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Research Paper

Exploring Phylogenetic Relationships and Genetic Variability of Date Palms (*Phoenix dactylifera* L.) in Ethiopia Using ISSR Markers

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Abstract

Date palms (Phoenix dactylifera L.) have socio-economic importance and ecological functions in arid regions, but are poorly studied in Ethiopia. This paper examines the hereditary framework and diversity of date palms native to Ethiopia. To achieve this, we analyzed 113 distinct samples through the application of 10 specific ISSR (Inter-Simple Sequence Repeat) primers. 241 distinct DNA fragments were successfully generated via PCR; notably, all employed ISSR primers revealed complete polymorphism (100%), indicating high genetic diversity. Among the tested markers, the AGG6 ISSR primer exhibited the highest degree of productivity, yielding a total of 37 distinct amplification products, whereas the $AG_{10}C$ primer produced the fewest (10) amplification bands. Diversity indices, including Shannon's Information Index (I) and Nei's genetic diversity (H), revealed significant variation among date palm populations. Analysis of Molecular Variance (AMOVA) revealed that 51% of the total genetic variation was attributable to differences among the sampled Ethiopian date palm populations. These differences were due to population-level variation, suggesting limited gene flow between populations and relative isolation, which contrasts with what is typically observed in long-lived plants. AMOVA analysis found that over half (51%) of the genetic differences between the results from the principal coordinate analysis (PCoA) and dendrogram (UPGMA clustering) showed that the different types of date palms grouped based on where they come from. The current research highlights special genetic resources for creating breeding programs that can withstand climate challenges, especially for date palms in the Horn of Africa, which can survive climate change and drought problems in the region.

1. Introduction

Date palm (*Phoenix dactylifera L.*) is a hallmark of agro-ecosystems in arid environments, offering unique tolerance to extreme environmental conditions (Abul-Soad et al., 2023). Date palms are dioecious monocots and are one of the first perennial crops to be grown by humans. Their cultivation now covers more than 1.3 million hectares around the world and produces about 10 million tons of fruit each year

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(Medany et al., 2023). It is among the most extensively cultivated perennial crops in the arid basins, an economically valuable fruit-bearing species in North Africa, the Middle East, and Southwestern Asia (Soomro et al., 2023). This fruit tree is considered native to Mesopotamia, an area that includes Syria, Kuwait, and portions of Turkey (Al-Karmadi & Okoh, 2024; Ali et al., 2025). Beyond its historical origin, the date palm plant also acts as a vital biological barrier against soil degradation and desertification (Alotaibi et al., 2023). However, despite its expanding global distribution exceeding 120 million trees (Hanieh et al., 2020), scientists continue to research the genetic basis of date palm adaptation to different ecological niches.

The date palm has socio-economic value because of its many uses, such as food, medicine, and industry (Ahmed et al., 2021; Latifian, 2026). It is an energy-rich food that contains vitamins, minerals, sugars, dietary fiber, and essential amino acids (Ali et al., 2025). Therefore, date palm is an excellent crop to grow in hot, humid, food-insecure regions (Al-Karmadi & Okoh, 2024). Additionally, the trunks of the date palm are used for building houses, making beehives, building bridges, and packaging materials (El-Mously & Darwish, 2020). Other uses include the terminal buds and leaves being used as vegetables and the rachis being used to produce paper (Al-Mssallem et al., 2024).

Ethiopia’s date palms constitute an exceptional and yet largely unstudied secondary center of diversity. Date palms were introduced to the Afar Region from the Awash River Basin approximately 200 years ago and have now become a critical part of the livelihoods of pastoralist communities living in the Afambo and Asayta districts (Ahmed et al., 2021). Recently, Ethiopia's agricultural industry has greatly expanded the importation of in vitro propagated date palms with the intent of enhancing date palm productivity. However, the introduction of foreign plant varieties into the country could put local varieties in jeopardy; therefore, a full and rapid investigation of the resources is needed to support conservation and breeding efforts to preserve the diagnostic features of Ethiopia's local date palm varieties for the emerging stability of Ethiopia's date palm industry.

Due to their long-lived and dioecious nature, date palm trees contain a high degree of heterozygosity, creating a large amount of variability both phenotypically and genotypically among individuals (Al-Karmadi & Okoh, 2024). To discern genetic relationships among these individuals, molecular markers serve as essential tools (Ahmed et al., 2021). Various molecular screening methods have been historically utilized to quantify genetic variation, including RAPD (Mirbahar et al., 2014), AFLP (Ibrahim et al., 2014), SSRs (Ahmed et al., 2021), and SNPs (Hazzouri et al., 2015). SSRs and SNPs markers are very informative; however, ISSR markers provide unique advantages for wide breadth of genomic screening in a cost effective and efficient manner due to their high reproducibility, given that no prior knowledge of the target species' DNA sequence is necessitated, thus making them an optimal choice for assessing population structure or genetic “fingerprinting” (Purayil et al., 2018; Khankahdani & Bagheri, 2019).

In Ethiopia, insufficient sample size has limited the ability of genetic researchers to fully assess the genetic diversity and to establish a comprehensive genetic inventory of indigenous date palm populations (Takele et al., 2021). Consequently, the present work aims to define the genetic landscape and create a credible molecular baseline for Ethiopian date palm groups. We have used a combination of ISSR markers and previous SSR-based research (Ahmed et al., 2021) to clarify the phylogenetic relationships between indigenous and introduced cultivars as well as the population structure within date palm populations. Using this combined marker system, we intend to develop a solid foundation for the sustainable management of and genetic improvements to date palm germplasm throughout East Africa.

2. Materials and Methods

2.1. Plant Sample Collection and Genomic DNA Isolation

A total of 113 date palm specimens were obtained from Ethiopia's Somali, Afar, and Dire Dawa regions (Figure 1, Table 1). Sampling of the plants was based on the presence of fruits or flowers and the availability of offshoots, permitting the easy identification of male and female plants. Young, yellowish tissue of the leaves of the plants was cut into pieces and placed in zip-lock plastic bags for preservation.

The extraction of genomic DNA for the samples took place at the Plant Genetics Laboratory, Addis Ababa University, which employs the CTAB (cetyltrimethylammonium bromide) method (Clarke, 2009). To facilitate efficient lysis, 100 mg of desiccated leaf tissue was subjected to mechanical homogenization using five 3.2 mm stainless steel beads within 2 ml microcentrifuge tubes. Homogenization into a fine powder was achieved via a Retsch Mixer Mill MM 400. Lysis was initiated by adding 1 ml of preheated 2% CTAB extraction buffer. This buffer, comprising 100 mM Tris-HCl (pH 8.0), 25 mM Na_2EDTA, and a high salt concentration (2 M NaCl), was optimized by adding 250 mg/ml of polyvinylpyrrolidone (PVP) and 2% β-mercaptoethanol. These additives were essential for neutralizing polyphenolic compounds and preventing sample oxidation, respectively (Ibrahiml et al., 2024).

Following the isolation and refinement phases, the resulting genomic DNA pellets were solubilized in 60 µl of 0.1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) supplemented with RNase (El-Ashram et al., 2016). The structural integrity and purity of the extracts were subsequently validated via 1% agarose gel electrophoresis using a Bio-Rad Gel Doc™ EZ imaging system. The total amount of genomic DNA per sample was quantified and assessed for purity using a NanoDrop 2000 spectrophotometer, and the samples all exhibited A260/A280 ratios (near 1.80) consistent with high-quality genomic DNA with little to no protein contamination. Samples with higher concentrations of genomic DNA were diluted in PCR-grade water to prepare a standard 100 µl volume for consistency in downstream applications (e.g., PCR or sequencing) (Hoorzook & Barnard, 2022).

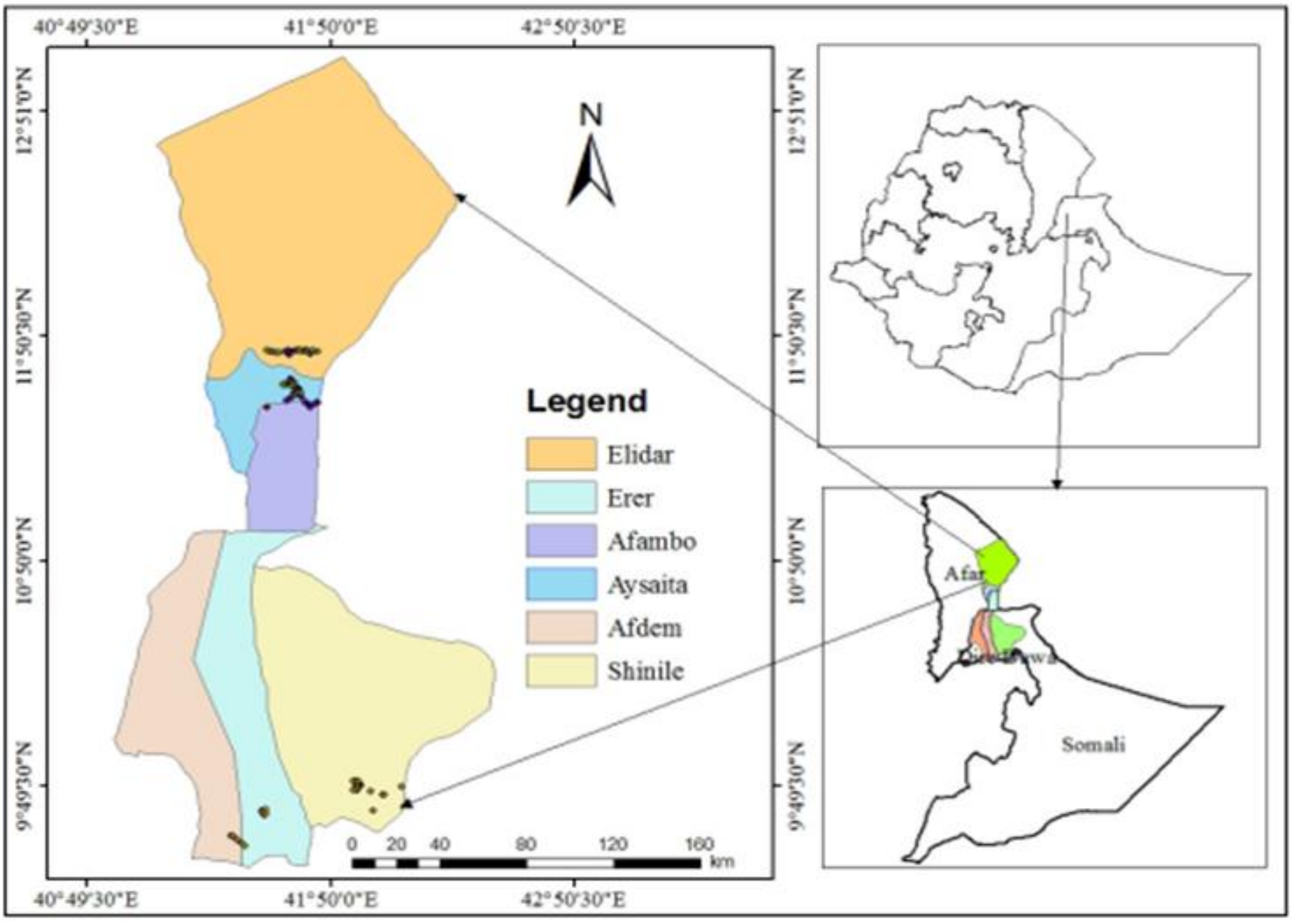

Figure 1. *The geographical distribution of date palm genotypes*

2.2. PCR processing and fragment analysis

A screen of fifteen ISSR primers (Table 1) was used in the assessment of genetic variation, with ten being provided by Karim et al. (2010) and five from Oumer et al. (2020). The ten chosen had high levels of reproducibility and clarity and were used for genotyping the 113 specimens. Each 10 µL PCR mixture comprised 100 ng template DNA, 1 µl of 10X PCR buffer, 0.6 µl of 25 mM $MgCl_2$, 0.2 µl of 10 mM dNTPs, 0.5 µl of 20 µM primer, and 0.25 µl of 5 U/µl Taq DNA polymerase, brought to volume with 6.95 µl of PCR-grade water (Hoorzook & Barnard, 2022).

The PCR thermal profile was initiated with a 5-minute denaturation phase at 94°C. The amplification consisted of 35 cycles, each comprising denaturation (94°C for 30 s), primer annealing (Ta for 30 s), and extension (72°C for 1 min). A final 10-minute elongation step at 72°C completed the reaction, after which samples were held at 4°C. To maximize both product yield and primer specificity, optimal annealing temperatures (Ta) were independently calibrated for each of the 10 ISSR primers (Ibrahiml et al., 2024).

An amplification using 1.5% agarose and 3.0 µl of ethidium bromide was used for the amplification (5 µl). Runs were performed in 0.5× TBE buffer at 100 V for 1 to 1.5 hours, using a 100 bp DNA ladder (BioTools) for size calibration. Results were visualized and recorded with a Bio-Rad Gel Doc™ EZ System (Arunachalam & Sasidharan, 2021). Figure 2 demonstrates the polymorphic diversity observed across the 113 samples using the optimized ISSR primers.

2.3. Molecular Profiling and Digitization

Band scoring was done based on the recommended criteria for dominant DNA markers (Bonin et al., 2004). This means that only unambiguous bands were considered, while bands of low intensity, as well as bands smaller than 100 base pairs or larger than 2000 base pairs, were not considered. Scoring was done sequentially from high to low molecular weight fragments.

The size of each fragment was determined by comparing it with a 100-base pair DNA ladder (BioTools). In accordance with the criteria for dominant markers (Lynch & Milligan, 1994), bands sharing the same relative position on the gel were considered homologous amplicons and assigned to a specific locus for comparative analysis.

Using Microsoft Excel, we made a binary data matrix that fit the input needs for downstream analytical software. Two formats were created, one with samples as columns and ISSR markers as rows, and the opposite arrangement as transposed, depending on the software required format. Each amplified fragment was named according to its primer number, followed by its approximate fragment size (in base pairs), for example, ISSR-807 and 450bp. Binary values were recorded for each locus; a value of one denoted the presence of the band, while a value of “0” denoted that a band was not present at that location. If it was unclear whether a band was present at that location, it was recorded as a '?' (ambiguity).

Table 1. *ISSR primers that were used in this study and their properties*

No.	Primer Sequences (5`-3`)	Types of motifs	Annealing Temperature °C	Chosen for PCR	Range of amplified bands in base pairs
1	(AG)10G	Dinucleotide	52	√	1000 – 230
2	(AG)10C	Dinucleotide	52	√	500 – 150
3	(AG)8YC	Dinucleotide	45	√	1350 – 180
4	(CT)8RC	Dinucleotide	43	√	1800 – 180
5	(AG)10T	Dinucleotide	57	√	1100 – 150
6	(CT)10G	Dinucleotide	52	√	900 – 120
7	(CT)10T	Dinucleotide	-	-	-
8	(AG)10	Dinucleotide	-	-	-
9	(AGG)6	Trinucleotide	55	√	1600 – 190
10	(ACC)6	Trinucleotide	-	-	-
11	(GACA)4	Tetranucleotide	45	√	1500 – 180
12	(CCCT)4	Tetranucleotide	-	-	-
13	(ACTG)4	Tetranucleotide	45	√	1800 – 250
14	(GGAGA)3	Pentanucleotide	-	-	-
15	(GACAC)4	Pentanucleotide	55	√	1750 – 200

2.4. Genetic Diversity Data Analysis

We used several analytical frameworks to characterize the genetic construction of sampled date palm populations. Partitioning of molecular variance across different hierarchical levels was determined via AMOVA within the GenAlEx 6.5 software environment (Peakall & Smouse, 2012). Similarly, population differentiation (PhiPT (Φ) statistics, a strong analogue of FST was calculated. In addition, a Principal Coordinate Analysis (PCoA) was carried out in the GenAlEx environment to show the spatial distribution of genetic variation as well as provide a multidimensional representation of inter-population relationships.

Estimates of genetic diversity were computed using the POPGENE 32 software package. The analysis included key indices such as observed (Na) and effective (Ne) allele numbers, Shannon’s information index (I), Nei’s gene diversity (H), the percentage of polymorphic loci, the total number of amplified bands, Nei's genetic identity, and distance matrix (Liu et al., 2025).

To determine the phylogenetic structure of the date palm populations examined in this study, cluster analysis using NTSYS-pc software was performed (Mirbahar et al., 2014). A dendrogram was constructed to illustrate the genetic relatedness of each of the date palm populations evaluated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and SAHN (Sequential, Agglomerative, Hierarchical, and Nested) algorithm, yielding a hierarchical representation of the genetic divergence and affinities among the diverse date palm populations sampled in this study (Ibrahimi et al., 2024).

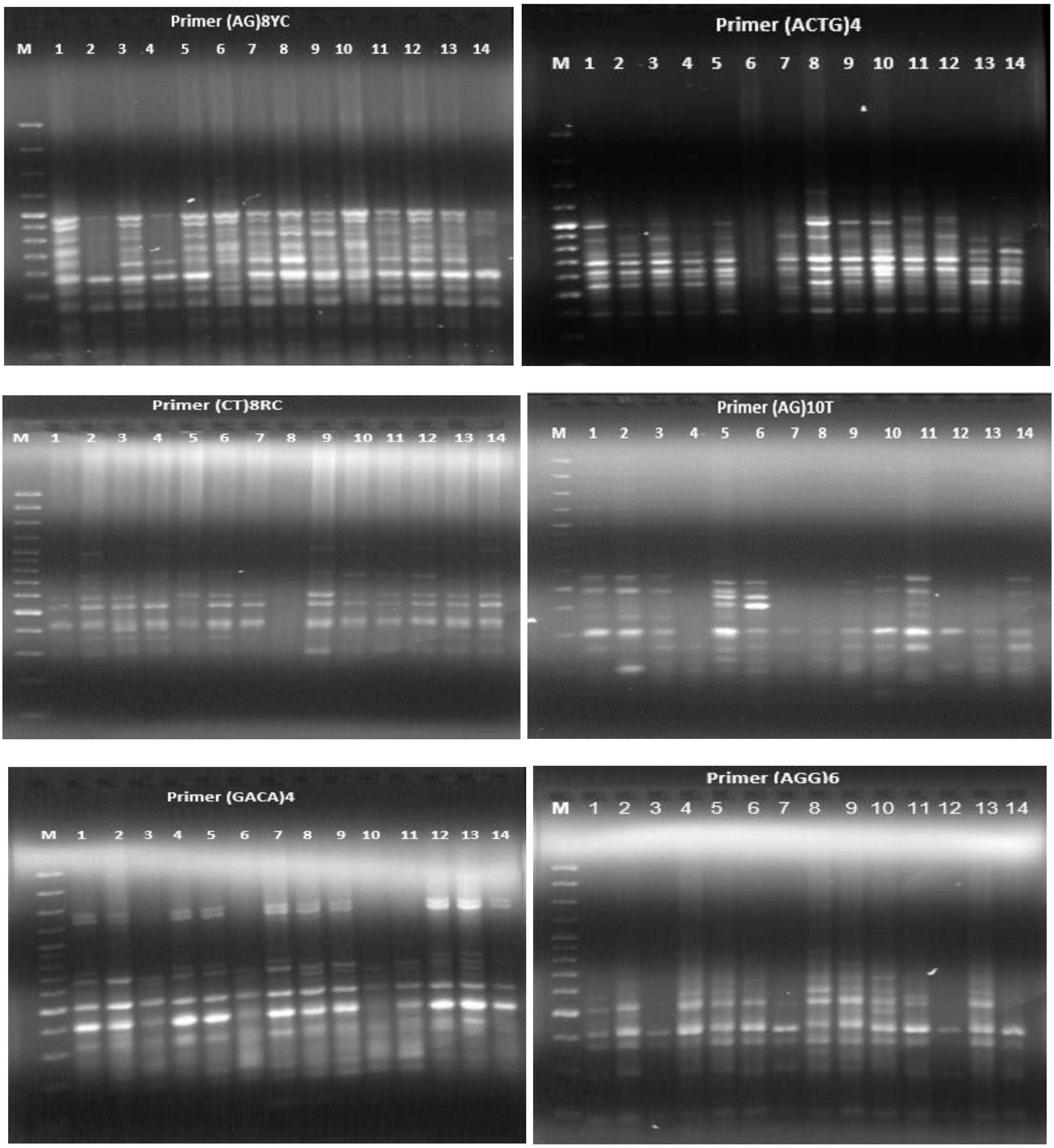

Figure 2. *Representative gel electrophoresis profiles of DNA fragments amplified in date palm genotypes using various ISSR markers*

3. Results

3.1. ISSR Assay and Polymorphism

Ten out of fifteen ISSR primers were selected based on initial screening tests that demonstrated their reliability in generating a clearly distinguishable DNA banding pattern (see Table 2, Figure 2). These primers generated a total of two hundred forty-one (241) scorable loci, representing a wide range of fragment sizes from 120 to 1800 base pairs. Each amplified locus generated by these primers exhibited a level of polymorphism of 100% (Table 2). Primer-specific performance was characterized by a broad spectrum of band counts, with the $(AG)_{10}C$ and $(AGG)_6$ markers defining the minimum (10) and maximum (37) thresholds, respectively. The average fragment density was calculated at 24.1 per amplification.

Analysis of the variation in the distance between the repeating units and the presence of anchors in the primer sequences yielded distinct amplification patterns. The anchored dinucleotide primers yielded the most loci (129), followed by tetranucleotide (52), trinucleotide (37), and pentanucleotide (23) primers, respectively. The genetic diversity (H) of each primer varied from 0.165± 0.157 for $(CT)_{10}G$ to 0.328 ± 0.136 for $(GACA)_4$, the overall mean genetic diversity value was 0.281 ± 0.154. Shannon's information index (I) reached a maximum of 0.76 ± 0.213 with $(CT)_{10}G$ and a minimum of 0.401 ± 0.193 with $(AGG)_6$, and had a combined mean of 0.436 ± 0.195. Gene flow (Nm) estimates obtained from individual primers demonstrated a wide range of migratory patterns; the highest gene flow (Nm = 3.066) was observed in domesticated plants amplified by $(CT)_{10}G$ and $(AG)_8YC$, while the lowest (Nm = 0.634) was observed in $(AG)_{10}C$

3.2. AMOVA and Pair-wise Genetic Distance Comparison

Molecular variance analysis (AMOVA) was employed to partition genetic diversity across the studied genetic resources (Table 4). The AMOVA results using a pooled ISSR dataset revealed significant genetic heterogeneity across the study populations ($P \leq 0.001$), with 51% and 49% of the total variation attributable to genetic variation between and within populations, respectively. Pairwise comparisons of ΦPT revealed that the least differentiated pair of populations was the Alasabolo and Mamulae populations (ΦPT = 0.389), whereas the most genetically distinct populations were Berga and early introductions. The repeat motif types provided differential resolution patterns when partitioned (Table 2).

Penta-nucleotide repeats primers captured the highest percentage of inter-population variability (63%), while tetra-nucleotide repeat primers had the lowest percentage of inter-population variability (46%). However, within populations, the maximum resolving capacity of genetic variation was found using di-nucleotide repeat primers (57%). Additionally, measures of Nei's genetic identity and distance (Table 5) aligned with the established results, with the populations of Alasabolo and Mamulae having the highest genetic identity (0.8903) and the lowest genetic distance (0.1162), whereas the populations of Errer Gota and Berga were the most exhibited the greatest molecular distance (0.7552 identity; 0.2807 distance, respectively).

Table 2. *Genomic attributes and polymorphic potential of ten ISSR markers utilized for fingerprinting Ethiopian date palm populations*

				Mean ± SD		
Primers	**Na**	**NPL**	**PPL (%)**	**H**	**I**	**Nm**
(AGG)6	37	37	100	0.249 ± 0.152	0.401 ± 0.193	1.424
(AG)10G	20	20	100	0.326 ± 0.157	0.491 ± 0.194	1.217
(AG)10C	10	10	100	0.305 ± 0.146	0.474 ± 0.146	0.634
(AG)10T	26	26	100	0.259 ± 0.149	0.411 ± 0.188	2.213
(CT)10G	20	20	100	0.165 ± 0.157	0.76 ± 0.213	3.066
(GACAC)4	23	23	100	0.305 ± 0.146	0.469 ± 0.178	1.265
(ACTG)4	28	28	100	0.305 ± 0.156	0.466 ± 0.195	1.772
(GACA)4	24	24	100	0.328 ± 0.136	0.498 ± 0.166	1.116
(AG)8YC	28	28	100	0.321 ± 0.167	0.481 ± 0.206	3.066
(CT)8RC	25	25	100	0.263 ± 0.162	0.413 ± 0.203	2.246
All mean average	24.1	24.1	100	0.281 ± 0.154	0.436 ± 0.195	**1.802**
Sum	241	241				
Predicated on a microsatellite core pattern						
Di-nucleotides	129	129	100	0.272 ± 0.162	0.423 ± 0.206	0.674
Tri-nucleotide	37	37	100	0.2493 ± 0.134	0.401 ± 0.173	0.468
Tetra-nucleotide	52	52	100	0.316 ±0.1461	0.481 ± 0.1810	0.473
Penta-nucleotide	23	23	100	0.305 ± 0.1458	0.469 ± 0.178	0.318
Based on terminal nucleotide anchoring						
Anchored	129	129	100	0.272 ± 0.1624	0.4231 ± 0.206	0.674
Unanchored	112	112	100	0.2914 ± 0.144	0.4518 ± 0.180	0.433

*Na = number allele, NPL = number of polymorphic loci, PPL = percentage of polymorphic loci, H = gene diversity, I = Shannon's Information index, Nm = gene flow (Nm = 0.5(1 - Gst)/Gst)

Table 3. *Hierarchical partitioning of molecular variance among and within nine populations of Phoenix dactylifera L., derived from ISSR marker analysis*

Source	**Df**	**SS**	**MS**	**Est. Var.**	**%**
All primers					
Among Pops	8	2370.247	296.281	21.992	51%
Within Pops	104	2158.275	20.753	20.753	49%
Total	112	4528.522		42.744	100%
Di-nucleotide repeat motif primers					
Among Pops	8	1131.601	141.450	10.341	47%
Within Pops	104	1237.116	11.895	11.895	53%
Total	112	2368.717		22.236	100%
Tri-nucleotide repeat motif primer					
Among Pops	8	372.170	46.521	3.501	57%
Within Pops	104	277.069	2.664	2.664	43%
Total	112	649.239		6.165	100%
Tetra-nucleotide repeat motif primers					
Among Pops	8	1131.601	141.450	10.341	54%
Within Pops	104	1237.116	11.895	11.895	46%
Total	112	2368.717		22.236	100%
Penta-nucleotide repeat motif primer					
Among Pops	8	279.613	34.952	2.663	63%
Within Pops	104	164.723	1.584	1.584	37%
Total	112	444.336		4.247	100%

Table 4. *Pairwise population PhiPT values and associated p-values among nine Ethiopian date palm populations based on 10 ISSR markers*

As	Bg	ErG	Hum	Ker	Leg	Meg	Mam	Intro	
0.000									As
0.541	0.000								Bg
0.535	0.581	0.000							ErG
0.514	0.584	0.514	0.000						Hum
0.504	0.574	0.549	0.568	0.000					Ker
0.480	0.550	0.420	0.497	0.473	0.000				Leg
0.500	0.575	0.486	0.541	0.544	0.406	0.000			Meg
0.389	0.520	0.494	0.492	0.482	0.408	0.447	0.000		Mam
0.518	0.612	0.558	0.597	0.566	0.470	0.551	0.478	0.000	Intro

Table 5. *Pairwise comparison of genetic distance and identity across nine populations of Phoenix dactylifera L. in Ethiopia, based on polymorphic ISSR markers*

Pop ID	As	Bg	ErG	Hd	Kr	Lg	Mg	Mm	Intro
As	****	0.8143	0.8050	0.8317	0.8362	0.8424	0.8281	0.8903	0.8482
Bg	0.2054	****	0.7552	0.7659	0.7910	0.7910	0.7746	0.8046	0.7865
ErG	0.2169	0.2807	****	0.8055	0.7827	0.8622	0.8188	0.8228	0.8114
Hd	0.1843	0.2668	0.2163	****	0.7865	0.8304	0.7999	0.8407	0.7941
Kr	0.1789	0.2345	0.2450	0.2401	****	0.8410	0.7943	0.8473	0.8308
Lg	0.1715	0.2345	0.1482	0.1859	0.1732	****	0.8712	0.8798	0.8633
Mg	0.1886	0.2554	0.2000	0.2233	0.2302	0.1379	****	0.8599	0.8194
Mm	0.1162	0.2174	0.1951	0.1735	0.1657	0.1280	0.1510	****	0.8716
Intro	0.1646	0.2402	0.2090	0.2306	0.1853	0.1470	0.1992	0.1374	****

3.3. Allelic Polymorphism within Phoenix dactylifera L. Germplasm

The ISSR-based assessment of genetic diversity across the investigated date palm populations (Table 6) revealed distinct levels of polymorphism. The number of polymorphic loci (NPB) fluctuated between 87 (Berga) and 127 (Legaharae), corresponding to a percentage of polymorphic loci (PPL) ranging from 36.1% to 52.0%. While the observed number of alleles (Na) averaged between 1.361 ± 0.481 and 1.527 ± 0.500 for these respective groups, the effective number of alleles (Ne) was lowest in the Introduced population (1.193± 0.321) and peaked in the Mamulae population (1.452 ± 0.354). The Legaharae population had the highest level of genetic diversity according to both Nei's mean diversity (H) 0.172 ± 0.195 and Shannon's information index (I) 0.259 ± 0.280 value. Conversely, the lowest mean values of genetic diversity (H = 0.115 ± 0.178) and Shannon’s index (I = 0.175 ± 0.257) were observed in the Introduced and Errer Gota populations, respectively.

Table 6. *Diversity and Genetic Variation within Ethiopian Date Palm Populations*

Population	Code	Sample size	NPL	PPL (%)	Mean ± SD			
					Na	Ne	H	I
Alasabolo	As	14	112	46.47	1.465 ± 0.4998	1.272 ± 0.359	0.159 ± 0.196	0.239 ± 0.2817
Bega	Bg	11	87	36.1	1.361 ± 0.481	1.234 ± 0.362	0.133 ± 0.195	0.196 ± 0.279
Erer Gota	ErG	14	126	52.28	1.523 ± 0.501	1.290 ± 0.358	0.172 ± 0.195	0.259 ± 0.280
Humodoyta	Hd	14	114	47.3	1.473 ± 0.500	1.271 ± 0.362	0.158 ± 0.196	0.238 ± 0.2804
Kerebuda	Ker	11	101	41.91	1.419 ± 0.494	1.227 ± 0.335	0.136 ± 0.184	0.206 ± 0.267
Legaharae	Lg	14	127	52.7	1.527 ± 0.500	1.249 ± 0.338	0.151 ± 0.184	0.233 ± 0.264
Mego	Mg	10	93	38.59	1.386 ± 0.488	1.253 ± 0.367	0.145 ± 0.199	0.214 ± 0.285
Mamulae	Mm	14	109	45.23	1.452 ± 0.499	1.452 ± 0.3541	0.153 ± 0.193	0.230 ± 0.278
Early introduction (Humodoyta)	Intro	11	89	36.93	1.369 ± 0.484	1.193 ± 0.321	0.115 ± 0.178	0.175 ± 0.257

*Na = number allele, Ne = effective number of alleles, NPL = number of polymorphic loci, PPL = percentage of polymorphic loci, H = gene diversity, I = Shannon's Information index

3.4. Principal co-ordinate analysis (PCoA)

Multivariate ordination via PCoA (Figure 3) revealed distinct grouping patterns that were largely consistent with the UPGMA dendrogram. Specifically, the first three principal coordinates accounted for 27.4% of the cumulative variation. Genotypes generally clustered according to their geographic origin, with the Erer Gota and Humodoyta populations exhibiting significant separation from the remaining cohorts.

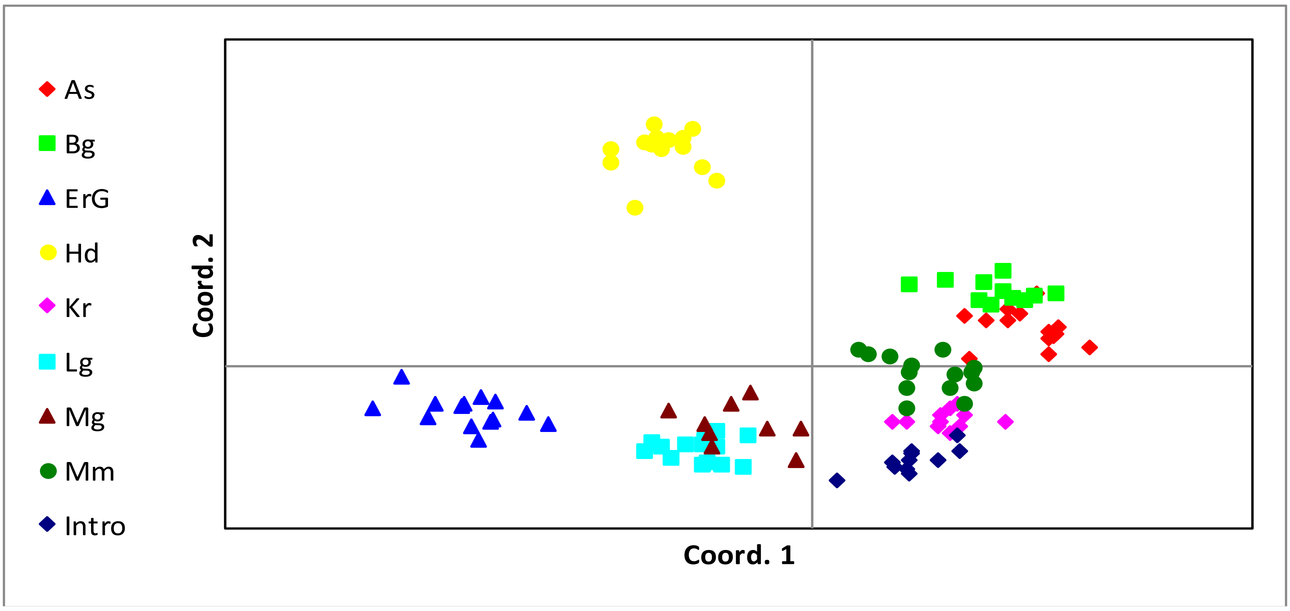

Figure 3: *Principal Coordinate Analysis (PCoA) of 113 date palm genotypes based on genetic distance. The plot illustrates the distribution of nine populations, where individuals from the same population are denoted by consistent colors and symbols*

3.5. Phylogenetic Relationships of Date Palm Populations

To visualize the genetic structure among the 113 date palm accessions, cluster analysis was performed using the SAHN module of NTSYS-pc. The resulting UPGMA dendrogram (Figure 4) partitioned the genotypes into two primary clades. The first major cluster, comprising 102 individuals, further bifurcated into two distinct sub-clusters: one containing 50 genotypes from the Alasabolo, Mamulae, Humodoyta (early introduced), and Berga populations, and a second encompassing 52 genotypes originating from Erer Gota, Legahare, Mego, and Humodoyta. The Kerebuda population formed a separate, exclusive lineage of eleven genotypes. This unique topology accounts for the significant genetic differentiation of the Kerebuda cohort and is entirely congruent with the multivariate patterns observed in the PCoA and the pairwise Nei's similarity coefficients (Table 4).

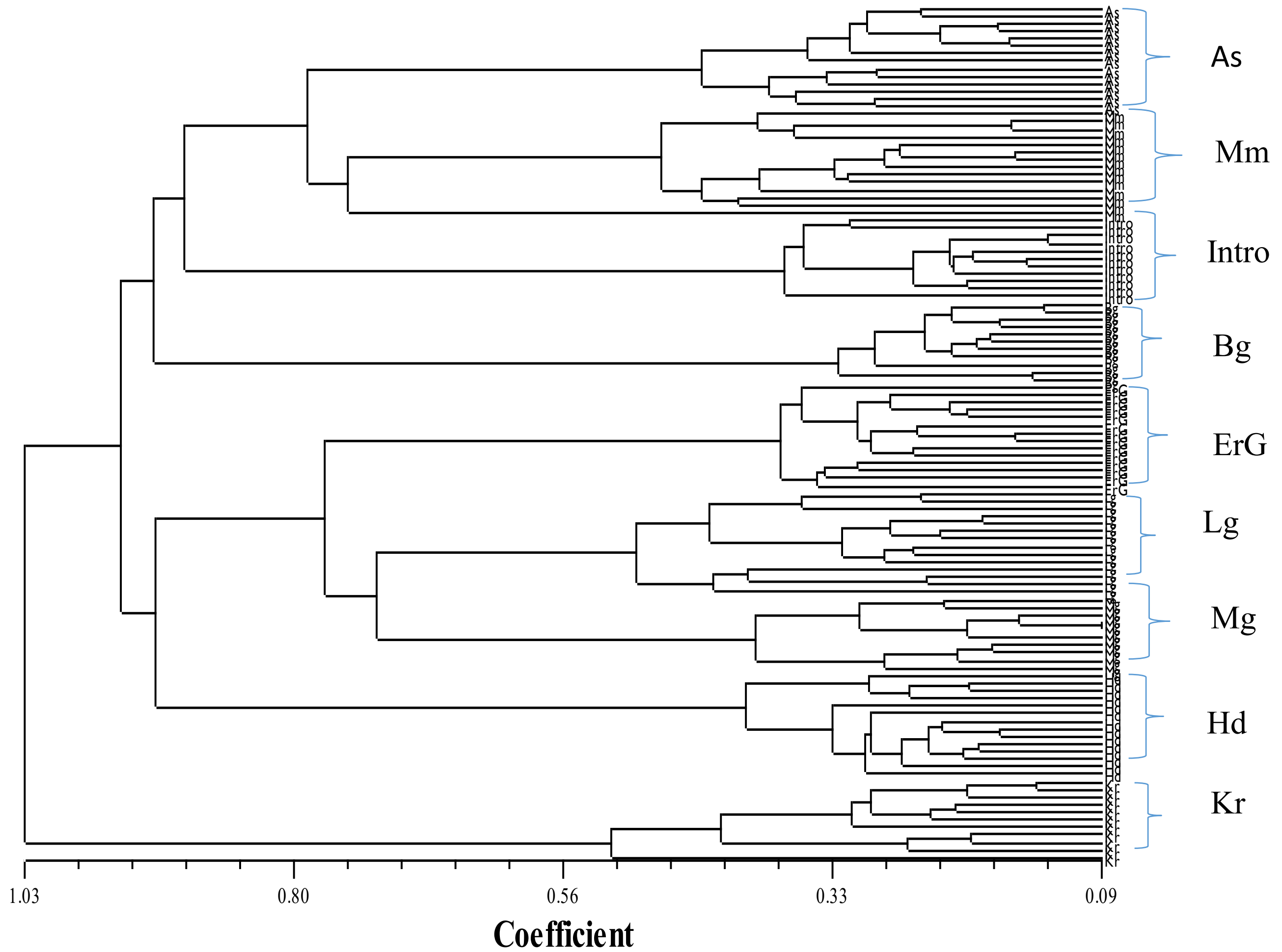

Figure 4: *UPGMA dendrogram illustrating the genetic relationships among nine date palm populations in Ethiopia based on ISSR markers. Population codes: As: Alasabolo; Mm: Mamulae; Intro: Early introduced; Bg: Berga; ErG: Erer Gota; Lg: Legahare; Mg: Mego; Hd: Hu*

4. Discussion

4.1. Polymorphic Potential and Fingerprinting Efficiency of Selected Primers

The 100 percent polymorphism rate found in Ethiopian date palm populations using ISSR markers indicates an extremely high level of genetic diversity, which is a much greater polymorphism rate than those reported from other parts of the world, such as Iran (67.6–90.9%) and Tunisia (60–66%) (Purayil et al., 2018; Indracanti et al., 2019). These results suggest that Ethiopian landraces have avoided the domestication bottleneck commonly seen in elite Middle Eastern varieties, maintaining extensive allelic richness likely because of geographic isolation and unique evolutionary pressures in East Africa (Zehdi-Azouzi et al., 2015; Takele et al., 2021). Studies conducted in Ethiopia's Afar region reported polymorphic measures approximately 69.9%, indicating the presence of a fair amount of diversity; however, it may represent less diversity than what was reported in this study, and the primary reason for the difference may be the disparity in sample amounts and marker types used for analysis (Indracanti et al., 2019; Takele et al., 2021). High levels of heterozygosity and allelic variation for Ethiopian date palm populations measured using microsatellite (SSR) techniques further indicate that the genetic resources of Ethiopian date palm populations are abundant and valuable to breeder and conservationists (Ahmed et al., 2021). In comparison, studies of date palm populations from the UAE and other Middle Eastern countries often show high levels of polymorphism, but generally less than full saturation, indicating greater selection pressure subjected to date palms in these regions and therefore the use of a narrower genetic base (Purayil et al., 2018; Amina et al., 2021).

The generation of 241 polymorphic fragments using 15 diverse ISSR motifs in Ethiopian date palm provides a detailed genetic profile and evidence of a complex, highly variable genome. The observed 100% polymorphism with certain primers such as $(AG)_{10}C$, $(AG)_{10}T$, $(GACAC)_4$, and $(GACA)_4$ is supported by Elmeer et al. (2017) and Karim et al. (2010) and proves these microsatellite motifs are conserved but hypervariable among all of the global P. dactylifera (Arabnezhad et al., 2012; Zhao et al., 2012). However, Hamza et al. (2012) reported much lower (60–66%) levels of polymorphism in Tunisian cultivars of the same motifs compared to Ethiopian samples, indicating great regional differences in genetic diversity due likely to different evolutionary histories and possibly due to differing pressures of domestication on date palms (Salem,2004). Furthermore, the strong amplification of $(AGG)_6$ with Ethiopian landraces, which failed in Persian cultivars, supports a hypothesis of unique genomic structures or null alleles specific to the Horn of Africa germplasm (Indracanti et al., 2019; Takele et al., 2021). These differences demonstrate Ethiopia's importance as a secondary centre of diversity for date palms, with distinct genetic signatures, requiring different regional-specific markers to be used for conservation and breeding strategies. The current findings exhibit an even higher level of allelic richness than reports from Afar region studies of moderate polymorphism (~62-70%) and further support the need for multiple marker systems to accurately determine the diversity of Ethiopian date palm (Abu-Afifeh et al., 2018; Indracanti et al., 2019; Takele et al., 2021).

The estimated genetic diversity (Nei's H = 0.281; Shannon's I = 0.436) suggests that there is moderate genetic diversity within Ethiopian date palm germplasm. For Shannon's I value, the maximum was for (CT)10G (0.76) and the minimum was for (AGG)6 (0.401). This study correlates with earlier ISSR marker studies showing both high levels of polymorphism and well-defined clustering of local landraces as seen in the example of both Harisa and Gewane, therefore confirming the presence of unique genotypes in the Afar region of Ethiopia (Indracanti et al., 2019; Takele et al., 2021). SSR markers in Ethiopia show high levels of heterozygosity and allelic richness but generate a different grouping than ISSR. ISSR may provide a better measurement of adaptive responses and localized genetic drift due to the dominance of multi-locus nature versus SSRs, where hypervariable co-dominant loci are more likely to show homoplasy (Hamza et al., 2013; Ahmed et al., 2021; Takele et al., 2021). Studies in Arab countries generally indicate higher levels of genetic diversity using SSRs, reflecting complex domestication processes using admixtures and introgression of wild relatives, such as those observed in date palms from the UAE and North Africa (Purayil et al., 2018; Ibrahimi et al., 2024).

The SSR indicator used in the Arab region also shows better discrimination of agronomic traits and population structure compared to ISSRs, demonstrating the complementary attributes of SSRs and ISSRs for characterizing germplasm (Hamza et al., 2013; Purayil et al., 2018). Thus, compared with SSR indicators, the ISSR indicators in this study may better document an individual's adaptive response to his or her site of origin or site of localized genetic drift, where the specific SSRs do not provide such evidence.

Most of the gene flow (Nm) values were greater than 1.0, except for the (AG)10C (Nm=0.634). This indicates that normal gene exchange provides enough support to counteract the effects of genetic drift on variation within Ethiopian date palm populations. The data reveal that the high level of connectivity in the Afar region results from both natural and human-induced dispersion mechanisms (Ahmed et al., 2021; Takele et al., 2021). The seasonal floods along the Awash River Basin contribute to the hydrochoric dispersal of date palm seeds throughout an increasingly fragmented landscape. In addition, semi-nomadic pastoralists provide human-mediated gene flow by transporting and exchanging date palms when they migrate between distant sites (Alasabolo to Mamulae) (Pérez-Escobar et al., 2021; Castillo et al., 2024). Although the use of AMOVA showed significant differentiation (51%) between inter-populations, the continual gene flow supports the integrity of the genetic structure of local landraces. Similar patterns of gene flow and population connectivity have been reported in invasive species studies within the Afar region, where landscape features did not impede dispersal, highlighting frequent long-distance movement likely aided by both natural processes and human activities (Castillo et al., 2024). These findings align with broader evidence from date palm populations across the Old World, where human-mediated diffusion has played a key role in shaping genetic structure alongside natural dispersal corridors (Zehdi-Azouzi et al., 2015).

4.2. Genomic structure of date palms in Ethiopia

The consistently high Nei's genetic identity values among date palm populations, along with relatively low net genetic distances ranging from 0.389 to 0.612 (Table 4), suggest a common ancestry or historical connectivity among the genotypes studied in Ethiopia (Ahmed et al., 2021; Takele et al., 2021). Moderate genetic divergence exists among date palm populations (<3% from 0.1-4.04) and was lower than in previous studies reporting a range greater than that of the current study in Ethiopian germplasm (Khierallah et al., 2011; Aladadi et al., 2018; Alqudah, 2024). Similarly, the same level of genetic distance and a higher level of intra-population variability were observed in date palm cultivars harvested from Iran and Tunisia using SCoT and SSR markers, which suggest high amounts of gene flow and admixture due to ecological separation (Kadri et al., 2019; Saboori et al., 2019). Conversely, regional differentiation linked to the origins of domestication was reported in studies that utilized AFLP marker analysis to investigate genetic distance among date palm cultivars, such as those collected from Tunisia and India. In terms of Ethiopia's date palm populations, relatively small amounts of genetic diversity can likely be attributed to both natural dispersal via ecological corridors (e.g., Awash River Basin) as well as human-mediated genetic transfer resulting from the activities of pastoralists, which have historically provided for the maintenance of genetic diversity among date palms (Alqudah, 2024). Overall, results from this study indicate moderate levels of meaningful genetic diversity among date palm populations that will contribute to long-term conservation and improvement by facilitating identification of lines with common ancestry as well as region-specific variation (Khierallah et al., 2011; Saboori et al., 2019; Alqudah, 2024).

Genetic divergence of local date palm populations in the Afar region ranges from genetically close together (0.389) to more genetically distant from one another (0.612). This structure of genetic divergence demonstrates a genetic landscape that is both structured and interconnected due to both gene flow and differentiation. The results of AMOVA indicate that there is significant differentiation between populations and between each population. This result is consistent with a study by Zehdi-Azouzi et al. (2015) and Takele et al. (2021), in which perennial, dioecious species where long-distance seed dispersal and human-mediated germplasm exchange promote genetic connectivity while local environmental selection maintains distinct population signatures. This duality within date palm population structures corresponds with previous findings that surmise that eastern and western date palm gene pools exhibit strong genetic structure; however, human and natural hybridization events create admixture among populations (Zehdi-Azouzi et al. 2015; Gros-Balthazard et al. 2018; Flowers et al. 2019). The Awash River Basin provides an ecological corridor for seed dispersal, while semi-nomadic pastoralist activity creates anthropogenic gene flow and retains cohesion between moderately genetically distinct populations (Takele et al. 2021). Genetic analysis of date palms has shown their evolutionary history consists of considerable genome diversity and complexity due to transitional processes that occurred throughout the process of their domestication (Flowers et al., 2019; Pérez-Escobar et al, 2021), including the transfer of wild populations into areas where cultivated populations now exist. The Afar date palm populations illustrate the role of both natural and anthropogenic processes in creating and maintaining genetic variation within a fragmented landscape (Takele et al., 2021; Zehdi-Azouzi et al., 2015; Gros-Balthazard et al., 2020).

Ethiopian date palm populations exhibit clustering patterns based on their geographic origins that correlate closely with their genetic differentiation as detected by inter-simple sequence repeats (ISSR). Thus, the discrepancies in population structure delineated by the two marker systems may be attributable to the fact that SSR markers target specific microsatellite regions while ISSR markers detect a broader range of genetic markers on the genome (Ahmed et al., 2021; Takele et al., 2021). Research using ISSR markers on date palms in the Afar Region indicated a high degree of polymorphism and unique clusters for individual landraces; confirmation of genetically differentiated populations correlated to their geographic locations (Indracanti et al., 2019; Takele et al., 2021). Conversely, SSR analyses identified allele variations and high heterozygosity but grouped genotypes into fewer clusters, suggesting a coarser resolution of population structure (Ahmed et al., 2021). This difference highlights the complementary nature of these marker systems: ISSRs provide finer resolution for detecting subtle regional differentiation, while SSRs are effective for assessing overall genetic diversity and heterozygosity. Overall, this work indicates that the Ethiopian date palm populations are undergoing active regional differentiation that is being best characterized by multiple-locus genome scanning techniques, such as ISSR markers (Indracanti et al., 2019; Ahmed et al., 2021; Takele et al., 2021).

According to Nei's genetic coefficients, PCoA and dendrogram clustering techniques, the Alasabolo and Mamulae populations and Legaharae and Mego populations share a high degree of genetic similarity with one another, indicating that they share an overall genetic identity in their respective geographic regions. These results are consistent with those reported by Indracanti et al. (2019) and, to some degree, by Ahmed et al. (2021). Evidence suggests that historical gene exchange occurred as a result of trade routes through the Awash River Basin and through traditional trade practices by pastoralists to exchange seed. Reports of greater genetic distance in previous studies may be due to low sample size (N=8), while the current studies with larger sample sizes provide a more accurate representation of the level of genetic diversity within these populations (Indracanti et al., 2019; Ahmed et al., 2021). The characterisation of these genetic baselines is also critical for flagging endangered indigenous landraces that are threatened by novel exotic in vitro-produced cultivars to preserve Ethiopia's biocultural history of date palms. The observed degree of connectivity results from the presence of both natural and human-created mechanisms strengthening gene exchange across frequently exchanged date palm populations, while maintaining unique local populations. The collective approach to assessing genetic input into conservation management and breeding programs is imperative in support of sustainable conservation and breeding efforts to protect local germplasm in Ethiopia (Jaradat, 2015; Takele et al., 2021).

5. Conclusion

This study uses an advanced form of genetic analysis (ISSR markers) to develop a comprehensive record of the genetic diversity of date palms in Ethiopia. 100% polymorphism across 113 genotypes was identified, revealing distinct geographical clustering, which significantly refines previous SSR-based frameworks. Genetic diversity was measured as high gene flow (Nm > 1), which was observed across the Awash River Basin; in addition, there was also a genetic association among the three geographical populations of date palms in the basin. The results of the study illustrate the role of both hydrologically based natural dispersal (hydrochory) and man's influence in exchanging/importing germplasm as the cause of gene flow across the Awash River Basin. Taken together, these studies provide scientists with the evidence needed to prevent the erosion of local allele frequencies currently faced by the expanding number of unqualified, imported in vitro-propagated cultivars (i.e., genetically homogeneous populations). Finally, the study provides the empirical evidence needed to support the establishment of national date palm conservation registry programs, as well as providing the base for future date palm breeding via marker-assisted selection (MAS) and the potential development of new climate-adapted date palm varieties.

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Conflict of interests

The authors disclose no potential conflicts of interest, financial or otherwise, relevant to the content of this manuscript.

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