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AI-Assisted Genetic Clustering Assessment of Date Palm (*Phoenix dactylifera L.*) Cultivars Through Mitochondrial DNA RAPD Markers

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Abstract

Understanding the genetic diversity of date palms is essential for improving and conservation of cultivars. In this study, we combined RAPD-PCR techniques with AI-powered data analysis to explore the genetic diversity of 15 date palm cultivars involving dry, semi-dry, and soft kinds. Using mtDNA as the template, ten random primers were employed to amplify DNA fragments, and AI algorithms assisted in band detection, binary matrix generation, and similarity index calculations. The integration of AI enabled accurate clustering (using Jaccard and Dice coefficients), identification of highly informative primers (notably OPA9), and pattern recognition in PCA space. Hierarchical clustering and PCA were performed for genetic relationships assesment. This human–machine collaboration revealed hidden genetic relationships and confirmed divergence between cultivars like Barhee and Ghazal. The study highlights how AI tools can elevate classical genetic fingerprinting, providing deeper insights for breeding, conservation, and cultivar management. The results revealed high polymorphism with notable discriminatory power by primers OPA9, OPA11, and OPA12. Hierarchical clustering grouped cultivars into three main genetic clusters, independent of fruit type, while PCA further clarified inter-cultivar relationships. The identification of genetically distinct cultivars like Samany and Ghazal provides valuable targets for genetic preservation, while close pairs like Hayany and Zaghloul suggest possible clonal or local lineage relationships.

Keywords: Date Palm; AI ; RAPD; Clustering a; mtDNA

1. Introduction

Date palm (*Phoenix dactylifera L.*) is one of the earliest dioecious perennial trees producing fruits with a long generation time. Date palm are members of the palm family (Arecaceae) and incorporates over 2500 types [1]. It is mostly abundant in the Middle East and the arid regions of North Africa [2]. Its origin speculated to be near Iraq. Date palm has holy importance for Muslims alongside its' importance as a nutritionally rich food and source of income [3]. Palm trees are recognized as a multi-beneficial source producing fruit, fiber, fuel, and sheltering material granting them as a nutritious and healthy benefactor for millions of people [4, 5]. However, date encounters substantial challenges such as Fungal and pest infestations, resulting in low yields and driving up prices [6]. The Middle East and Africa are estimated to subsidize 72% to the growth of date global market. In 2020, the top exporters of dates, fresh or dried were Tunisia ($296 million), Saudi Arabia ($252 million), United Arab of Emirates ($235 million), Israel ($168 million), and Islamic Republic of Iran ($141 million) [7]. Egypt is considered the top date palm producing about 1.71 million tonnes harvested from14.86 million palm trees grown in 134.1 thousand acres in 2020 with an average yield of 115.07 kg/palm [8]. Global trade dates to USD $1.2 billion in 2016, thereby contributing to millions of rural incomes in the AP and neighboring areas [7].

The global palm market was valued at around USD 13 billion in 2018 and increased to USD 18 billion by 2023 [9, 10]. As of 2018, The worldwide consumption of date fruit was 9.36 million mt in 2018 and expected to extent to 13.48 mt in 2025 [2]. The global production of dates amounts to 6.7 million metric tons

(Mt) globally and 2,379,370 Mt in Africa. The five most producing nations, which are Egypt, Saudi Arabia, Iran, Algeria, and Iraq, account for 75% of the overall production. These countries also make up about 69% of the total production [11].

The global palm population exceeds 150 million trees, encompassing around 5,000 distinct cultivars. [12, 13] with an intra-species diversity across the different geographical regions [14] and an annual global production of dates exceeding 8 million tons [15]. Date palm cultivars and varieties are usually distinguished by shapes, sizes, and organoleptic properties [16, 17].

The mitochondrial DNA and chloroplast DNA (cpDNA and (mtDNA) in higher plant carry important genetic information for respiration, photosynthesis, growth, development and reproduction. These genetic features include genome sizes, arrangements of genome, low gene density, high intron density within genes, RNA editing, gene transfer or loss [18]. MtDNA are considered as a source of original markers which are very useful in phylogeny and in population genetics because they are haploid and evolve clonally [19].

Biochemical and molecular marker is widely used in the characterization of date palm and its genetic diversity. Special Molecular markers use rDNA or mtDNA with random amplified polymorphic DNA (RAPD). RAPD is rapid, not expensive and easy method that depends on selecting specific set of primer(s) [20]. The universality of RAPD primers was tested for cattle breeds differentiation using 12 oligonucleotide primers, relatedness among the selected breeds was confirmed by the selected markers [21].

Date palms have wide variability in phenotypic characteristics such as length, length uniformity, strength, taste, maturity and fruit quality. These phenotypic characters are largely genetically controlled, with limited influence from the environment [22]. Thus, date palm cultivars are the main determinant of their type and quality, which, in turn, determine the end use [23]. In addition, the identification of the date palm cultivars is essential for breeding, germplasm maintenance, and quality control [24]. Further it has an important role in plant protection breeders' rights. Accordingly date palm cultivars recognition as the correct identification is usually delayed until the full growth of plant. The characterization of cultivars requires a large set of phenotypic data that is often difficult to assess and sometimes variable due to environmental influences. RAPD-PCR are powerful techniques, which could be used to identify and determine specific genomes or to estimate the phylogenetic relationship among the individual genomes of date palm [25].

The use of AI in plant phenotyping through digital image analysis, extracting the results using data classification to predict trends patterns [26]. The application of AI has been implanted in phenotyping systems for different plants with desired characteristics [27].

The current study aimed to identify and characterize some of the Egyptian date palm cultivars (dry, semi-dry, and soft cultivars) using different DNA molecular markers such as RAPD and AI for data analysis.

2. Materials

2.1 Plant Materials

In the present study fifteen Egyptian Date palm cultivars were used. Seven cultivars (soft, semi dry and dry cultivars) were collected from Siwa Oasis, Matroh Governorate, while seven cultivars (soft cultivars) were collected from El-Meadia, El-Behera Governorate and one cultivar (soft cultivar) was kindly provided by the Ministry of Agriculture, Agricultural Research Center (ARC), Cairo. Samples were collected from the leaves surrounding the meristem of palm trees.

First Date palms cultivated in Siwa; Oshikagbil (semi-dry), Ghorm Ghazal)Dry) Ghazal (Dry), Seady (Semi-dry) , Ferehy (Dry), Tagtagt (Soft), Kramte (semi-dry), Second Soft Date Palm cultivated in El Madina; Aom El-Frakhe (Soft), Zaghloul, Hayany, Samany, Bent Aisha, Halawy, Uraby, Third Soft Date Palm cultivated in ARC; Barhee. is

2.2 Primers Employed in the RAPD-PCR

All primers used in this study were synthesized by MWG, Germany. Primers Sequences: OPA 03 5´ AGGGGTCTTG 3´[28], OPA 07 5´ GAAACGGGTG 3´[29], OPA 09 5´ GGGTAACGCC 3´[30], OPA 011 5´ CAATCGCCGT 3´[31], OPA012, 5´ TCGGCGATAG 3´[32], OPA 013, 5´ CAGCACCCAC 3´[33], OPA 014, 5´ TCTGTGCTGG 3´[34], OPA 015, 5´ TTCCGAACCC 3´[35], P1, 5´ GGCACTGAGG 3´, [36], 2005) P2, 5´ CGCTGTCGCC3´[37].

2.3 Extraction of Mitochondrial DNA (mtDNA)

Mitochondrial DNA was extracted from plant tissues according to (Scotti et al. 2001) [38]: Two grams of each plant leave sample were ground in 10 ml of cold homogenization buffer A and then filtrated through a nylon filter (100 um mesh). After filtration, the filtrate was centrifuged at 500 g for 10 min to eliminate cell debris. To eliminate plastid, the supernatant was centrifuged twice at 2600 g for 15 and 10 min, respectively. To sediment mitochondria the supernatant was centrifuged at 14500 g for 15 min (Supernatant containing lysed nuclei components was discarded). The mitochondrial pellet was resuspended carefully in 10 ml of cold homogenization buffer A,

was centrifuged at 14500 g for 15 min. The mitochondrial pellet was resuspended carefully in 1 ml of lysis buffer. Protienase K was added to a final concentration of 50 µg/ml and incubated at 37□C for 1 h with gentle shaking to avoid mechanical breaking of DNA. After the addition of 0.1 volume of 2M ammonium acetate and TE-saturated phenol the mixture was centrifuged at 14500 g for 15 min. The aqoues phase was transferred into new eppendorf tube, equal volume of chloroform was added, mixed and centrifuged at 14500 g for 15 min. This was repeated twice. DNA was then precipitated by the addition of 2 volumes of absolute ethanol and incubation overnight at -20°C. After centrifugation at 18000 g for 20 min, ethanol (70%) was added to the pellet. After centrifugation at 18000 g for 20 min the pellet was dissolved in 20 µl of Milli-Q water.

2.4 RAPD PCR

The PCR reaction mixture was prepared in a total volume of 25.0 µl. The components included 13.9 µl of sterile Milli Q water, 2.5 µl of 10× Taq buffer without $MgCl_2$ to achieve a final concentration of 1.0X, and 2.5 µl of 50 mM $MgCl_2$ to reach a final concentration of 5 mM. Additionally, 2.5 µl of 4 mM nucleotide mix was added, resulting in a final concentration of 0.2 mM. For primer annealing, 2.5 µl of primer solution (10.0 pmol/µl) was included, giving a final concentration of 25.0 pmol per reaction. Taq DNA polymerase (5.0 U/µl) as 0.5 U per reaction. mtDNA solution (10.0 ng/µl) was added to provide 10 ng of template DNA.PCR products were then separated on agarose gel electrophoresis using 1.5% (w/v) agarose in 0.5x TBE buffer. The size of each band was estimated by using DNA molecular weight marker. Finally, the gel was photographed using gel documentation system.

2.5 RAPD Phylogenetic Analysis

Bands of mtDNA RAPD pattern were scored manually for all samples studied as 0.0 and 1.0. Level of marker polymorphism according to the various molecular techniques used with calculations. A Similarity dendrogram for each was produced.

2.6 Clustering Based on Jaccard and Dice Similarity Index Analysis by AI

The Jaccard coefficient is used extinsevely in various sciences where binary or binarized data are required. It is a ratio of Intersection over union, taking values between 0 and 1, where 0 indicates no similarity and 1 indicates

complete similarity.

Similar to the Jaccard index, the Dice similarity coefficient is a measure of the overlap between two samples. For image segmentation, it was calculated by AI [39] as twice the area of overlap between the predicted segmentation and the ground truth divided by the sum of the areas. It is a common metric for evaluating the performance of image segmentation algorithms, particularly in medical imaging.

Jaccard similarity index (based on presence/absence of RAPD bands) (Klang 2023, Qumsiyeh 2023).

Distance Metric:

Distance=1−Jaccard similarity\text{Distance} = 1 - \text{Jaccardsimilarity}Distance=1−Jaccard similarity

Clustering Algorithm:

Average linkage (UPGMA). Binary Matrix Input: 1 = band present, 0 = band absent (from the gel image & marker matrix).

2.7 Binary Matrix of RAPD-PCR Bands in 15 Date Palm Cultivars:

This binary matrix visually represents the presence (green squares = 1) or absence (white squares = 0) of 40 RAPD-PCR bands (Band_1 to Band_40) across 15 different date palm cultivars was analyzed by AI [39]. This matrix is the basis for calculating genetic similarity among cultivars and serves as the input for cluster and PCA analyses.

3. Results

3.1 RAPD PCR

The RAPD method is useful when applied specifically to mtDNA for plant genotyping and for characterization of associated organelles. The mtDNA was isolated from fifteen date palm cultivars (dry, semi-dry, and soft) and then subjected to the RAPD-PCR technique using 10 different random primers. The results revealed that all used primers showed different levels of polymorphism.

The primers were classified based on similarity in band patterns across the cultivars using a binary presence/absence matrix. Since the band presence (1) and absence (0) were reported per primer for each cultivar, each primer can be treated as a feature vector across cultivars.

Group 1: Broad-spectrum, high polymorphism, this group includes primers OPA11, OPA12, OPA15, P1. These primers amplified bands across a broad size range (300–2000 bp) and showed strong polymorphism among cultivars.

Group 2: Intermediate polymorphism and mid-size bands. This group includes primers OPA3, OPA13, OPA14, and P2. These primers produced bands ranging from 300 to 1200 bp, with some shared patterns.

Group 3: Lower polymorphism or conserved bands, this group includes primers OPA7, OPA9. These primers tended to amplify similar band sizes (~1000

bp, ~700 bp), with less diversity across cultivars.

Group 1 (Broad-spectrum, high polymorphism) OPA11, OPA12, OPA15, P1

Analysis of cultivars amplified by OPA11

High Genetic polymorphisms were observed in Lanes 3, 4, 7, 8, 10 — multiple distinct bands, indicating polymorphisms. Moderate Diversity was observed in Lanes 1, 2, 5, 9, 12, and 13, displaying several common and unique bands. The Lowest Diversity was observed in Lanes 6, 11, 14, 15, where faint bands or fewer loci were amplified, possibly due to low-quality DNA or primer mismatch (Figure 1).

Analysis for cultivars amplified by OPA12

High genetic diversity was observed in lanes 3, 4, 7, 8, and 10, showing multiple distinct bands that indicate polymorphisms. Moderate diversity was noted in lanes 1, 2, 5, 9, 12, and 13, which displayed several common and unique bands. The lowest diversity was detected in lanes 6, 11, 14, and 15, where faint bands or fewer loci were amplified, possibly due to low-quality DNA or primer mismatch.

The amplification profiles produced using the primer OPA012 showed polymorphic profile among all cultivars (Figure 1), the primer could amplify ten bands in cultivars 1, 4, 7, 13 and from 3 to 8 bands in cultivars 2, 3, 5, 8, 9, 10, 11, 12, 14, 15 while in cultivar 13 the primer amplified only one band. Also, cultivar number 6 had no amplicon, which also confirmed the identity of this cultivar when compared with the others.

Analysis for cultivars amplified by OPA15

High genetic Diversity was observed in Cultivars like **Ferehy (Lane 5), Oshikagbil (Lane 1), Hayany (Lane 10), and Ghazal (Lane 3)** showing **strong band richness**, reflecting high genetic diversity. **Lower diversity** or weaker amplification is seen in Tagtagt (Lane 6), Uraby (Lane 14), and Barhee (Lane 15). Many cultivars share bands in the ~1000 bp and **~1500 bp** regions, indicating some conserved loci amplified by OPA-015.

The amplification profiles produced using the primer OPA015 produced mostly polymorphic profile among all cultivars (Figure 1). It could be observed that 3-12 bands could be amplified using this primer. On the other hand, cultivars 12 and 15 showed no amplicones, which also confirm the identification of cultivars comparing with each others.

Analysis for cultivars amplified by P1

Primer P1 produced bands that are mostly conserved across cultivars, with strong band intensity around 150–250 bp and ~500 bp in nearly all lanes. **Conserved Bands** at **~110 bp** was detected in **all cultivars**, likely a **monomorphic marker**. Bands at **~240–300 bp** are also shared across most cultivars.

Ferehy (Lane 5), Oshikagbil (Lane 1), and Hayany (Lane 10) still show relatively rich and intense profiles, consistent with earlier gels. Tagtagt, Kramte, and Barhee showed low complexity, supporting the pattern of lower genomic diversity or weaker amplification.

Primer P1 was also used to discriminate the examined cultivars and the results showed its ability to categorize them into 13 groups (Figure 1). Each of the cultivars 2, 4, 5, 6, 7, 8, 9, 10, 13, 14 and 15 form one group, and there are two other groups, the first one comprised cultivar 1 and 3 and the second one include cultivars 11 and 12. The band patterns were so high in band numbers and low in molecular weights.

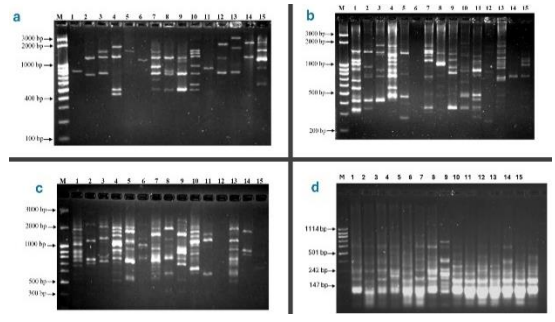

Figure 1. RAPD-PCR using the primer a OPA11, b OPA12,c OPA15, d P1 ; M: (100 bp+2 kb+3kb) DNA ladder, Lanes1-15: Oshikagbil, Ghorm Ghazal, Ghazal, Seady, Ferehy, Tagtagt, Kramte, Aom El-Frakhe, Zaghloul, Hayany, Samany, Bent Aisha, Halawy, Uraby, and Barhee cultivar, respectively.

Group 2 Intermediate polymorphism and mid-size bands OPA03, OPA13, OPA14, P2

Analysis of cultivars amplified by OPA03

RAPD-PCR using the OPA03 primer showed polymorphism among the fifteen examined cultivars (Figure 2).The obtained band pattern also grouped the examined cultivars into 12 groups. Group 1 contains two cultivars (2 and 5). Group 2 consists of three cultivars (1, 6 and 13) and each one of the cultivars (3, 4, 7, 8, 9, 10, 11, 12, 14 and 15) makes one separate group.

The amplification profiles with the primer OPA03 are shown in Figure 2 revealing a highly polymorphic profile with no similarity among all the cultivars. The primer can amplify band numbers ranging from 3 to 13 bands with molecular weights ranging from 200-2000 bp. Unique profiles distinguished the Bahree and Tagtag cultivators (Lane 15 and 6), confirming high diversity for the Bahree cultivator with strong and multiple bands. Conversely, fewer bands could be detected for the Tagtag cultivator (lane 6), indicating less variation and lower amplification. A similar

amplification profile was observed for Ghazal and Ghorm Ghazal cultivators (Lanes 2 and 3) sharing similar banding.

Analysis for cultivars amplified by OPA13

High genetic Diversity was observed in lanes 1, 3, 5, 7, and 10, displaying Multiple well-defined bands. Moderate Diversity was detected in lanes 2, 4, 8, 9, 11 and lane 13, revealing an Intermediate number of bands. Low Band Amplification was observed in Lanes 6, 14 and 15, exposing a Possible poor primer binding (Figure 2).

The data presented showed the band profile obtained by RAPD-PCR using the primer OPA013. Approximately 125 reproducible polymorphic bands in all lanes with a high range of molecular weight, starting with 2000 bp till 200 bp were obtained. Each cultivar showed its unique profile that differed from the others.

Analysis for cultivars amplified by OPA14

High genetic Diversity was observed in Lanes 1 (Oshikagbil), 5 (Ferehy), and 10 (Hayany), displaying diverse and intense banding patterns and indicating high polymorphism. Moderate Diversity: Lanes 2, 3, 4, 7, 8, 9, 11, and 13 — good resolution, medium complexity. Low Diversity was observed in Lanes 6, 12, 14, and 15 exposing fewer bands and weak intensity.

Approximately 15 amplified bands were obtained by the primer OPA014 in each lane that succeeded in differentiation among the examined fifteen date palm cultivars (Figure 2).The primer showed the ability of separating the cultivars into 15 different groups; each group consists of one cultivar. OPA014 primer gave characteristic band patterns for each cultivar. The amplified bands have different molecular weights ranging from 300-2000 bp.

Analysis for cultivars amplified by P2

Amplification of cultivars using primer P2 shows moderate polymorphism (62.5%). Most cultivars show bands at ~150–500 bp, making them monomorphic. The most informative/polymorphic bands are around 700, 900, and 350 bp. PIC values suggest ~700, ~1100, and ~350 bp are useful for diversity studies. (Figure 2).

The results revealed that the primer P2 grouped the cultivars into 10 groups. Each of the cultivars 9, 12, 13, 14 and 15 make one separate group, whenever the other cultivars formed five different groups. Group one consists of the cultivars (1 and 4), group two contains (2 and 3), group three comprised (5 and 6), group four contains (7and 8) and cultivars 10 and 11 formed the fifth group.

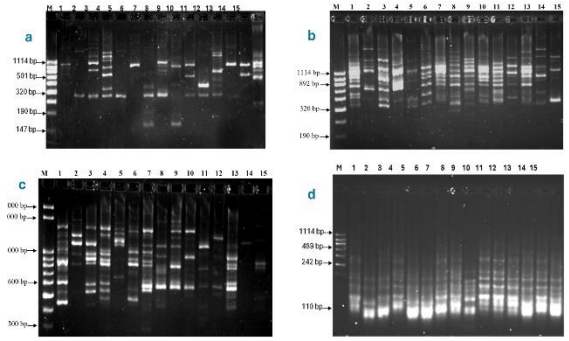

Figure 2. RAPD-PCR using the primer a OPA03, b OPA13,c OPA14, d P2 ; M: (100 bp+2 kb+3kb) DNA ladder, Lanes1-15: Oshikagbil, Ghorm Ghazal, Ghazal, Seady, Ferehy, Tagtagt, Kramte, Aom El-Frakhe, Zaghloul, Hayany, Samany, Bent Aisha, Halawy, Uraby, and Barhee cultivar, respectively.

Group 3 Lower polymorphism or conserved bands OPA07, OPA09

Analysis of cultivars amplified by OPA07

-Genetic diversity was tested among cultivators using primer OPA0,7 showing some common strong bands across cultivars of 1000 bp and 2000 bp, indicating some shared loci (Figure 3).

A High polymorphism (variation) could also be detected through Band differences, especially in Ghazal (Lane 3), Bent Aisha (Lane 12), and Barhee (Lane 15), showing high polymorphism, useful for cultivar differentiation. Some cultivators yielded Similar profiles Ghorm Ghazal, Ferehy, and Uraby (Lanes 2, 5, and 14) with similar amplification patterns. Zaghloul and Hayany (Lanes 9, 10) are also close in profile.

Alternatively, unique profiles could be detected through amplifying Ghazal cultivar (Lane 3) showing the most complex banding. Tagtagt (Lane 6) shows fewer visible bands, suggesting distinctiveness or weaker amplification (Figure 3).

Analysis of cultivars amplified by OPA09

-RAPD-PCR was also performed using the primer OPA09 (Fig. 1). The results showed that there are no similarities among different cultivars. However, each cultivar has its own band pattern, which is completely different from those of other cultivars. The amplified band numbers ranged from 5 to15 bands with different molecular weights ranged from 200-3000 bp.

Most cultivars share strong common bands around 1000 bp and 2000 bp. The most highly Polymorphic Cultivars amplified by OPA09 was Seady (Lane 4) and Uraby (Lane 14), showing a wide range of band sizes and intensity.

Alternatively, Unique Patterns was detected through amplification of Tagtagt (Lane 6) and Ghazal (Lane 3) exhibiting fewer or weaker bands.

The Most Distinct Profiles amplified by OPA9 primer was Ghorm Ghazal (Lane 2) with upper strong bands

near 2500 bp and Barhee (Lane 15) with sharp, well-separated bands (Figure 3).

The previous results of RAPD-PCR used mtDNA as template with the ten primers indicated that the primers OPA07, OPA09, OPA011, OPA012, OPA013, OPA014, and OPA015 showed the ability of separating the cultivars into 15 different groups; each group consists of one cultivar. While the primers OPA03, P1, and P2 could divide the examined cultivars into 12, 13, and 10 groups respectively.

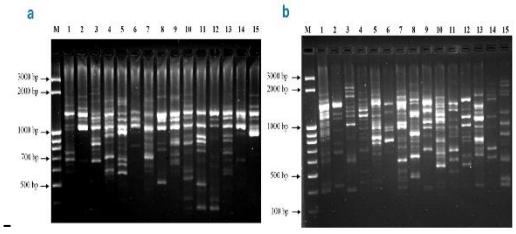

Figure 3. RAPD-PCR using the primer a OPA07, b OPA09; M: (100 bp+2 kb+3kb) DNA ladder, Lanes1-15: Oshikagbil, Ghorm Ghazal, Ghazal, Seady, Ferehy, Tagtagt, Kramte, Aom El-Frakhe, Zaghloul, Hayany, Samany, Bent Aisha, Halawy, Uraby, and Barhee cultivar, respectively.

3.2 Effectiveness of Primers in Discrimination Using AI Elecetrophoresis Analysis

Our data (Table 1) showed that OPA15 primer generated the highest diversity with 15 unique bands, indicating high polymorphism among cultivars for this primer. OPA11 and OPA12 followed closely with 14 unique bands each. (Figure 4) .OPA3 and OPA7 showed the least diversity, each producing only 5 unique bands. Primers P1 and P2 (likely custom or less common) produced relatively few unique bands (8 and 6, respectively), suggesting limited discriminatory power.

These results showed that OPA15, OPA11, OPA12 primers are most effective for distinguishing between cultivars due to their higher polymorphism. Additionally, Primers OPA3, OPA7 may still be useful for confirming similarity or lack of variation in specific cultivar comparisons.

Table 1 Effectiveness of Primers on discriminating cultivar diversity according to RAPD band sizes.

Cultivar Name	OPA3	OPA7	OPA9	OPA11	OPA12	OPA13	OPA14	OPA15	P1	P2
Oshikagbil	500, 320, 190	2000, 1000, ~700, 500	~2000, 1000, 700, ~300	~250, 500, 750, 1000, 1500, 2000	~250, 500, 750, 1000, 1500, 2000	300, 500, 700, 900, 1100	400, 600, 900, 1100, 1600	500, 850, 1000, 1200, 1500, 2200	150, 250, 350, 500, 1000	110, 240, 300, 450
Ghorm Ghazal	~600, 320, 190	2000, 1000, 700, 500	~2500, ~1200, 900, ~400	~300, 500, 900, 1200, 1800	~300, 500, 900, 1200, 1800	350, 600, 850, 1050	500, 700, 1000, 1300	400, 700, 1000, 1600, 2000	150, 250, 400, 500	110, 240, 300, 450
Ghazal	~700 , 320 , 190	2000, ~1300, 1000, 700, 500	~1800, 1000, 500	~400, 600, 850, 1000, 1300	~400, 600, 850, 1000, 1300	300, 500, 750, 1000 bp	400, 650, 800, 1200	600, 900, 1100, 1400, 1700	150, 300, 500, ~900	110, 240, 400, 480
Seady	500 , 320 , 190	2000, 1000, ~600	~2200, 1500, 1000, 500	~400, 600, 700, 900, 1100, 1400	~400, 600, 700, 900, 1100, 1400	320, 400, 700, 950	450, 700, 950, 1100	500, 800, 1000, 1200, 2000	150, 250, 400, 500	110, 250, 350, 450
Ferehy	~500, 320	2000, 1000, ~700, 500	~2000, 1100, 700, ~500	~500, 700, 1200, 1800	~500, 700, 1200, 1800	250, 600, 800, 1100	500, 600, 800, 1500	450, 750, 1000, 1300, 1600, 2100	150, 250, 350, 450, 500	110, 250, 350, 450, 500
Tagtagt	320	~1800, 1000	~1000, 800, 600, weak ~300	~400–900	~400–900	300, 700	300, 600, 900	500, 950, 1400	150, 250, 300	110, 240, 300, 450
Kramte	~500 , 320	2000, ~1100, ~700	~1800, 1000, 500	~300, 600, 800, 1000, 2000	~300, 600, 800, 1000, 2000	200, 400, 600, 800, 1000	400, 700, 950, 1200	450, 900, 1150, 1700	150, 250, 500	110, 240, 300
Aom El-Frakhe	~500 , 320 , ~200	2000, ~1000, ~700	2000, 1000, 500	~450, 600, 850, 1100, 2000	~450, 600, 850, 1100, 2000	250, 500, 850, 1000	350, 600, 850, 1000	400, 600, 1000, 1350, 1900	150, 200, 300, 400, 500	110, 240, 300, 450
Zaghloul	500 , 320	2000, 1000, 700	~1900, 1200, 700, ~300	~300, 700, 1000, 1200	~300, 700, 1000, 1200	300, 450, 700, 1050	450, 700, 1000	550, 800, 1000, 1500	150, 250, 350, 450	110, 250, 300, 450
Hayany	~500 , 320	2000, 1000, 700, 500	2000, 1000, 600	~300, 400, 800, 900, 1500	~300, 400, 800, 900, 1500	200, 450, 600, 850, 1000	500, 750, 1000, 1200	500, 850, 1050, 1300, 2000	150, 250, 350, 450, 500	110, 240, 300, 420
Samany	~700 , 320	2000, 1000, ~650	~2100, 1100, ~800	~500, 800, 1100	~500, 800, 1100	250, 500, 900, 1100	400, 650, 900, 1150	400, 650, 950, 1200, 1600	150, 200, 300, 500	110, 240, 300, 450
Bent Aisha	320 , 190	~1900, 1000, 700	~2000, 1000, 500	~400, 700, 1000, 1300	~400, 700, 1000, 1300	300, 700, 950	350, 600, 900	350, 700, 950, 1150	150, 250, 500	110, 240, 300
Halawy	~500 , 320	2000, 1000, 700	~1900, ~1000, 600	~300, 600, 900, 1000	~300, 600, 900, 1000	320, 600, 850, 1000	300, 700, 850, 1100	450, 900, 1200, 1400, 1900	150, 250, 350, 500	110, 240, 300, 480
Uraby	~600 , 320	2000, 1000, 700, 500	~2100, 1200, 800, 300	~300, 600, 800	~300, 600, 800	350, 700, 900	600, 950	400, 700, 1000	150, 250, 300, 400	110, 240, 300, 450
Barhee	700 , ~500 , 320	2000, 1000, 700, 500	~1800, 1000, ~700, 400	~500, 700, 900	~500, 700, 900	300, 600, 950	400, 900	500, 800, 1000	150, 250, 300, 500	110, 240, 300, 450

The Top Performing Primers was OPA9 showing the highest differentiation power with 33 unique bands, indicating it is highly polymorphic and effective in distinguishing between genotypes. OPA11 and OPA12 primers followed closely with 30 unique bands each, making them also strong candidates for genetic diversity analysisThe Moderate Performance primers were OPA3 and OPA15, producing 27 unique bands, indicating moderate but consistent differentiation power. OPA13, OPA14, and OPA7 were also considered moderate-producing bands, ranging between 20–24 bands, showing usable but lower variability.

The Low Differentiation Primers (P1 and P2)

produced the fewest unique bands (9 and 7, respectively), suggesting limited usefulness for diversity studies.

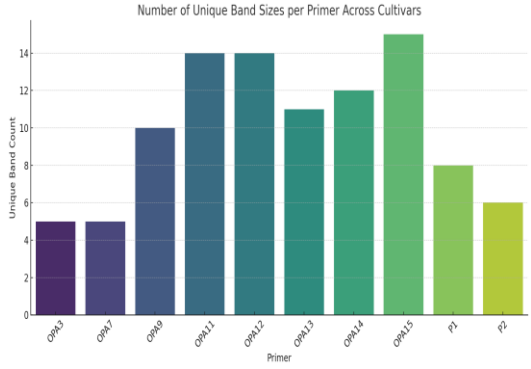

Figure 4. Effectiveness of Primers on discriminating cultivar diversity according to RAPD band sizes

3.3 Effectiveness of Primers in Discrimination Using AI Elecetrophoresis Analysis (Jaccard Simiarity)

The binary matrix was derived from key bands (presence = 1, absence = 0). The clustering used average linkage and 1 − Jaccard similarity as the distance metric.

Cultivars with similar banding patterns (e.g., Zaghloul, Hayany, Barhee) are clustered together.Outliers like Tagtagt and Bent Aisha show more distinct profiles.

Based on the Cluster Dendrogram of the 15 Date Palm Cultivars (Figure 5) generated from RAPD-PCR data using Jaccard similarity and average linkage method, we can interpret the following detailed results and insights:

The dendrogram divides the 15 cultivars into three main clusters, with subgroups indicating varying degrees of genetic similarity:

-Cluster I: Highly Related Cultivars (leftmost)F

This group shows the highest similarity (shortest linkage distances). All above cultivars form Cluster I, possibly representing traditional or regionally conserved cultivars.

Subcluster A: Including Bent Aisha, Ghorm Ghazal, Tagtagt. These three cultivars share very similar RAPD banding patterns, possibly indicating a close genetic lineage or regional adaptation.

Subcluster B: Grouped closely, suggesting a moderate similarity, but distinct from Subcluster A. Uraby, Ferehy. Grouped closely, suggesting a moderate similarity, but distinct from Subcluster A.

-Cluster II: Intermediate Genetic Diversity (middle group):

These cultivars are genetically more diverse than those in Cluster I. They cluster at a higher linkage distance, indicating more banding differences: Kramte, Oshikagbil, Aom El-Frakhe, Zaghloul, Hayany. This cluster likely represents a genetically broader group, possibly with hybrid characteristics or derived from different breeding sources.

-Cluster III: Most Divergent Cultivars (rightmost):

This cluster contains cultivars with the lowest similarity to the rest.

Subcluster A: Halawy, Barhee, Seady. These show a moderate to high degree of similarity among themselves.

Subcluster B:This pair is genetically distinct even from the rest of Cluster III, indicating high divergence, especially Ghazal which branches late in the dendrogram. Samany and Ghazal

Table 2: Summary of Cluster Groups according to Jaccard Similarity:

Cluster	Cultivars
I	Bent Aisha, Ghorm Ghazal, Tagtagt, Uraby, Ferehy
II	Kramte, Oshikagbil, Aom El-Frakhe, Zaghloul, Hayany
III	Halawy, Barhee, Seady, Samany, Ghazal

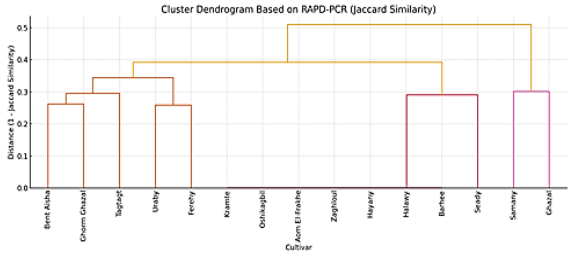

Figure 5. Cluster Dendogram of the 15 cutivars based on RAPD-PCR (Jaccard Similarity).

3.4 Effectiveness of Date cultivars Clustering based on AI analysis of RAPD-PCR data using (Dice similarity):

Based on Dice Similarity coefficient the cultiars was claiified into Close clusters indicating cultivars with more similar RAPD profiles ; Oshikagbil, Ghorm Ghazal, and Ghazal cluster together, confirming earlier insights, Hayany and Zaghloul also appear closely related. Tagtagt and Bent Aisha are more distant, showing distinct banding patterns (Figure 6, Table 3).

Table 3 Most similar cultivar pairs based on Dice similarity coefficients

Cultivar 1	Cultivar 2	Dice Similarity
Aom El-Frakhe	Halawy	0.923
Kramte	Hayany	0.909
Zaghloul	Hayany	0.909
Hayany	Halawy	0.909
Hayany	Barhee	0.909
Oshikagbil	Zaghloul	0.857
Ferehy	Kramte	0.833
Kramte	Zaghloul	0.833
Kramte	Halawy	0.833
Kramte	Barhee	0.833

These values (closer to 1) indicate a high degree of similarity in RAPD profiles between the pairs.

3.5 Effectiveness of Date Cultivars AI Analysis of the Binary Matrix of RAPD-PCR Bands in 15 Date Palm Cultivars

This binary matrix visually represents the presence (green squares = 1) or absence (white squares = 0) of 40 RAPD-PCR bands (Band_1 to Band_40) across 15 different date palm cultivars. This matrix was the basis for calculating genetic similarity among cultivars and serves as the input for cluster and PCA analyses (Figure 6).

1. Polymorphism Across Bands:

•A high level of polymorphism is evident, with many bands showing differential presence/absence across cultivars.

•Some bands (e.g., Band_7, Band_10, Band_21, Band_26) appear to be present in a large number of cultivars, suggesting conserved genetic regions.

•Others (e.g., Band_5, Band_11, Band_37) show very limited distribution, indicating more unique or cultivar-specific markers.

2. Cultivar-Level Diversity:

- Certain cultivars like Barhee, Uraby, Halawy, and Bent Aisha exhibit relatively more bands, suggesting higher genetic diversity or more marker amplification.
- Others like Oshikagbil and Ghazal show fewer positive bands, potentially indicating a more conserved or less amplified RAPD profile.

3. Potential Diagnostic Markers:

- Bands uniquely present in one or two cultivars (monomorphic or rare polymorphic bands) can be used as diagnostic or distinguishing markers for those cultivars in fingerprinting or breeding programs.

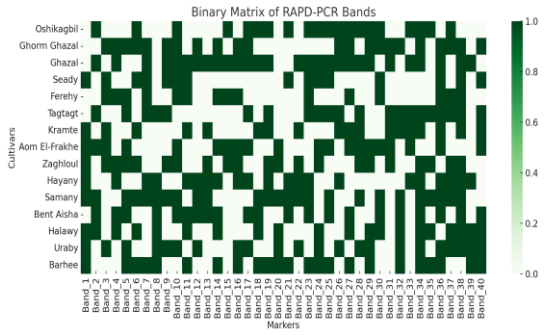

Figure 6. The image represents a binary matrix heatmap of RAPD-PCR banding profiles across 15 date palm cultivars and 40 RAPD markers (bands).

3.6 Effectiveness of Date cultivars AI Analysis of PCA (Principal Component Analysis) plot

The PCA (Principal Component Analysis) plot above visualizes the genetic variation among 15 date palm cultivars based on their RAPD-PCR (Random Amplified Polymorphic DNA - Polymerase Chain Reaction) banding patterns, represented as a binary matrix.

Explained Variance:

- PC1 accounts for 16.87% of the total genetic variation.
- PC2 explains 14.03%, giving a combined variance of approximately 30.9% captured in the two dimensions. While this is modest, it still reveals significant clustering and differences.

Distinct Cultivar Groupings:

1. Barhee and Samany are positioned far from others, suggesting a distinct genetic profile.
2. Seady, Hayany, and Kramte group somewhat closely, indicating potential genetic similarity.
3. Zaghloul, Halawy, and Oshikagbil are clustered in the lower left quadrant, possibly sharing related allelic profiles.
4. Ghorm Ghazal, Ghazal, and Tagtagt are separated along PC1 and PC2, indicating genetic diversity among them despite possible nomenclature similarity.

Potential Clonal Relationships or Shared Ancestry:

o Cultivars such as Zaghloul and Halawy being in proximity may reflect similar origin or breeding background.

o Those further from the origin (0,0), like Barhee, may possess unique alleles or have been less interbred.

4. Discussion

RAPD markers are polymorphic DNA separated by gel electrophoresis after PCR using short random oligonucleotide primers [40, 41, 42]. It has been particularly used for genomic and molecular studies as it is a simple and rapid method for determining

genetic diversity and similarity in various organisms.

In the present study, the mtDNA samples of the fifteen date palm cultivars (Dry, Semi-dry, and soft cultivars), were used as a template for amplification with ten arbitrary sequences decamer primers. A number of studies used mtDNA as templates for amplification with arbitrary sequence decamer primers [43, 44, 45, 46]. Primers were grouped by their ability to distinguish date palm cultivars based on RAPD band patterns. Group 1 primers showed high polymorphism and broad band ranges, making them highly effective for differentiating cultivars. Group 2 had moderate diversity with mid-size bands, offering limited discrimination. Group 3 primers showed low polymorphism with mostly conserved bands, contributing little to cultivar distinction. This highlights the importance of using highly polymorphic primers for effective genetic analysis.The obtained results showed a high number of polymorphic bands and mostly polymorphic profiles generated among the mtDNA of all cultivars. With primers OPA07, OPA09, OPA012, OPA013, OPA014, and OPA015, a high number of bands and highly different fingerprint patterns were obtained, while with primers OPA03, and OPA011 a few number of bands were obtained. These findings are in agreement with the results of Sane et al. (1997) [47], who used mtDNA as a template to generate RAPD profiles in rice. They found that 6 primers from 25 primers gave mostly polymorphic profiles, two primers revealed highly polymorphic profiles with no similarity, and one primer produced mostly monomorphic profile. Moreover, Lorenz et al. (1997) [48] stated that "PCR fingerprint analysis with 18 random decamer primers between two types of male sterility in sugar beat revealed a high number of polymorphic bands of mtDNA". While Nakajima et al. (1997) [49] detected 150 stable amplified bands from mtDNA of eight carrot cultivars by using 16 RAPD primers, 16 bands were common to all eight lines. In China, 47 pomegranate were clustered by11 RAPD primers with the employment of the cultivar identification diagram CID strategy [50-51].

In the current study, the high level of polymorphism among the examined cultivars were obtained may be due to the variability of mtDNA among all cultivars. Thereby, RAPD using mtDNA as a template is able to detect the variation among the examined date palm cultivars. It has been reported that the intergenic regions of the mitochondrial gene show tremendous variability due to the highly recombinative nature of the mitochondrial genome. This variability in the mitochondrial genomes is more likely detected by RAPD due to the random nature of binding with the random primers, thereby screening a much larger portion of the genome and leading to a better characterization of the organelle genome diversity [47]. They also added that RAPD profiles obtained with isolated mtDNA templates enable the distinction between two or more types of cytoplasms in rice. MtDNA could use a RAPD marker to distinguish two types of cytoplasmic male sterility in rice [32].

The genetic similarities among the examined cultivars based on RAPD results were estimated. These similarities ranged from 8 to 38% revealing low percentage of genetic similarity, which may be due to the different locations from which these cultivars were collected (Siwa, El-Meadia and ARC). In addition, they are also differed in fruit characterization (dry, semi dry and soft). Thus, the highest genetic similarity (38%) was obtained between the cultivars Bent Aisha / Uraby, and Hayany / Samany, which collected from the same location (El-Meadia) and they have soft fruit. In contrary, the lowest genetic similarity (8%) was observed between the cultivars Kramte /Barhee and Ferehy / Samany, that is may be due to the fact that they collected from different locations: Kramte cultivar collected from Siwa as well as it has semi dry fruit, while Barhee collected from (ARC) and soft fruit. Ferehy collected from Siwa and it has a dry fruit, while Samany from El-Meadia and its fruit is soft.

A phylogenetic tree was generated from the diverse fifteen cultivars based on RAPD patterns using mtDNA as a template. The results separated cultivars Uraby, Bent Aisha, Samany, Hayany, Zaghloul and Aom El-Frakhe in one subcluster (A2), perhaps because they are adapted to similar environmental conditions in El-Meadia. In contrast the RAPD technique was not able to divide the examined cultivars according to dry, semi dry and soft fruit perhaps, because the fruit characterization controlled by several genes.

Nakajima et al. (1997) reported that the genetic distances were calculated to generate a dendrograme; this dendrograme classified the eight carrot cultivars into four clusters [49].

Using AI for band discrimination analysis revealed that Primer OPA9 showed the highest polymorphism with 33 unique bands, making it the most effective for distinguishing date palm cultivars. OPA11 and OPA12 also demonstrated strong discriminatory power with 30 unique bands each. In contrast, primers like OPA3 and OPA7 showed low diversity and are better suited for confirming genetic similarity. Overall, OPA9, OPA11, and OPA12 are the most suitable primers

for genetic diversity analysis in this study.

The AI cluster analysis of 15 date palm cultivars based on RAPD-PCR data using Jaccard similarity revealed three major genetic groupings. Cluster I included closely related cultivars with minimal genetic divergence, such as Bent Aisha, Ghorm Ghazal, and Tagtagt, suggesting a shared genetic background or regional adaptation. Cluster II represented cultivars with intermediate diversity, including Kramte, Oshikagbil, and Hayany, indicating broader genetic variability likely due to hybridization or distinct breeding origins. Cluster III comprised the most genetically divergent cultivars, with Halawy, Barhee, and Seady forming a moderately similar subgroup, while Samany and Ghazal were the most distinct, suggesting unique genetic profiles. This clustering reflects the underlying genetic relationships and supports the utility of RAPD markers in assessing cultivar diversity.

The AI Dice similarity analysis further supports the genetic relationships observed among the date palm cultivars. High similarity coefficients (≥0.90) were found among several cultivar pairs, such as Aom El-Frakhe and Halawy (0.923), and Hayany with Kramte, Zaghloul, Halawy, and Barhee (all ~0.909), indicating strong genetic resemblance and possibly shared ancestry. Notably, Kramte appears as a central cultivar, showing high similarity with multiple others. In contrast, Tagtagt and Bent Aisha remained genetically distinct, consistent with earlier clustering results. Overall, Dice similarity coefficients effectively quantify cultivar relatedness and reinforce RAPD analysis as a valuable tool for genetic diversity assessment.

AI observations from the Matrix showed that for Band Distribution: Some bands (e.g., Band_5, Band_25, Band_27) appear across many cultivars → likely monomorphic markers. Others (e.g., Band_2, Band_7, Band_12) are present in only a few cultivars → likely polymorphic markers. Cultivar Uniqueness: Oshikagbil and Barhee exhibit distinct patterns. Cultivars like Seady, Ferehy, and Tagtagt share similar band profiles. Highly Informative Bands: Markers that split cultivars distinctly (present in ~50% of them) have high PIC values, indicating greater discriminative power.

The PCA results provide valuable insights into the genetic structure and diversity among the 15 date palm cultivars analyzed using RAPD markers. The first two principal components (PC1 and PC2) explain approximately 30.9% of the total genetic variation, which, although not very high, is sufficient to reveal meaningful patterns of clustering and divergence. The clear separation of cultivars such as Barhee and Samany from the rest suggests these genotypes possess unique alleles or may have been subject to different selective pressures or breeding histories, highlighting their genetic distinctiveness. On the other hand, the close grouping of Seady, Hayany, and Kramte indicates a higher level of genetic similarity, potentially due to shared ancestry or limited divergence. Similarly, the proximity of Zaghloul, Halawy, and Oshikagbil supports the hypothesis of related genetic backgrounds, possibly arising from common regional origins or propagation practices. The distinct placement of Ghorm Ghazal, Ghazal, and Tagtagt along both principal components further underscores their genetic variability, which could be due to differing evolutionary paths or environmental adaptations. Overall, PCA has proven to be an effective tool for visualizing genetic relationships and supports the clustering patterns observed through similarity coefficients and hierarchical clustering, contributing to a comprehensive understanding of the genetic diversity within these cultivars.

In this study, the genetic diversity of 15 date palm cultivars was assessed using Random Amplified Polymorphic DNA (RAPD) markers amplified from mitochondrial DNA (mtDNA). The analysis began with PCR amplification using ten random decamer primers. Each primer produced DNA bands of varying sizes (300–2000 bp), and these bands were resolved by gel electrophoresis. The presence (1) or absence (0) of each band in every cultivar was recorded, resulting in a binary data matrix, which formed the foundation for all subsequent analyses. This matrix represented the RAPD fingerprint profiles of each cultivar across multiple primers and was critical for identifying polymorphic loci, estimating similarity, and generating dendrograms and principal component plots.

The binary matrix allowed for the calculation of Dice similarity coefficients and Jaccard similarity indices, two distance metrics used to quantify genetic similarity between cultivars. The Dice coefficient, being more sensitive to shared bands, emphasized genetic closeness; Jaccard was slightly more conservative and used in the hierarchical clustering process. Both approaches enabled the identification of highly similar cultivar pairs (e.g., Aom El-Frakhe/Halawy and Hayany/Zaghloul) and the detection of genetically distinct cultivars (e.g., Tagtagt, Samany, and Ghazal).

The binary matrix also facilitated hierarchical clustering (UPGMA and average linkage methods), using 1 − similarity indices to construct a dendrogram that divided cultivars into three main

clusters. These clusters reflected genetic relationships potentially shaped by cultivar origin, fruit type (dry, semi-dry, soft), or breeding history. For example, cultivars such as Bent Aisha, Tagtagt, and Ghorm Ghazal grouped together due to similar band patterns, whereas Samany and Ghazal formed a highly divergent clade.

Furthermore, Principal Component Analysis (PCA) was applied to the binary matrix to reduce dimensionality and visualize genetic structure. The first two principal components (PC1 and PC2) accounted for 30.9% of the total variance. The PCA scatterplot revealed distinct groupings consistent with clustering and similarity coefficients, while also highlighting genetically unique cultivars like Barhee and Samany that were placed far from the main groups.

Each method played a unique role:

•Dice and Jaccard coefficients quantified genetic similarity and enabled pairwise comparison.

•Hierarchical clustering organized cultivars into evolutionary or breeding-related groups.

•PCA visualized genetic structure and detected outliers or unique genotypes.

•The binary matrix served as the unifying data structure, enabling consistent comparison across all analyses and supporting identification of highly polymorphic primers and cultivar-specific markers.

5. Conclusion

The combined use of RAPD markers, binary matrix analysis, similarity coefficients (Dice and Jaccard), hierarchical clustering, and principal component analysis (PCA) provided a comprehensive assessment of genetic diversity among the 15 date palm cultivars. The results revealed a moderate to high level of polymorphism, indicating substantial genetic variation among the cultivars, particularly when using highly informative primers such as OPA9, OPA12, and OPA15.

The binary matrix derived from RAPD banding patterns enabled consistent and comparative analysis across all cultivars. Similarity coefficients identified cultivar pairs with high genetic resemblance (e.g., Hayany–Zaghloul, Aom El-Frakhe–Halawy) and also highlighted genetically distinct cultivars (e.g., Tagtagt, Ghazal, and Samany).

Hierarchical clustering divided the cultivars into three major clusters, reflecting varying degrees of genetic relatedness. Cultivars from the same geographic region often clustered together, suggesting shared ancestry or local adaptation. However, fruit type (dry, semi-dry, soft) did not strictly correspond to genetic grouping, indicating that such traits may not be strongly linked to mitochondrial genome variation.

PCA results supported these findings, separating genetically unique cultivars from closely related groups and confirming patterns observed in clustering and similarity matrices. Cultivars like Barhee and Samany showed strong divergence, while others like Seady, Hayany, and Kramte grouped closely, reinforcing the presence of both shared and distinct genetic backgrounds.

The integrated molecular and statistical analyses confirmed the presence of both high similarity and substantial genetic divergence among the studied date palm cultivars. These findings are valuable for guiding breeding programs, conservation strategies, and the management of genetic resources in date palm cultivation.

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