leaves and trunk to the fibres has diverse uses, including construction, fuel, and handicrafts. Its economic and ecological value is significant, contributing to the sustainability of agriculture in fragile oasis ecosystems (Elmeer *et al.,* 2020).

Date palms are cultivated for high-quality fruit, classified into soft, semidry, and dry types, each meeting different market needs. Dry dates, prized for their low moisture and high sugar content, are ideal for long storage and have historically supported desert trade routes (Racchi *et al.,* 2014). By 2020, Libya's date palm cultivation covered 32,868 hectares, producing 177,629 metric tons, a crucial contribution to the country's agricultural output (FAOSTAT, 2020).

Globally, date palms contribute to food security and economic stability, while also supporting biodiversity and ecosystem services. Molecular tools like DNA fingerprinting and SSR (Simple Sequence Repeat) markers are essential in advancing date palm research, enhancing genetic diversity, fruit quality, and addressing challenges such as climate change, pests, and diseases (Jubrael *et al.,* 2005; Elmeer and Mattat, 2015). This study hypothesizes that SSR markers will provide insights into the genetic diversity of date palm cultivars in Southern Libya. By analyzing elite cultivars, the research aims to identify superior varieties for enhancing the quality and sustainability of date palm production in the region.

Despite the importance of date palms to local economies, there is a lack of comprehensive genetic studies on Southern Libyan cultivars. A clear understanding of genetic diversity is essential for improving fruit quality, yield, and resilience to environmental stressors. Without this knowledge, selecting and propagating high-performing cultivars remains challenging.

This study proposes using SSR markers to assess the genetic diversity of date palm cultivars in Southern Libya. By profiling elite cultivars (Taghyat, Tafsert, and Talis) from regions like Sabha, Ubari, and Murzuq, the research aims to generate genetic fingerprints that will help identify cultivars with the highest potential for cultivation and improvement.

Recent studies have demonstrated the effectiveness of SSR markers in assessing genetic relationships in date palms across various regions, including GCC countries (Elmeer *et al.,* 2020), Algeria (Abdelkrim *et al.,* 2023), Morocco (Khoulassa *et al.,* 2023), Tunisia (Abdallah *et al.,* 2020), Oman (Reddy *et al.,* 2022), and Pakistan (Faqir *et al.,* 2021). Additionally, microsatellite markers have been

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successfully used for sex determination in *Phoenix dactylifera L.* (Salameh *et al.*, 2024)

In Libya, date palm cultivars are often categorized by texture and storage capacity, but genetic studies in the southern regions remain scarce. The Southern Libyan Region, with its diverse date varieties, holds significant potential for global date production if its genetic resources are fully understood and utilized.

This study focuses on the Southern Libyan Region, particularly the oases of Sabha, Ubari, and Murzuq, known for their unique date cultivars. By using SSR markers, the research aims to provide the first comprehensive genetic profile of elite cultivars from this area. The findings are expected to support conservation efforts, and strategies to improve date palm production and quality. Given the growing global demand for high-quality dates and challenges posed by climate change, this research is timely and essential for the future of date palm cultivation in Libya and beyond.

2. Materials and Methods

2.1. Plant materials

Leaf samples were collected from 27 female date palm trees, representing three distinct cultivars: Tafsert (F), Talis (T), and Taghyat (G). The trees were sourced from three different farms, each located in a unique region: Ubari (26.5810° N, 12.7940° E), Sabah (27.0365° N, 14.4290° E), and Murziq (25.9182° N, 13.9260° E), as shown in Figure 1. These particular cultivars were selected for their status as elite date palm genotypes within the SLR plantation. Young leaves were carefully harvested from randomly selected mature trees, then dried and processed for DNA extraction.

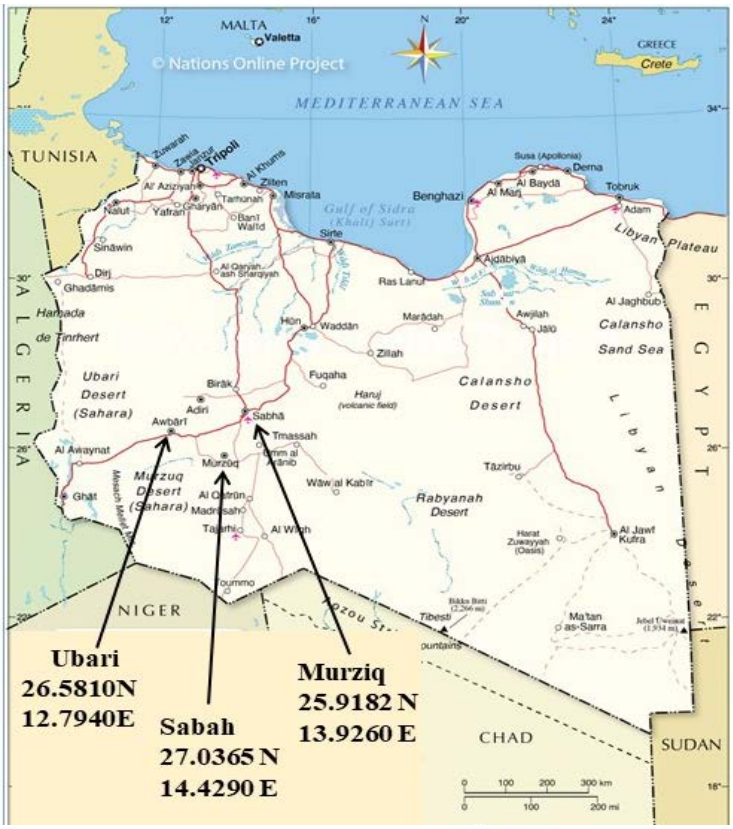

Figure 1 Map illustrating the three study locations within the Southern Libya region.

2.2. DNA extraction and genotyping

One gram leaf sample was finely ground using liquid nitrogen. DNA extraction was performed using the DNeasy Plant Maxi Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. The extracted DNA was quantified using a Nanodrop spectrophotometer.

Twenty labeled primer pairs were synthesized by Applied Biosystems (Life Technologies BV, Kwartsweg, Bleiswijk, Netherlands). Eleven of these primers were previously described by Billotte *et al.*, (2004), and the remaining nine were outlined by Elmeer *et al.*, (2011). The list of primers can be found in Table 1. PCR amplification was conducted in a 25 µL reaction volume, consisting of 2 µL (5 ng) DNA, 12.5 µL of AmpliTaq Master Mix, 1 µL of each forward and reverse primer (at a concentration of 5 µM), and 8.5 µL of nuclease-free water.

DNA amplification was carried out using a Veriti 96 Thermal Cycler (Applied Biosystems) under the following conditions: an initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, primer-specific annealing temperatures (as detailed in Table 1) for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 7 minutes.

Microsatellite analysis was conducted using an ABI 3130 Genetic Analyzer (Applied Biosystems). For this, 1 µL of PCR product was mixed with 10 µL of Hi-Di formamide and 0.3 µL of GS500LIZ size standard, then denatured at 95°C for 3 minutes and cooled on ice. The samples were analyzed on the ABI 3130 Genetic Analyzer, and allele scoring was performed using the GeneMapper Software v4.0 (Applied Biosystems).

Table 1. Date palm-specific microsatellite primers (primer pairs 1-11 developed by Billotte et al., 2004, and primer pairs 12-20 developed by Elmeer et al., 2011).

No.	Primer code	Repeat motif	Primer sequences (5'–3')	Optimal Tm °C
1	mPdCIR010	$(GA)_{22}$	F: ACCCCGGACGTGAGGTG	55.9
			R: CGTCGATCTCCTCCTTTGTCTC	
2	mPdCIR015	(GA)15	F: AGCTGGCTCCTCCCTTCTTA	51.6
			R: GCTCGGTTGGACTTGTTCT	
3	mPdCIR016	(GA)14	F: AGCGGGAAATGAAAAGGTAT	51.7
			R: ATGAAAACGTGCCAAATGTC	
4	mPdCIR032	(GA)19	F: CAAATCTTTGCCGTGAG	51.5
			R: GGTGTGGAGTAATCATGTAGTAG	
5	mPdCIR035	(GA)15	F: ACAAACGGCGATGGGATTAC	53.9
			R: CCGCAGCTCACCTCTTCTAT	
6	mPdCIR057	(GA)20	F: AAGCAGCAGCCCTTCCGTAG	55.4
			R: GTTCTCACTCGCCCAAAAATAC	
7	mPdCIR070	(GA)17	F: CAAGACCCAAGGCTAAC	48.7
			R: GGAGGTGGCTTTGTAGTAT	
8	mPdCIR078	(GA)13	F: TGGATTTCCATTGTGAG	49.6
			R: CCCGAAGAGACGCTATT	
9	mPdCIR085	(GA)29	F: GAGAGAGGGTGGTGTTATT	50.4
			R: TTCATCCAGAACCACAGTA	
10	mPdCIR090	(GA)26	F: GCAGTCAGTCCCTCATA	48.6
			R: GCAGTCAGTCCCTCATA	
11	mPdCIR093	(GA)16	F: CCATTTATCATTCCCTCTCTTG	51.8
			R: CTTGGTAGCTGCGTTTCTTG	
12	DP151	(AC)37	F TTGCTGGTTGAAATGGTGTT	53.3
			R GCAACAGATGCTCTTGCTCA	
13	DP157	(TC)19	F TGGACAATGACACCCCTTTT	54.6
			R GCCCACACAACAACCTCTCT	
14	DP159	(TC)27	F AGCTCCAATTTGCTGCAGAG	54.3
			R GCTGACCTGGAGTCCAAAAC	
15	DP160	(GAAA)5	F AAGAGCGACAATCATGACCA	57.7
			R GGAAATTGAAGGGCATCTTG	
16	DP169	(AAT)12	F GCATGGACTTAATGCTGGGTA	57.1
			R GGTTTTCCTGCCAACAACAT	
17	DP170	(AGGG)5	F TCTTTGGGCTTACGACAACC	55.9
			R GTATGGCCCAAGATGCAGAT	
18	DP171	(TTC)10	F GTGGGAGTAGCGAGGTATGG	56
			R GTCCGGCACTTTAGGAAGTT	
19	DP172	(AGG)11	F GGTGTTTGGGCCTATTTCCT	54.2
			R GTCCTCCTCCTCCTCTGTCC	
20	DP175	(CA)19	F ACACACACACACACACACACC	57.6
			R GTGGCTTCTTTTTGGCTGTC	

2.3. Data analysis

The evaluation included the analysis of allele size (in base pairs), number of alleles per locus, major allele frequency, number of genotypes per locus, genetic diversity, and polymorphic information content, all performed using PowerMarker software v3.25 (Liu and Muse, 2005), with the Hamming similarity index and 100 bootstrap replications applied. Phylogenetic diagrams were constructed using Past software version 1.91 (Hammer, 2001). Molecular variance (AMOVA), genetic variation within and among populations, expected and observed heterozygosity, inbreeding coefficient, F-statistics, and genetic distances of southern Libyan date palm cultivars were analyzed using GenAlEx 6.5 software (Excoffier *et al.*, 2005).

3. Results

Date palms reproduce through offshoots, utilizing vegetative propagation to produce offspring that are genetically similar to the parent plant. However, the genetic diversity of date palms in the Southern Libyan Region (SLR) remains insufficiently explored at the molecular level. Previous studies on genetic diversity have primarily focused on phenotypic traits, such as fruit and leaf characteristics, and certain chemical properties. These approaches are influenced by environmental factors and variations across different growth and developmental stages of the plant.

Microsatellite marker analysis of various palm samples revealed polymorphism, attributed to variations in the number of alleles identified. In this study, 27 palm samples from three distinct cultivars and three regions were tested using 20 primers. Most primers successfully amplified specific regions of the palm samples, with the exception of two samples: the Taghyat cultivar from the second farm in the Sabha region (SG2) and the Tafsert sample from the third farm in the Sabha region (SF3).

3.1. Genetic Diversity

The analysis of genetic diversity encompassed the quantification of alleles and genotypes across twenty microsatellite loci within the three cultivars sourced from (SLR). The outcomes of this analysis are showcased in Table 2, revealing a cumulative count of eighty-four alleles, translating to an average of 4.2 alleles per locus. Allelic distribution per locus ranged from two alleles in mPdCIR010 and DP159 to seven alleles in DP157 (Table 2).

The study assessed the average genetic diversity of the Taghyat, Tafsert, and Thales cultivars grown across three regions of the Southern Libyan Region (SLR)—Sabha, Ubari, and Murzuq. Using twenty microsatellite markers, the analysis revealed an average genetic diversity value of 0.61, with a range from 0.17 (associated with the mPdCIR090 locus) to 0.80 (corresponding to the mPdCIR070 locus).

The heterozygosity index varied across the three cultivars, ranging from zero (indicating the absence of heterozygous alleles) at the mPdCIR035 locus to 100% at both the mPdCIR085 and DP160 loci. The average heterozygosity index for the cultivars was 0.62. Most of the loci analyzed exhibited heterozygosity greater than 50%, as expected for date palms, given their predominantly outcrossing nature.

Table 2. Genetic diversity analysis using 20 microsatellite polymorphism loci of southern Libyan date palm cultivars.

Marker	Az (bp)	Na	Maf	Ng	GD	PIC	He	Ho	Fis	Fst
mPdCIR010	124-134	2	0.68	2	0.44	0.34	0.42	0.64	-0.52	0.03
mPdCIR015	126-138	3	0.54	3	0.58	0.50	0.49	0.92	-0.87	0.16
mPdCIR016	126-132	4	0.38	6	0.72	0.67	0.50	0.83	-0.67	0.30
mPdCIR032	288-300	3	0.38	4	0.66	0.59	0.52	0.67	-0.29	0.22
mPdCIR035	181-187	3	0.56	3	0.57	0.49	0.18	0.00	1.00	0.68
mPdCIR057	250-258	3	0.54	4	0.59	0.52	0.44	0.33	0.25	0.25
mPdCIR070	186-206	6	0.28	5	0.80	0.77	0.43	0.74	-0.72	0.47
mPdCIR078	117-177	5	0.41	5	0.73	0.69	0.65	0.67	-0.03	0.12
mPdCIR085	168-182	6	0.33	6	0.75	0.71	0.55	1.00	-0.82	0.27
mPdCIR090	148-166	3	0.91	3	0.17	0.16	0.16	0.11	0.30	0.08
mPdCIR093	161-179	6	0.36	8	0.75	0.71	0.56	0.68	-0.21	0.25
DP151	170-174	3	0.54	3	0.54	0.45	0.47	0.79	-0.68	0.13
DP157	198-214	7	0.56	9	0.65	0.63	0.45	0.44	0.01	0.31
DP159	160-162	2	0.63	2	0.47	0.36	0.47	0.74	-0.59	0.00
DP160	116-122	4	0.39	4	0.67	0.60	0.55	1.00	-0.81	0.17
DP169	200-214	6	0.59	6	0.61	0.58	0.39	0.37	0.05	0.36
DP170	214-224	4	0.67	6	0.52	0.48	0.43	0.56	-0.30	0.17
DP171	198-214	5	0.39	4	0.73	0.68	0.65	0.96	-0.49	0.11
DP172	206-214	4	0.57	4	0.59	0.53	0.51	0.48	0.06	0.13
DP175	208-224	5	0.54	5	0.65	0.62	0.42	0.52	-0.24	0.36
Mean		4.2	0.51	4.6	0.61	0.55	0.46	0.62	-0.28	0.23

Az: allele size base pair, Na: number of alleles per locus, Maf: major allele frequency, Ng: number of genotypes per locus, GD: genetic diversity, Pic: polymorphic information content, He: expected heterozygosity, Ho: observed heterozygosity, Fis: inbreeding coefficient and Fst Wright's analysis of hierarchical F-statistics.

The Polymorphism Information Content (PIC) values, which assess the discriminatory power of markers and the quality of marker genotype data, were determined. The average PIC value for the primer sets was 0.55, with values ranging from 0.16 for the mPdCIR090 locus to 0.77 for the mPdCIR070 locus.

3.2. Cluster Analysis and Genetic Structure

The dendrogram (Fig. 2) illustrates the genetic divergence among the three date palm cultivars from the Southern Libyan Region (SLR) and reveals two main clusters. Cluster (A) includes the Taghyat and Talis cultivars, which, despite exhibiting varying degrees of dissimilarity, remain grouped together regardless of their geographical origins, indicating a closer genetic relationship. In contrast, Cluster (B) contains the Tafsert cultivar, which is distinctly separated from the other two cultivars.

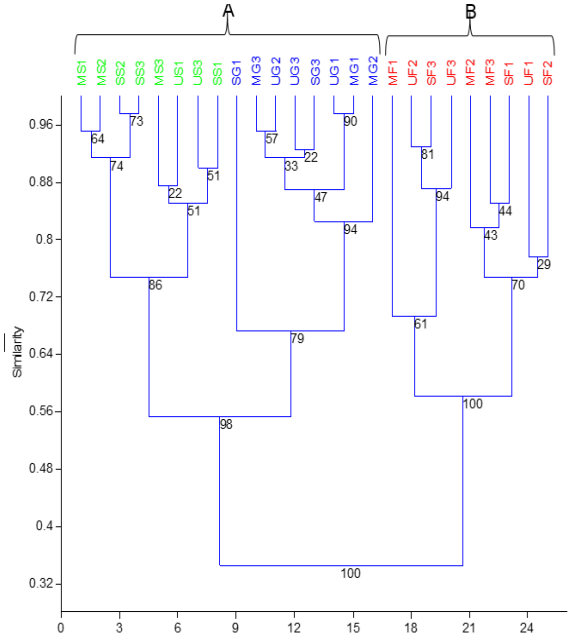

Figure 2. Dendrogram based on Hamming coefficient analysis depicting the genetic relationships among three date palm cultivars: Taghyat (G), Tafsert (T), and Talis (S), originating from Ubari (U), Sabha (S), and Murzuq (M) in southern Libya.

Genetic relationships among the three date palm cultivars from the Southern Libyan Region (SLR) were examined. The average number of alleles per locus ranged from 2.5 for Tafsert, 2.65 for Taghyat, and 2.8 for Talis, with an overall average of 2.65 alleles per locus. The expected heterozygosity was nearly identical across the cultivars, with an average of 0.46, while the observed heterozygosity had an average of 0.62. The percentage of polymorphic loci was 100% for Taghyat, and 95% for both Tafsert and Talis (Table 3). The similarity matrix computed for the three cultivars revealed an average similarity coefficient ranging from 20% to 97.5%

Table 3. Mean and standard error (SE) across loci for each population of the three date palm cultivars.

Population		N	Na	He	Ho	Fis	%P
Taghyat	Mean	8.70	2.65	0.43	0.60	-0.20	100
	SE	0.11	0.15	0.04	0.10	0.16	
Talis	Mean	8.65	2.80	0.50	0.69	-0.38	95
	SE	0.11	0.28	0.04	0.07	0.10	
Tafsert	Mean	9.00	2.50	0.46	0.58	-0.19	95
	SE	0.00	0.17	0.04	0.09	0.16	
Average		8.78	2.65	0.46	0.62	-0.26	96.67

N: number of accessions, **Na**: number of alleles per locus, **He**: expected heterozygosity, **Ho**: observed heterozygosity, **Fis**: fixation index values and **%P**: Percentage of Polymorphic Loci.

The dendrogram based on the Hamming genetic similarity coefficient among the date palm cultivars in the Southern Libyan Region (SLR) supports the findings of the principal coordinate analysis (PcoA), with the cultivars clearly divided into two distinct clusters. The first cluster includes the Taghyat and Thales cultivars, represented in blue and green in Fig. 3, while the second cluster, shown in red, distinctly separates the Tafsert cultivar from the other two. These relationships are further emphasized by the genetic distance analysis presented in Table 4.

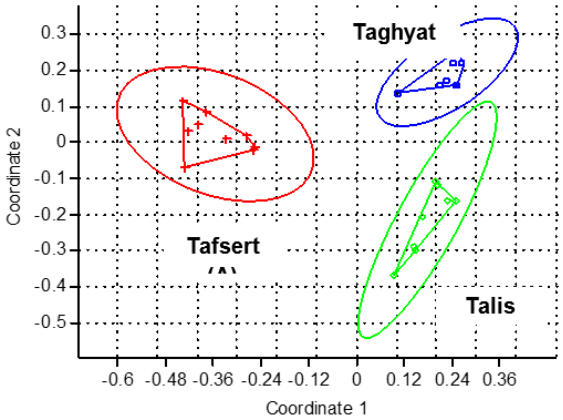

Figure 3. Scatter plot of Principal Coordinate Analysis (PCoA) based on 20 microsatellite loci among three date palm cultivars: Taghyat (G), Tafsert (T), and Talis (S), from Ubari (U), Sabha (S), and Murzuq (M) in southern Libya.

Table 4. Genetic distance of southern Libyan date palm cultivars.

Nei Genetic Distance			
	Taghyat	Tales	Tafsert
Taghyat	0	0.67	0.33
Tali s	0.67	0	0.63
Tafsert	0.33	0.63	0

The AMOVA results indicate that 24% of the total variance is attributable to differences between the cultivars, reflecting underlying structural genetic variation. The remaining 76% of the genetic variation is due to differences within each individual cultivar (Table 5).

Table 5. AMOVA table of southern Libyan date palm cultivars.

Source	df	Sum of Square	Estimated Variability	% of variation
Among Pops	2	76.944	1.912	24%
Among Indiv	24	97.222	0.000	0%
Within Indiv	27	163.500	6.056	76%
Total	53	337.667	7.968	100%

4. Discussion

A similar study involving Tunisian date palms reported successful amplification with sixteen primer pairs, although only fourteen primers produced positive results (Zehdi *et al.*, 2004). In Oman, 62.5% of primer pairs (10 out of 16) yielded successful amplification (Al-Ruqaishi *et al.*, 2008). The mean allele count in our study (2.65 alleles per locus) exceeded the 3.09 alleles per locus observed in a study of 72 wild date palms from Jordan (Al Asasfa *et al.*, 2015), likely due to the use of fewer primers in the latter. Our findings align with those from Qatar (4 alleles per locus), Saudi Arabia (4.1 alleles per locus), and Iran (4.8 alleles per locus) (Ahmed *et al.*, 2011; Al-Faifi *et al.*, 2016; Arabnezhad *et al.*, 2012). In contrast, higher allele counts have been reported in Libya, Iraq, Nigeria, Morocco, and Tunisia. For example, a Libyan study of 377 palms revealed 6.88 alleles per locus (Racchi *et al.*, 2014), while studies in Iraq, Nigeria, Morocco, and Tunisia reported averages ranging from 7 to 8.5 alleles per locus (Khierallah *et al.*, 2011; Yusuf *et al.*, 2015; Bodian *et al.*, 2012; Zehdi *et al.*, 2004).

The observed genetic diversity in this study suggests significant genetic variation in the Southern Libyan date palms (SLR), comparable to the 0.66 gene diversity in Qatari date palms (Elmeer and Mattat, 2015), though lower than the 0.75 reported in GCC date palms (Elmeer *et al.*, 2020). This diversity may stem from cross-pollination, where progeny retains the cultivar name despite potential genetic differences. This genetic variation is crucial for maintaining healthy populations and mitigating the risks of genetic bottlenecks. The observed heterozygosity (0.62) in this study was higher than in Moroccan palms (0.11-0.30) (Khoulassa *et al.*, 2023), but aligned with Saudi (0.68) and GCC palms (0.62) (Al-Faifi *et al.*, 2016; Elmeer *et al.*, 2020), though lower than the 0.72 found in Iraqi and Iranian date palms (Arabnezhad *et al.*, 2012).

The majority of primers in this study exhibited PIC values above 0.5, indicating their effectiveness in assessing genetic diversity, surpassing the 0.22 reported for Jordanian palms (Al Asasfa *et al.*, 2015) and matching the 0.68 for Saudi palms (Al-Faifi *et al.*, 2016), but falling short of the 0.72 observed in GCC palms (Elmeer *et al.*, 2020). Interestingly, the dendrogram analysis revealed that genetic structure is largely unaffected by geographical location, despite distances of 120-200 km between regions. This supports the genetic similarity observed between Taghyat samples from the Murzuq and Ubari regions, highlighting significant intra-cultivar variation despite geographical separation.

Inbreeding coefficients (Fis) ranged from 0.01 (DP157) to 1.0 (mPdCIR035), with genetic divergence noted in the highest similarity values (97.5%) between certain Taghyat and Talis cultivars across regions. The lowest similarity (20%) was found between Taghyat (Ubari) and Tafsert (Ubari). This divergence mirrors findings from studies in Oman and Morocco, where higher variance was observed within populations compared to among populations (Ibrahimi *et al.*, 2023; Reddy *et al.*, 2022), possibly due to the widespread practice of cultivating date palms via cross-pollinated offshoots. This approach increases genetic diversity and can contribute to disease resistance and improved genetic resilience. These results are consistent with findings by Bodian *et al.* (2012) on Moroccan germplasm.

5. Conclusion

This study is an initial exploration aimed at assessing the genetic diversity of date palms in southern Libya. The recorded genetic diversity rate of 0.61 is moderately high. Notably, 76% of the observed genetic variation originates from differences among cultivars. The current results could be attributed to the prevalence of cross-pollination among collected samples, resulting in the generation of novel and diverse genotypes. This process enriches the genetic traits of date palm cultivars in southern Libya, simultaneously diminishing the susceptibility to genetic issues like vulnerability, genetic drift, and disease susceptibility.

Acknowledgments

The authors extend their gratitude to Omar AlMukhtar University in Bayda, Libya, as well as the Ministry of Municipality and Environment in Doha, Qatar, for generously providing the necessary facilities that enabled the successful completion of this research endeavor.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics approval and consent to participate

This article does not contain any studies involving animals or human participants performed by any of the authors.

Consent for publication

Not applicable

Availability of data and materials

All data analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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