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Original Article

Exploring Egyptian date palm cultivars using morphological traits and molecular markers

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Abstract

This study used 38 morphological characteristics, IRAP and SCoT markers, and DNA barcoding to assess the relationships among seven date palm cultivars commonly grown in North Sinai, Egypt—five classified and two unknowns. Results of morphological characterization analysis within date palm cultivars showed significant variability ($P \leq 0.05$) in tree, leaf, fruit, and seed morphological traits. Principal component analysis revealed strong relationships among certain morphological traits and cultivars, forming four major homogeneous variable groups. Cluster analyses revealed that Beid El Gamal exhibited the lowest genetic similarity among the seven cultivars, while the highest similarity index was observed between Amri and Yellow Majhal, Hayani and Red Majhal, and Khedri and Kapoushi. The evaluation was performed using IRAP and SCoT markers; IRAP markers generated 97 amplicons with 36% polymorphism, while SCoT markers produced 89 amplicons with 45% polymorphism. Genetic similarity coefficients ranged from 0.83 to 0.93, with the highest similarity (0.93) observed between Amri and Yellow Majhal cultivars. DNA barcoding using rbcL, matK, and trnH genes successfully differentiated the cultivars into distinct phylogenetic groups. Molecular analyses revealed that Amri and Yellow Majhal cultivars were most closely related, while Beid El Gamal showed the lowest similarity. We concluded that analyzing morphological traits can distinguish date palm cultivars, but it is time-consuming and requires specific traits to identify each cultivar. By integrating morphological and genetic characteristics, we achieved more reliable identification. Molecular markers, such as IRAP and SCoT—particularly using IRAP-2204 or SCoT-09 primers—along with DNA barcoding, allow for quick and accurate differentiation of closely related cultivars, even unknown ones. This approach enhances sustainable date palm production by conserving genetic resources, improving breeding programs, verifying identities, tracking genetic diversity, and protecting local cultivars.

Keywords: Date palm, Morphological identification, DNA barcoding, Genetic diversity, IRAP markers, SCoT markers

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Introduction

The date palm (*Phoenix dactylifera* L.) is famous for its resilience to harsh environmental conditions, cultivated across over 1.3 million hectares globally, producing nearly 10 million tons annually, with Egypt contributing 19% (FAOSTAT, 2023). Dates are a staple food in arid regions, including Mediterranean countries. It has a highly nutritional value—providing sugars, amino acids, fiber, vitamins, and minerals. Palm trees are crucial for sustainable agriculture development, particularly in addressing food security challenges from climate change (Mihi et al., 2019; Alotaibi et al., 2023; Al-Karmadi and Okoh, 2024). Therefore, date palm trees can grow in all types of lands and difficult climatic conditions where other fruit tree species cannot and also help protect the environment. They reduce soil degradation and desertification (Al-Khalifah et al., 2006; FAO and AOAD, 2023). For this reason, countries located in the palm-growing belt around the world have increased the number of date palms and now have about 160 million trees (FAOSTAT, 2023).

In Egypt, over 79 date palm cultivars have been identified, including common, rare, and exotic ones, alongside some unclassified (unknown) cultivars (Rizk and El-Sharabasy, 2019). However, selecting high-quality cultivars that can adapt to environmental changes remains a challenge. The date palm reproduces in two ways: sexually, through seeds, and asexually, through offshoots. Seed propagation produces unclassified or unknown cultivars, known as "Majhal" that may resemble classified ones, increasing variability in date palms (Elsafy et al., 2015). Another issue involves sharing names among classified and unclassified cultivars due to their similar traits, or they are known by different names in various regions (Al-Khalifah et al., 2012). Environmental variation can change the expression of morphological traits within a species and introduce ambiguity in identifying a species, especially in diversity-rich ecosystems (Endara et al., 2018). In addition, the phenotypic plasticity that allows organisms to change their phenotypes with environmental conditions can lead to characteristic differences within the same species, making identification more complex (Liu et al., 2013). Morphological identification of the date palm is a traditional method commonly used for this purpose. Date palm cultivars can be distinguished by their vegetative characteristics, such as the trunk, crown, leaf, and spines, and their reproductive characteristics, which include flowers, fruits, and seeds. These characteristics can be categorized into quantitative traits, such as leaf length, the number of pinnae, spine area length, and fruit weight, and qualitative traits, including crown shape, fruit shape, fruit color, and seed apex shape (Rizk and El-Sharabasy, 2007). Many researchers have reported that morphological traits play a significant role in distinguishing between date palm cultivars. They utilized these traits to explore diversity among various date palm cultivars, including 11 from Egypt (Aly et al., 2019), 12 from Iraq (Abd et al., 2019), 26 from Algeria (Bedjaoui and Benbouza, 2020), 11 from Ethiopia (Ahmed et al., 2023), 50 from Pakistan (Ahmad et al., 2023), 18 from Saudi Arabia (Al Rashidi et al., 2023), and 10 from Tunisia (Kadri et al., 2025). Data collected on morphological characteristics provides essential information to scientists, breeders, and farmers for effectively managing and utilizing date palm germplasm collections (Ahmad et al., 2023).

Molecular techniques are now widely used in genetic diversity studies due to their simplicity, speed, and reasonable cost, offering new opportunities for precise cultivar identification (Fatima et al., 2019). Many studies have utilized traditional techniques such as RAPD (random amplified polymorphic DNA), ISSR (Inter-simple sequence repeat), and SSR (simple sequence repeats) markers (Elmeer et al., 2017; Abdelkrim et al., 2023). RAPD markers typically use shorter and less specific primers, which often leads to variable amplification patterns (Vivodík et al., 2016; Miler et al., 2023; Collard and Mackill, 2008). ISSR marker production is more reproducible than RAPD and is often used in genetic diversity studies of medicinal plants like Memecylon (Ramasetty et al., 2016). In contrast, techniques like IRAP (Inter-retrotransposon amplified polymorphism) and SCoT (Start Codon Targeted), as well as chloroplast DNA barcodes (rbcL, matK, and trnH), remain largely unexplored for the characterization of date palms.

IRAP marker is an effective tool for genetic studies due to its cost-efficiency, high resolution, and applicability across a wide range of species. It is particularly useful for evaluating natural genetic variability because of its high level of polymorphism and the genomic loci associated with retrotransposon long terminal repeats. SCoT marker is a specialized fingerprinting technique that enables higher polymorphism rates, allowing for detailed comparisons of closely related cultivars and assessing genetic variability (Al-Khayri et al., 2022; Hromadová

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et al., 2023). It targets conserved regions near the ATG start codon, featuring longer primer sequences that enhance the reproducibility and stability of functional genes. This method increases the detection of genetic variation and aids in genetic characterization and cultivar identification (Rhouma-Chatti et al., 2020). It has been used to effectively identify and analyze the Echinacea plant (Jedrzejczyk, 2020). Additionally, it can complement traditional genetic mapping to improve marker density in specific chromosomal regions, offering advantages over older techniques like RAPD (Thakur et al., 2016). This approach enhances our understanding of evolutionary relationships and helps in selecting desired traits (Li et al., 2011). The unique features and clear genetic reproducibility of SCoT and IRAP markers make them valuable tools in plant genetics. They help in understanding genetic diversity and support conservation and breeding programs (Al-Khayri et al., 2022).

DNA barcoding has emerged as another promising method for accurately identifying plant species, offering distinct advantages over traditional morphological identification techniques (Saddhe and Kumar, 2018). This method is also essential for analyzing cultivar identification and conserving genetic diversity. It can differentiate among closely related species that look similar (Jain, 2015). Additionally, DNA barcoding provides precise species identification as well as valuable insights into genetic diversity and relationships (Nadin-Davis, 2012; Ghorbani et al., 2017). Among the common markers used in plant systematics and taxonomy are the rbcL and matK genes. These genes come from rapidly mutating regions of plant DNA and provide a clear phylogenetic signal. The trnH marker complements these by offering more information on plastid genome variation, allowing for better species differentiation within closely related groups. Together, these molecular markers significantly enhance date palm germplasm management, facilitating improved conservation, breeding, and agricultural productivity (Yu et al., 2011).

In general, taxonomic identification using morphological characters is often labor-intensive and prone to errors, influenced by environmental factors, and requires reliable expertise and resources (Elmeer and Mattat, 2012; Simozrag et al., 2016). Our results align with more recent molecular studies by Abdulla and Gamal (2010), who found close genetic relationships between cultivars with similar fruit characteristics despite different local names.

So, this study utilized both morphological and molecular methods to improve the accuracy of date palm cultivar identification, given their broad genetic background. The present research attempts to assess the relationships among seven date palm cultivars commonly grown in North Sinai, Egypt, five classified and two unknowns, by examining their morphological characteristics and genetic diversity using IRAP and SCoT molecular markers. We also evaluated three chloroplast DNA barcodes for cultivar identification and established reliable genetic markers to differentiate the unknown cultivars. Together, these molecular markers significantly enhance date palm germplasm management, facilitating improved conservation, breeding, and agricultural productivity. This combined approach not only enhances identification but also supports effective conservation of date palm cultivars, ensuring their genetic resources are preserved for future generations.

Material and Methods

Plant materials

This study was conducted on seven Egyptian date palm cultivars, which we categorized into two groups: classified (Amri, Hayani, Khedri, Kapoushi, and Beid El Gamal) and unclassified or of unknown origin (Red Majhal and Yellow Majhal) (Table 1). The research was conducted across three date palm orchard locations in the Bir Al-Abd region of North Sinai Governorate, Egypt: Rabaa Valleys, Qatia Desert Plains, and the El-Sadat Coastal Strip (Figure 1). All three date palm orchards were similar in age, soil type, and agronomic management. Trees were approximately 25 years old, growing in sandy soil under rainfed conditions, and received uniform annual care, including fertilizers, pesticides, and superficial tillage. In September 2021, three trees of each cultivar with similar growth patterns and fruit loads were selected in each orchard to serve as one of three experimental replicates (3 trees/orchard/replicate). Trunk measurements were recorded in the orchards, and leaf and fruit samples (at the Besser and Rutab stages) were randomly collected and transported to the laboratory for analysis.

Table-1. List of studied Egyptian cultivars.

No.	Date palm cultivars	Group	Notes
1	**Amri**	Group I (classified cultivars): The morphology of trees and fruits is well-known among farmers, researchers, and consumers in Egypt.	Trees are propagated through offshoots or the tissue culture technique.
2	**Hayani**		
3	**Khedri**		
4	**Kapoushi**		
5	**Beid El Gamal**		
6	**Red Majhal**	Group II (unclassified or unknown cultivars): These are not distinctly named and are referred to as "Majhal" or "Majhol" by farmers, researchers, and consumers in Egypt.	These cultivars may have been grown from seed, resulting in genetic variations, or may have been genetically affected by environmental factors.
7	**Yellow Majhal**		

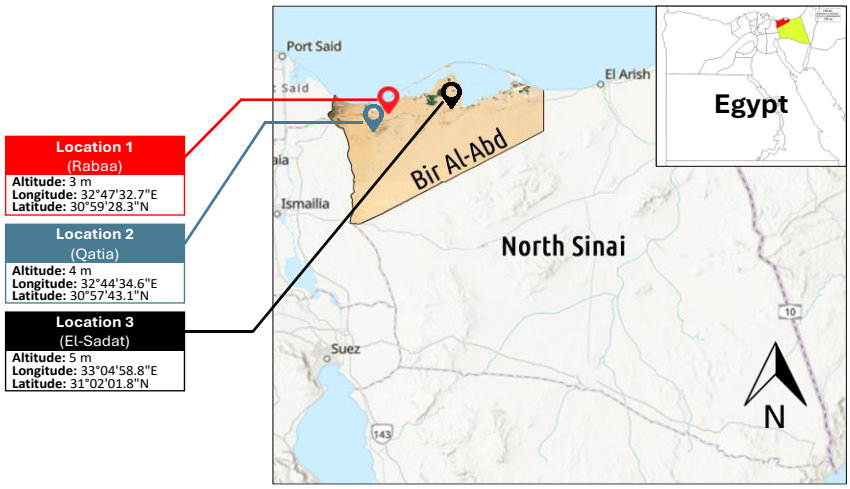

Figure-1. The three study date palm orchards are located in Bir Al-Abd region, North Sinai, Egypt.

Morphological traits

Morphological characteristics for seven date palm cultivars were conducted using 38 traits (Table 2), as described in recent studies (Simozrag et al., 2016; El kadri et al., 2019; Rizk and El-Sharabasy, 2019). Ordinal and nominal scales were employed following the standard date palm descriptors developed by Rizk and El-Sharabasy, (2007). The trunk diameter of each date palm was measured with a millimeter scale, and the crown shape was assessed using an ordinal scale with the following categories: 1) dense, 2) moderately dense, 3) loose and flat above, and 4) loose and open from the middle.

One mature leaf from each tree were used to measure various morphological leaf traits as; leaf length (cm), leaf width (cm), petiole length (cm), petiole/leaf ratio, blade length (cm), blade/leaf ratio, pinnated part length (cm), pinnae/ leaf ratio, number of pinnae, pinnae density was recorded using an ordinal scale (1:very dense, 2:dense, 3:lax, and 4:very lax), pinnae types were scored based on (1:Introse and antrose, 2:Antrose and retrose, and 3:Antrose, introse and extrose), spine part length (cm), spine part/leaf ratio, maximum spine length (cm), minimum spine length (cm), number of spine and spine type was scored as scale (1: Single, 2: Twin, and 3: Mixed).

Fruit samples (100 random fruits per tree) were collected at the Besser ripening stage. According towing data were recorded: fruit length (cm), fruit width (cm), fruit weight (g), fruit volume (cm^3), fruit shape was calculated using a nominal scale (1:Cylindrical, 2:Elliptical, 3:Flaccid-elongate, 4:Ovate-elongate, 5:Obviate-elongate, 6:Ovate, 7:Obviate, 8:Sub-spherical, and 9:Global), fruit apex according scale (1:Obtuse, 2:Blunt, 3:Retuse, and 4:Truncate), fruit base according scale (1:Obtuse, 2:Retuse, 3:Truncate, 4:Truncate and emarginated), fruit color according scale (Besser; 1:Pale red, 2:Shiny red, 3:Dark red, 4:Pale Yellow, 5:Yellow, 6:Yellowish red, 7:Yellow-brown, 8:Yellow orange, 9:Orange), fruit color according scale (Rutab; 1:Red, 2:Dark red, 3:Orange-yellow, 4:Yellow-orange mottled pale red, 5:Brown-yellow, 6:Pale brown, 7:Brown, 8:Brownish black), flesh color according scale (1:White, 2:Whitish creamy, 3:Whitish yellow, 4:Cream, 5:Cream-brown), flesh thickness (cm), flesh weight (g), flesh texture according scale (1:Soft, 2:Firm, and 3:Fibrous), flavor according scale (1:Poor, 2:Good, and 3:Excellent), flesh taste according scale (1:Palatable, 2:Delicious, and 3:Delicious-sweet).

Seeds extracted from the fruits were used to determine seed dimensions (length and width) using Vernier calipers, seed weight (g), and the seed weight to fruit weight ratio (%).

Table-2. List of morphological traits and their codes that were used for analysis.

Vegetative traits	Parameter	Code
Tree	Crown shape	VP1
	Trunk diameter (cm)	VP2
Leaf	Leaf length (cm)	VP3
	Leaf width (cm)	VP4
	Petiole length (cm)	VP5
	Petiole: leaf ratio (%)	VP6
	Blade length (cm)	VP7
	Blade: leaf ratio (%)	VP8
Pinnies	Pinnated part length (cm)	VP9
	Pinnae: leaf ratio (%)	VP10
	Number of pinnae	VP11
	Pinnae density	VP12
	Pinna types	VP13
Spines	Spine part length (cm)	VP14
	Spine part: leaf ratio (%)	VP15
	Max. spine length (cm)	VP16
	Min. spine length (cm)	VP17
	Number of spines	VP18
	Spine type	VP19

Fruit traits	Parameter	Code
Fruit	Fruit length (cm)	FP1
	Fruit width (cm)	FP2
	Fruit weight (g)	FP3
	Fruit volume (cm^3)	FP4
	Fruit shape	FP5
	Fruit apex	FP6
	Fruit base	FP7
	Fruit color (Besser)	FP8
	Fruit color (Rutab)	FP9
	Flesh color	FP10
	Flesh thickness (cm)	FP11
	Flesh weight (g)	FP12
	Flesh texture	FP13
	Flesh Flavor	FP14
	Flesh taste	FP15
Seed	Seed length (cm)	FP16
	Seed width (cm)	FP17
	Seed weight (g)	FP18
	Seed weight: fruit weight (%)	FP19

Molecular and DNA barcoding analyses

Extraction of genomic DNA

From each date's cultivars, one-year-old and healthy leaves were collected and stored at -80°C before DNA isolation. Genomic DNA was isolated from the leaf by grinding with liquid nitrogen. Lyophilized powdered leaves were used for DNA extraction using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) (Sambrook et al., 1989). The purity and concentration of the DNA were assessed using an ND-1000 spectrophotometer (Nano-Drop Technologies, Thermo Fisher Scientific Inc.). The extracted DNA was stored at -20°C for PCR amplification.

IRAP/SCoT primers and PCR amplification

To achieve the IRAP and SCoT fractions, eight IRAP and ten SCoT primers were selected (Table 3). The PCR amplification for IRAP was carried out, as described in Badr et al. (2020), while the PCR amplification for SCoT was carried out, as described in Al-Qurainy et al. (2015). PCR mixture was carried out in a 20 µl reaction volume containing 10 µl Master Mix (GeneDireX), 2 µl primer (10 pmol/µl), 5 µl dH_2O, and 3 µl of genomic DNA (200 ng) in each sample tube. Amplification for IRAP primers was performed with the cycler programmed as follows: 1 cycle of 94 °C for 5 min, 40 cycles (94 °C for 40 sec, 45 °C for 40 sec, and 72 °C for 1 min), 1 cycle of 72 °C for 7 min. Amplification for SCoT primers was performed with the cycler programmed as follows: 1 cycle of 94 °C for 5 min, 40 cycles (94 °C for 40 sec, 50 °C for 50 sec, and 72 °C for 1 min), 1 cycle of 72 °C for 7 min. The degrees of primer annealing varied according to the melting point of each primer. Resolved amplified products on 1.5% Agarose gels, buffered with 1x TAE and stained with Ethidium bromide (EtBr). The fragments were visualized using a gel documentation system. The molecular size of the DNA bands was detected using a 100-bp ladder to size DNA fragments of 100 – 3000 bp in length.

Table-3. List of IRAP/SCoT primers.

SCoT primer list			IRAP primer list		
No.	**Primer name**	**Sequence (5'→3')**	**No.**	**Primer name**	**Sequence (5'→3')**
1	SCoT-01	ACGACATGGCGACCACGC	**1**	IRAP-2175	TTAGACCCGGAACCGCCGTG
2	SCoT-02	ACCATGGCTACCACCGGC	**2**	IRAP-2198	ATCCTTCGCGTAGATCAAGCGCCA
3	SCoT-03	ACGACATGGCGACCCACA	**3**	IRAP-2197	GAAGTACCGATTTACTTCCGTGTA
4	SCoT-05	CAATGGCTACCACTAGCG	**4**	IRAP-2200	ATGTGACAGTCGACTAACCAC
5	SCoT-07	ACAATGGCTACCACTGAC	**5**	IRAP-2202	TGGCGCTTGATCTACGCGAAGGA
6	SCoT-08	ACAATGGCTACCACTGAG	**6**	IRAP-2204	AACTTGATCCAGATCATCTCC
7	SCoT-09	ACAATGGCTACCACTGCC	**7**	IRAP-4334	CCATGGCGAGCAGATGTGCT
8	SCoT-11	ACAATGGCTACCACTACC	**8**	IRAP-4370	ATGCCGTATTCTCAGCATCC
9	SCoT-12	CAACAATGGCTACCACCG			
10	SCoT-13	ACCATGGCTACCACGGCA			

c. *rbc*L, *mat*K, and trnH chloroplast gene barcoding

Sequences of primers forward and reverse used for barcoding are given in Table 4. The PCR amplification of the rbcL and matK genes is described in Ghareb et al. (2020). The PCR amplification of the trnH/psbA gene is described in Lee et al. (2007). PCR mixture was carried out in a 20 µl reaction volume containing 10 µl Master Mix (GeneDireX), 2 µl primer (10 pmol/µl), 5 µl dH2O, and 3 µl of genomic DNA (200 ng) in each sample tube. Amplification for rbcL primers was performed with the cycler programmed as follows: 1 cycle of 94 °C for 5 min, 40 cycles (94 °C for 30 sec, 52 °C for 30 sec, and 72 °C for 50 sec), 1 cycle of 72 °C for 7 min. Amplification for Matk primers was performed with the cycler programmed as follows: 1 cycle of 94 °C for 5 min, 40 cycles (94 °C for 48 sec, 30 °C for 50 sec, and 72 °C for 50 sec), 1 cycle of 72 °C for 7 min. Amplification for trnH primers was performed with the cycler programmed as

follows: 1 cycle of 94 °C for 5 min, 40 cycles (94 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 50 sec), 1 cycle of 72 °C for 7 min. Resolved amplified products on 1.5% Agarose gels, buffered with 1x TAE and stained with Ethidium bromide (EtBr). The fragments were visualized using a gel documentation system. The molecular size of the DNA bands was detected using a 100-bp ladder to size DNA fragments of 100 – 3000 bp in length.

Table-4. Primer codes, sequences, and size for barcoding the rbcL, matK, and trnH/psbA genes.

Primer Code	Sequence (5'→3')	Product Size	Reference
rbcL-F	ATGTCACCACAAACAGAAAC	700bp	Fay et al. (1997)
rbcL-R	TCGCATGTACCTGCAGTAGC		
matK-F	CGATCTATTCATTCAATATTTC	800bp	Yu et al. (2011)
matK-R	TCTAGCACACGAAAGTCGAAGT		
trnH/psbA-F	GTTATGCATGAACGTAATGCTC	600bp	Lee et al. (2007)
trnH/psbA-R	CGCGCATGGTGGATTCACAATCC		

Purification and sequencing of DNA barcode analysis

EZ-10 spin column PCR product purification was used to clean all amplified products. Following the manufacturer's instructions, the resultant PCR was sequenced using Big Dye TM Terminator Cycle Sequencing Kits in an automated sequencer, the ABIPRISM3730XL Analyser. Single-pass sequencing was carried out on each template using the rbcl, matK, and trnH forward primers. An ethanol precipitation process separated the fluorescently tagged fragments from the unincorporated terminators. The samples were reconstituted in distilled water and electrophoresed on a Microgen Company ABI3730xl sequencer.

Statistical analysis

All collected data of morphological characteristics were statistically analyzed by the one-factor analysis of variance (ANOVA), and differences among the means were determined using the LSR test at $P \leq 0.05$ using Co-STAT software, V.6.13 (CoHort software, Berkeley, CA 94701). Principal component analysis (PCA) analysis was performed using XLSTAT version 20, as well as dissimilarity dendrogram and heat map with hierarchical clustering were performed using R software v.4.4.3.

The gel images were analyzed using the Gel Analyzer-3 software to determine the molecular weights of the amplified fragments. The amplified fragments were scored as present (1) or absent (0). Jaccard's coefficient estimated the similarity coefficient. Cluster analysis was carried out with PAST software Version 4.03 to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA), according to Hammer et al. (2001). Polymorphic Information Content (PIC) and resolving power (RP) values for each primer were determined using the online program to calculate polymorphic information content and resolving power (https://irscope.shinyapps.io/iMEC/).

The alignment of each generated sequence was completed by employing the Basic Local Alignment Search Tool (BLAST), comparing them with existing sequences available in the GenBank. DNA sequences corresponding to the above-mentioned molecular markers for other Sideritis taxa were molecular markers mentioned above and subsequently aligned for each marker using Mega-11 software version 11.0.13 (Tamura et al., 2021). A unified phylogenetic tree was constructed using the neighbor-joining statistical method and the maximum composite likelihood substitution model. To ensure accessibility and transparency, all newly generated sequences were submitted to GenBank, obtaining the accession numbers OR909054–OR909060. The evaluation of evolutionary divergence between sequences for each molecular marker involved the calculation of pair-wise distances, conducted within the Mega-11 software version 11.0.13 (Tamura et al., 2021).

Results

Morphological traits

ANOVA analysis

Results of morphological data analysis revealed significant differences ($P \leq 0.05$) among seven date palm cultivars—Amri, Kapoushi, Khedri, Hayani, Beid El Gamal, Red Majhal, and Yellow Majhal—collected from three localities with similar climatic conditions. This analysis covered various characteristics of the trees, leaves, fruits, and seeds (Tables 5 and 6).

In this regard, the VP1 varied among different cultivars, ranging from loose and flat at the top to loose and open in the middle to a medium-density structure. Regarding leaf traits, Amri and Yellow Majhal had significantly greater VP2, VP11, and VP15. The cultivars Amri and Red Majhal had greater VP5 and VP6. Kapoushi, Hayani, and Red Majhal cultivars showed superiority over other palm tree cultivars in VP3, VP7, and VP9 traits. Khedri had the widest VP4, while the Hayani outperformed the others in VP8 and VP10. The VP12 characteristic was found to be very dense in both Khedri and Hayani, whereas the other cultivars were classified as dense. The VP13 exhibited antrose and retrorse types in four cultivars, introse and antrose in two cultivars, and a combination of antrose, introse, and extrose in one cultivar. Notably, Yellow Majhal cultivars recorded the longest VP14 and VP17, with no significant difference between Red Majhal in VP18. The VP19 differs among cultivars as either a single, twin, or mixed type. Beid El Gamal gave the lowest significant values for leaf characteristics compared to the other cultivars.

The fruits of Amri, Kapoushi, and Khedri cultivars showed the longest FP1 trait compared to the other cultivars. Amri and Yellow Majhal cultivars had higher values for FP2, FP3, FP4, FP11, and FP12 than the other studied cultivars. Additionally, the date palm cultivars showed highly significant variation in FP5, FP6, FP7, FP8, FP9, FP10, FP13, FP14, and FP15. Regarding the FP5 trait, Amri fruits were elliptical, Kapoushi was flaccid-elongate and obtuse, Khedri, Hayani, and Red Majhal were cylindrical, while Beid El Gamal and Yellow Majhal exhibited an ovate shape. In terms of the FP6 trait, three cultivars had a blunt shape, whereas four cultivars were obtuse. The FP7 trait was observed as obtuse in the Kapoushi and Khedri cultivars, while other cultivars displayed truncated and emarginated shapes. Based on FP8 at the Besser stage, cultivars were classified into five groups: yellow (2 cultivars), yellowish-red (2 cultivars), pale-yellow (1 cultivar), pale-red (1 cultivar), shiny red (1 cultivar), and pale-red (1 cultivar). At the Rutab stage, cultivars were divided into three groups based on FP9: pale brown (2 cultivars), brown (3 cultivars), and brownish-black (2 cultivars). The FP10 trait was characterized as whitish-creamy in Amri, Kapoushi, Khedri, Red Majhal, and Yellow Majhal cultivars, while Hayani and Beid El Gamal showed a cream-brown color. Significant differences were noted among cultivars based on the FP13 trait, which included soft, firm, and fibrous textures. For FP14, cultivars ranged from poor to excellent in flavor, and for FP15, their tastes ranged from palatable to delicious and delicious-sweet.

In terms of seed characteristics, the Beid El Gamal cultivar had the smallest FP16, while the Kapoushi cultivar recorded lower FP17 values among the studied cultivars. There was no significant difference in the FP18 trait across all the date palm cultivars, however, Hayani and Red Majhal achieved higher values for the FP19 trait.

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Table-5. Vegetative morphological traits of seven date palm cultivars.

Cultivars	Amri	Kapoushi	Khedri	Hayani	Beid El Gamal	Red Majhal	Yellow Majhal
VP1	Moderately dense	Loose and flat above	Loose and open from the middle	Moderately dense	Loose and flat above	Loose and open from the middle	Loose and flat above
VP2	170.33 a	134.00 c	154.66ab	131.66 c	148.33bc	137.33bc	169.33 a
VP3	368.00 ab	390.66 a	351.33 b	371.33 ab	235.66 c	388.66 a	353.66 b
VP4	12.50 b	11.66 b	15.00 a	12.16 b	6.16 d	9.00 c	9.00 c
VP5	33.66 a	26.33 b	24.66 b	20.33 c	11.00 d	34.66 a	8.33 d
VP6	9.14 a	6.73 bc	7.00 b	5.48 cd	4.67 d	8.93 a	2.34 e
VP7	252.66 c	292.00 a	279.33 ab	294.00 a	166.33 d	298.00 a	260.33bc
VP8	68.65 e	74.75 cd	79.51 a	79.12 ab	70.59 e	76.66 bc	73.66 d
VP9	171.00 b	219.66 a	232.00 a	237.00 a	108.00 c	242.00 a	175.33 b
VP10	46.45 c	56.23 b	66.04 a	63.74 a	45.86 c	62.26 a	49.68 c
VP11	177.66 a	162.33 b	170.33 ab	158.66 b	120.66 c	170.33 ab	170.00 ab
VP12	Dense	Dense	Very dense	Very dense	Dense	Dense	Dense
VP13	Introse and antrose	Antrose and retrose	Antrose and retrose	Antrose and retrose	Introse and antrose	Antrose and retrose	Antrose, introse and extrose
VP14	81.66 ab	72.33 b	47.33 c	57.00 c	58.33 c	56.00 c	85.00 a
VP15	22.20 a	18.51 b	13.47 c	15.39 c	24.73 a	4.40 c	23.99 a
VP16	19.00 ab	17.66 b	15.66 c	20.33 a	13.33 d	15.33 c	19.33 ab
VP17	7.16 b	6.00 bc	6.66 b	6.66 b	6.33 b	4.33 c	9.00 a
VP18	23.00 d	23.00 d	16.33 e	25.33 c	28.66 b	33.33 a	31.33 a
VP19	Twin	Single	Single	Twin	Twin	Mixed	Mixed

- Means followed by a different letter(s) are significantly different at a $P \leq 0.05$.
- Vegetative Parameter: Crown shape (VP1), Trunk diameter (VP2), Leaf length (VP3), Leaf width (VP4), Petiole length (VP5), Petiole: leaf ratio (VP6), Blade length (VP7), Blade: leaf ratio (VP8), Pinnated part length (VP9), Pinnae: leaf ratio (VP10), Number of pinnae (VP11), Pinnae density (VP12), Pinnae types (VP13), Spine part length (VP14), Spine part: leaf ratio (VP15), Max. spine length (VP16), Min. spine length (VP17), Number of spine (VP18), Spine type (VP19).

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Table-6. Fruit and seed morphological traits of seven date palm cultivars.

Cultivars	Amri	Kapoushi	Khedri	Hayani	Beid El Gamal	Red Majhal	Yellow Majhal
Morphology							
FP1	5.42 a	5.11 ab	5.63 a	4.11 c	3.65 cd	3.55 d	4.70 b
FP2	3.02 a	2.28 c	2.83 ab	2.25 c	2.68 b	2.18 c	3.00 a
FP3	27.16 a	16.53 c	22.10 b	10.24 d	16.20 c	9.80 d	26.51 a
FP4	27.16 a	16.53 c	22.10 b	10.27 d	16.20 c	9.80 d	26.51 a
FP5	Elliptical	Flaccid-elongate	Cylindrical	Cylindrical	Ovate	Cylindrical	Ovate
FP6	Blunt	Obtuse	Blunt	Obtuse	Obtuse	Obtuse	Blunt
FP7	Truncate& emarginated	Obtuse	Obtuse	Truncate& emarginated	Truncate& Emarginated	Truncate& emarginated	Truncate& emarginated
FP8	Yellowish-red	Yellowish-red	Yellow	Shiny red	Yellow	Pale-Red	Pale-Yellow
FP9	Brownish black	Brown	Brown	Brownish black	Pale brown	Brown	Pale brown
FP10	Whitish creamy	Whitish creamy	Whitish creamy	Cream-brown	Cream-brown	Whitish creamy	Whitish creamy
FP11	0.89 a	0.71 bc	0.63 c	0.61 c	0.92 a	0.63 c	0.80 ab
FP12	24.85 a	14.85 c	20.22 b	13.48 c	13.82 c	7.54 d	24.78 a
FP13	Firm	Firm	Soft	Soft	Firm	Fibrous	Fibrous
FP14	Poor	Good	Poor	Poor	Excellent	Good	Good
FP15	Palatable	Delicious	Delicious-sweet	Palatable	Delicious	Delicious	Delicious
FP16	3.06 ab	3.28 a	2.85 ab	2.95 ab	2.43 b	2.61 ab	2.91 ab
FP17	1.08 a	0.96 b	1.05 ab	1.14 a	1.16 a	1.08 a	1.10 a
FP18	1.96 a	1.71 a	1.66 a	2.33 a	2.37 a	2.11 a	1.67 a
FP19	7.27 c	10.34 bc	7.41 c	22.93 a	14.69 b	21.80 a	6.30 c

- Means followed by a different letter(s) are significantly different at $P \leq 0.05$.
- Fruit Parameter: Fruit length (FP1), Fruit width (FP2), Fruit weight (FP3), Fruit volume (FP4), Fruit shape (FP5), Fruit apex (FP6), Fruit base (FP7), Fruit color, Besser (FP8), Fruit color, Rutab (FP9), Flesh color (FP10), Flesh thickness (FP11), Flesh weight (FP12), Flesh texture (FP13), Flesh Flavor (FP14), Flesh taste (FP15), Seed length (FP16), Seed width (FP17), Seed weight (FP18), Seed weight: fruit weight (FP19).

Principal component analysis

Principal Component Analysis (PCA) was performed, and data matrices were standardized to examine the relationship between the seven date palm cultivars and key morphological traits. PCA categorized the palm tree, leaf, fruit, and seed morphological data of the date palm cultivars into 38 components. The first six components explain a total variance of 100% (Table 7). A biplot was created using the first two components, which collectively accounted for 60.70% of the variance (F1: 31.79%, F2: 28.91%) and grouped the cultivars based on morphological similarities (Figure 2).

The PCA Biplot, based on the analysis of all morphological trait data for the seven studied date palm cultivars, revealed strong relationships among certain morphological traits and cultivars, which were situated in the same area of the PCA plane. In contrast, uncorrelated traits and cultivars were located in separate regions of the PCA plane. The fruit apex, fruit width, fruit weight, fruit volume, spine part length, min. spine length and trunk diameter are positively correlated with Amri and Yellow Majhal date palm cultivars; seed weight: fruit weight, crown shape, petiole length, petiole: leaf ratio, blade length, blade: leaf ratio, pinnated part length, and pinnae: leaf ratio with Hayani and Red Majhal; fruit length, fruit color at besser and rutab stage, leaf length and width, number of pinnae, pinna types, and max. spine length are correlated with Kapoushi and Khedri, and fruit base, flesh color, flesh flavor, seed width, number of spines, and spine type with the Beid El Gamal cultivar.

Table-7. Principal component analysis of date palm cultivars using morphological traits.

	F1	F2	F3	F4	F5	F6
Eigenvalue	12.08	10.99	4.69	4.49	3.42	2.33
Variability (%)	31.79	28.91	12.35	11.81	9.01	6.14
Cumulative %	31.79	60.70	73.05	84.86	93.86	100.00

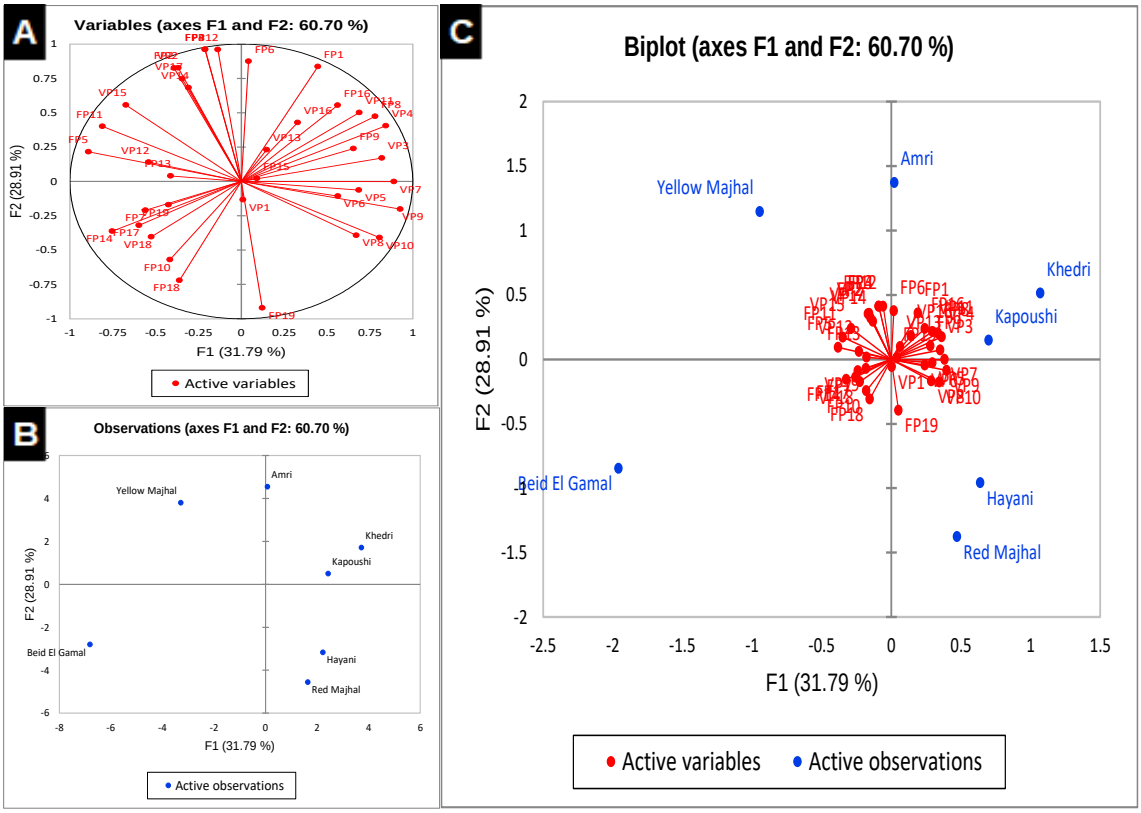

Figure-2. PCA analyses of seven date palm cultivars based on 38 morphological traits (A) variable plot, (B) observations plot, and (C) biplot.

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Cluster analyses

Heatmap identified distinct relationships among date palm cultivars and all morphological traits (Figure 3). The cultivars based on vegetative traits were grouped into three clusters: Cluster 1 was split into three subgroups. One included Red Majhal, the second included Khedri, and was linked with the third subgroup of Hayani and Kapoushi, which joined together. Cluster 2 contained the least similar cultivar, Beid El Gamal, and Cluster 3 featured the highly similar cultivars Amri and Yellow Majhal. Vegetative traits were organized into four row clusters: the first included VP3, VP4, VP5, VP6, and VP11; the second consisted of VP1, VP7, VP8, VP9, and VP10; the third contained VP13 and VP16; and the fourth cluster was divided into two subgroups, with VP2, VP15, and VP17 in the first subgroup, and VP12, VP14, VP18, and VP19 in the second subgroup (Figure 3-a). Figure 3-b illustrated the relationship between date palm cultivars and their fruit traits, categorizing them into four clusters based on similarity. Cluster 1 includes Kapoushi and Khedri, Cluster 2 has Amri and Yellow Majhal, Cluster 3 has Beid El Gamal, and Cluster 4 contains Hayani and Red Majhal. Fruit traits were grouped into four row clusters based on their correlations: the first cluster includes FP2, FP3, FP4, FP6, and FP12; the second cluster consists of FP1, FP8, FP9, and FP16; the third cluster includes FP7, FP10, FP17, FP18, and FP19; and the fourth cluster consists of FP5, FP11, FP13, FP14, and FP15.

In the hierarchical cluster analysis aimed at determining the genetic similarity and relationships among the studied date palm cultivars based on morphological traits, four main clusters were identified (Figure 4). Cluster I included only Beid El Gamal, which showed the longest Euclidean distance (11), indicating significant dissimilarity with other date palm cultivars. Cluster II comprised Amri and Yellow Majhal as they shared many morphological traits. Cluster III featured Hayani and Red Majhal, which were closely related. Lastly, Kapoushi and Khedri were in Cluster VI, showing the shortest Euclidean distance (6) among the cultivars. The variability within clusters was 7.40%, compared to 92.60% between clusters (Table 8).

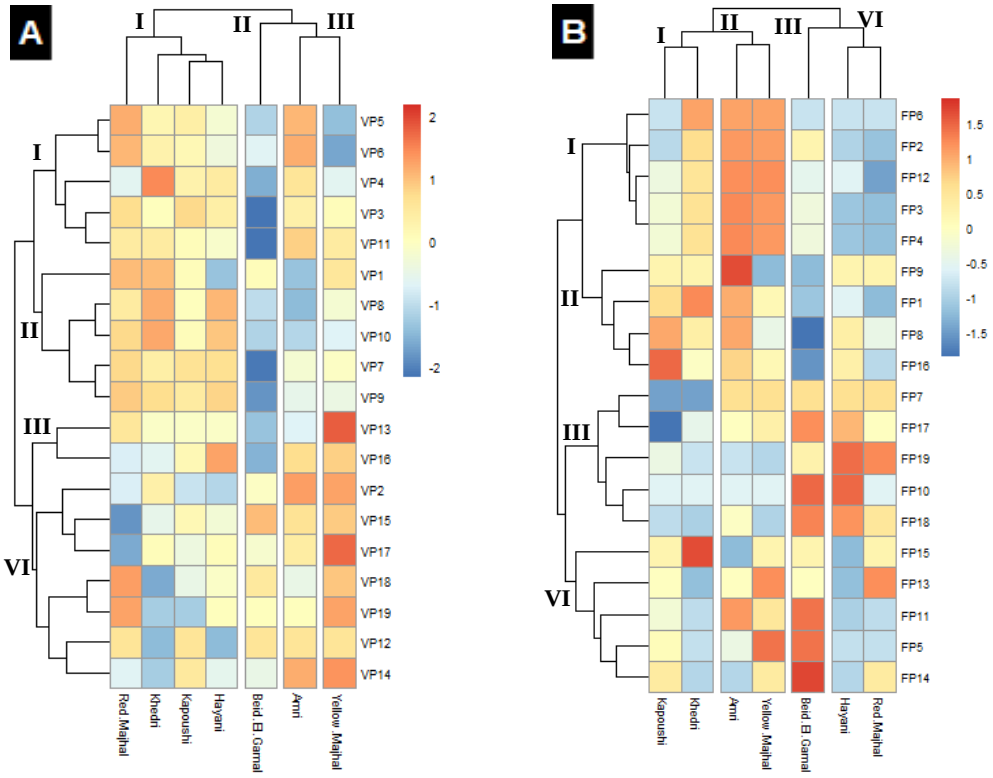

Figure-3. Heatmap of hierarchical clustering analysis of A) vegetative, and B) fruit and seed, morphological traits (rows) for seven date palm cultivars (columns). Dendrogram above displays the cultivars, while the left side lists

the morphological traits. The abundance scale ranges from blue (low value) to red (high value) to indicate relationship levels.

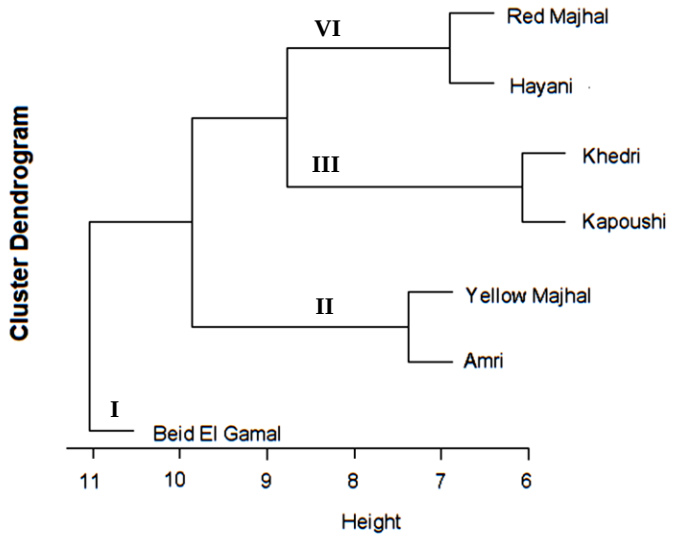

Figure-4. Dendrogram illustrating the genetic relationships among seven studied palm tree cultivars based on their morphological traits.

Table-8. Variance decomposition for the optimal classification of the studied date palm cultivars.

	Absolute	Percent
Within-class	644.494	7.40%
Between-classes	8059.386	92.60%
Total	8703.880	100.00%

Molecular analyses

IRAP and SCoT Fingerprinting

Data in Table 9 showed that (TNAs, MAs, PAs, P%, PIC, and RP). Using 8 IRAP primers, 97 amplicons were generated, with 35 (36%) being polymorphic. Each primer amplified an average of 12.20 fragments, ranging from 11 to 13. Polymorphic bands varied from 2 for IRAP-4334 to 6 for IRAP-2204 and IRAP-2200, with percentages between 15% (IRAP-4334) and 46% (IRAP-2204 and IRAP-2200). PIC values ranged from 0.13 (IRAP-4334) to 0.34 (IRAP-2204), while RP values spanned from 1.08 (IRAP-4334) to 4.46 (IRAP-2204) (Figure 5).

The SCoT fingerprinting profiles from the 10 primers are shown in Figure 6. A total of 89 amplicons were produced, with 42 being polymorphic markers (45%). The number of amplified PCR amplicons ranged from 7 for primer SCoT-07 to 11 for primer SCoT-09. Polymorphic bands varied from 0 for SCoT-07 to 9 for SCoT-09, with an average of 4.20 polymorphic bands per primer. The percentage of polymorphism ranged from 0% (SCoT-07) to 82% (SCoT-09). PIC values ranged from 0 for SCoT-07 to 0.37 for SCoT-09, while SCoT-03 had the highest Resolving Power (RP) at 5.50.

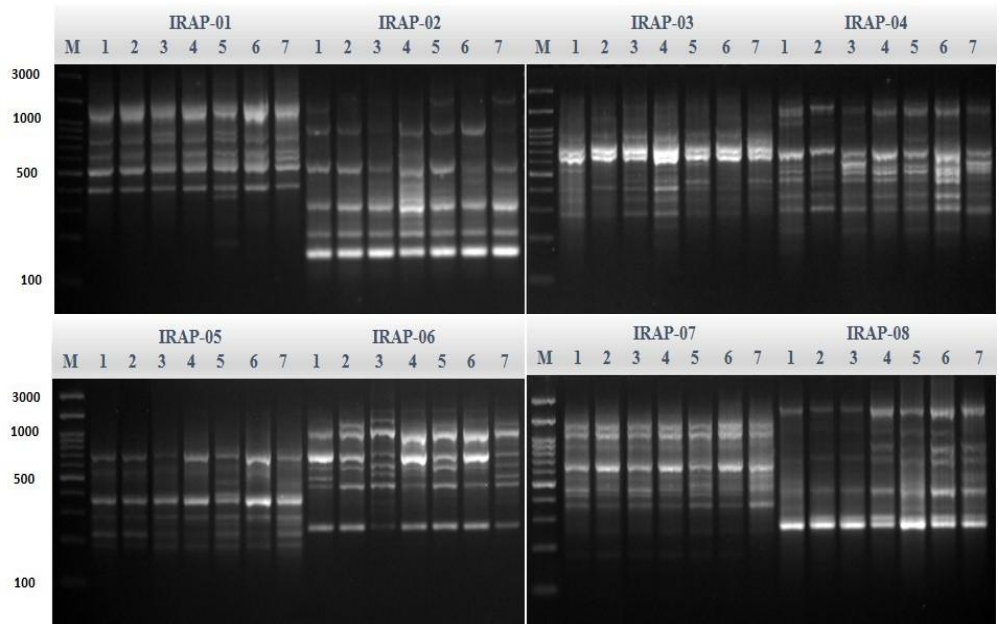

Figure-5. The PCR patterns of the seven date palm cultivars using the 8 IRAP Primers.

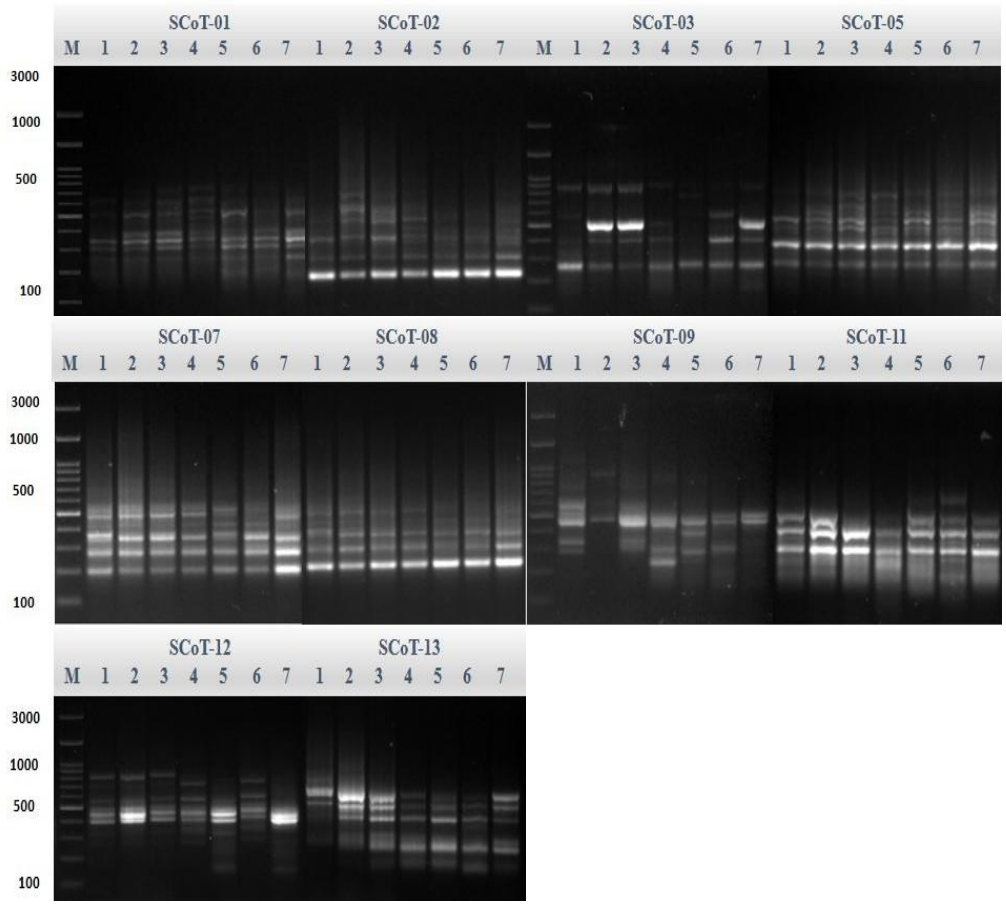

Figure-6. The PCR patterns of the seven date palm cultivars using the 8 SCoT Primers.

Table-9. List of the IRAP (A) and SCoT (B) primers; name, sequence, GC%, TM°C, total number of amplicons (TNAs) per primer, monomorphic amplicons (MAs), polymorphic amplicons (PAs), percentage of polymorphism (P%), polymorphic information content (PIC), resolving power (RP), and marker index (MI) as revealed by IRAP and SCoT profiles in the 7-date palm cultivars. The primer sequences were synthesized by the HVD Egypt Company.

A) IRAP primer							
Ser	**Primer name**	**TNAs**	**MAs**	**PAs**	**P%**	**PIC**	**RP**
1	IRAP-2175	11	7	4	36	0.32	4.00
2	IRAP-2198	12	8	4	33	0.25	2.50
3	IRAP-2197	12	7	5	42	0.33	4.16
4	IRAP-2200	13	7	6	46	0.33	4.15
5	IRAP-2202	11	6	5	45	0.33	4.18
6	IRAP-2204	13	7	6	46	0.34	4.46
7	IRAP-4334	13	11	2	15	0.13	1.08
8	IRAP-4370	12	9	3	25	0.20	1.83
	Total	**97**	**62**	**35**	**-**	**-**	**-**
	Mean	**12.20**	**7.80**	**4.40**	**36.00**	**0.30**	**3.30**
B) SCoT primer							
Ser	**Primer name**	**TNAs**	**MAs**	**PAs**	**P%**	**PIC**	**RP**
1	SCoT-01	9	5	4	44	0.29	3.11
2	SCoT-02	10	5	5	50	0.35	5.00
3	SCoT-03	8	3	5	63	0.36	5.50
4	SCoT-05	10	7	3	30	0.16	1.40
5	SCoT-07	7	6	1	14	0.10	1.00
6	SCoT-08	10	6	4	40	0.28	3.00
7	SCoT-09	11	2	9	82	0.37	4.91
8	SCoT-11	7	4	3	43	0.30	3.42
9	SCoT-12	9	3	6	67	0.35	4.44
10	SCoT-13	8	5	3	38	0.28	3.00
	Total	**89**	**47**	**42**	**-**	**-**	**-**
	Mean	**8.90**	**4.70**	**4.20**	**45.00**	**0.27**	**3.38**

Genetic similarity and cluster analysis based on IRAP and SCoT markers

Table 10 and Figure 7 illustrate the genetic similarity and clustering structure among seven date palm cultivars, consisting of five classified cultivars and two that are unknown. The UPGMA method and Dice coefficients were utilized to assess genetic similarity, which ranged from 0.83 to 0.93, indicating a high level of similarity overall. Notably, the highest genetic similarity (0.96) was found between the cultivars Amri and Yellow Majhal, followed by a similarity of 0.92 between Amri and Beid El Gamal. The lowest genetic similarity recorded was 0.83 between Kapoushi and Beid El Gamal. The dendrogram revealed two main clusters: the first cluster contained four date palm cultivars—Hayani, Kapoushi, Khedri, and Red Majhal—while the second cluster comprised three cultivars—Amri, Yellow Majhal, and Beid El Gamal.

Table-10. Similarity matrix for the seven date palm cultivars constructed from IRAP and SCoT data according to Dice coefficient.

	Amri	Kapoushi	Khedri	Hayani	Beid El Gamal	Red Majhal
Kapoushi	0.84					
Khedri	0.85	0.91				
Hayani	0.86	0.89	0.89			
Beid El Gamal	0.92	0.83	0.89	0.84		
Red Majhal	0.85	0.89	0.90	0.88	0.88	
Yellow Majhal	0.93	0.84	0.85	0.87	0.90	0.87

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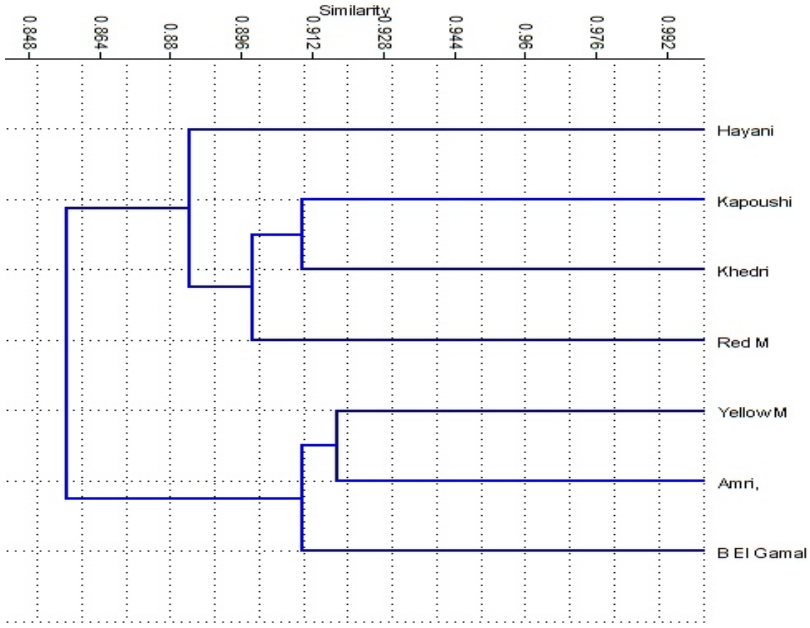

Figure-7. Phylogenetic tree for the seven date palm cultivars constructed from IRAP and SCoT data using UPGMA according to Dice coefficient.

To show the genetic relationships among date palm cultivars based on IRAP and SCoT markers, a principal coordinate analysis (PCA) was conducted using Dice's similarity matrix (Figure 8). Unit variance scaling was applied to the rows, and singular value decomposition (SVD) with imputation was utilized to calculate the principal components. The X and Y axes represent principal component 1 and principal component 2, which account for 32.00% and 20.40% of the total variance, respectively. The relationships observed in the PCA were consistent with and confirmed the results of the clustering analysis.

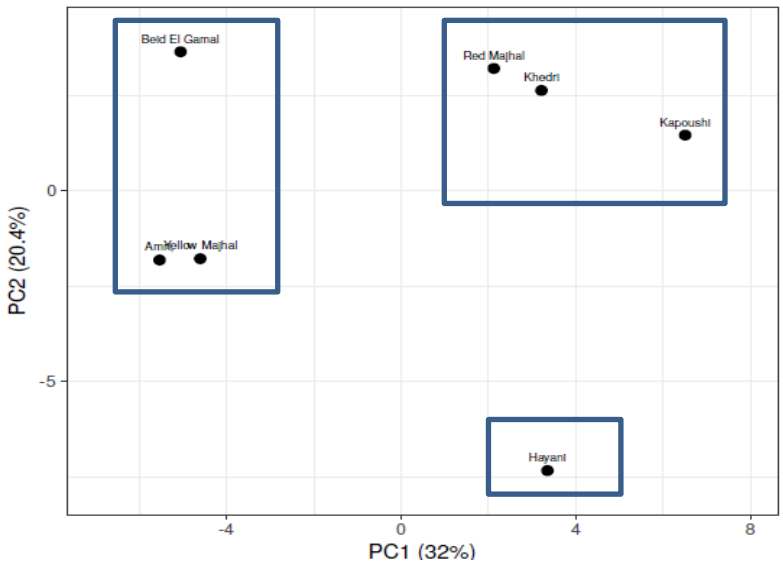

Figure-8. Principal coordinate analysis based on the calculation of the first three coordinates was performed according to the analysis of IRAP and SCoT markers of the studied seven date palm cultivars.

DNA Barcoding Loci of *rbcL, matK,* and *trnH* Sequencing

In our study, we utilized three genes—rbcL, matK, and trnH—because these are the most commonly used genes for plant DNA barcoding. We compared the amplification, sequencing, and effectiveness of these chloroplast DNA barcoding loci in measuring genetic diversity, identification, and phylogeny among seven date palm cultivars from the Bir Al-Abd region in North Sinai, Egypt. The cultivars analyzed include five classified cultivars: Amri, Hayani, Khedri, Kapoushi, and Beid El Gamal, as well as two unknown cultivars: Red Majhal and Yellow Majhal. The PCR primers for rbcL, matK, and trnH successfully amplified and produced PCR products with expected band sizes of approximately 650 bp, 900 bp, and 600 bp, respectively, as shown in Figure 9. Furthermore, we successfully sequenced the barcode genes (rbcL, matK, and trnH) for the seven date palm cultivars. These sequences have been submitted to NCBI using the BankIt tool and have been assigned corresponding GenBank accession numbers, as listed in Table 11.

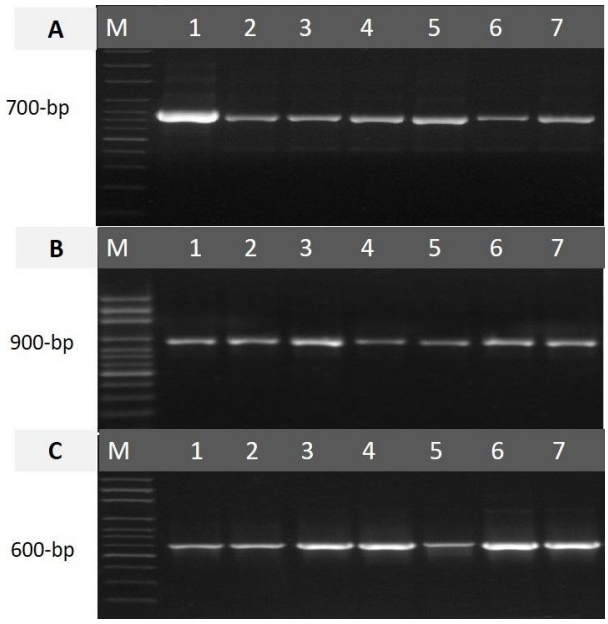

Figure-9. Amplification of DNA barcoding loci of rbcL, matK and trnH genes. Agarose gel electrophoresis of amplified PCR products of A) rbcL (750bp), B) matK (900bp) and C) trnH (600bp) [indicate the molecular size of amplified DNA barcodes].

Table-11. NCBI GenBank accession numbers for rbcL, matK, and trnH for the seven date palm cultivars produced by this study.

SN	Cultivars name	rbcL	matK	trnH
1	Amri	PP412556	PP412549	PP537596
2	Kapoushi	PP412557	PP412550	PP537597
3	Khedri	PP412558	PP412551	PP537598
4	Hayani	PP412559	PP412552	PP537599
5	Beid El Gamal	PP412560	PP412553	PP537600
6	Red Majhal	PP412561	PP412554	PP537601
7	Yellow Majhal	PP412562	PP412555	PP537602

A tree rbcL analysis distinguished the date palm cultivars (Figure 10). The Khedri formed its own cluster, while the Red Majhal was in a sub-cluster, separating it from the remaining five cultivars. The Hayani created a sub-sub-cluster with four other cultivars in a separate group. The Kapoushi also formed a distinct group, which was further divided: one subgroup included Beid El Gamal, and the other contained the genetically similar cultivars Yellow Majhal and Amri.

A matK tree separated the date palm cultivars into clusters. Yellow Majhal and Amri showed the highest similarity, followed by Beid El Gamal, Khedri, Hayani, Kapoushi, and Red Majhal. Meanwhile, a trnH tree identified the seven studied date palms into two main clusters: the first included Khedri and Hayani, while the second had two sub-clusters—one with Kapoushi and Red Majhal, and the other with Beid El Gamal, Amri, and Yellow Majhal.

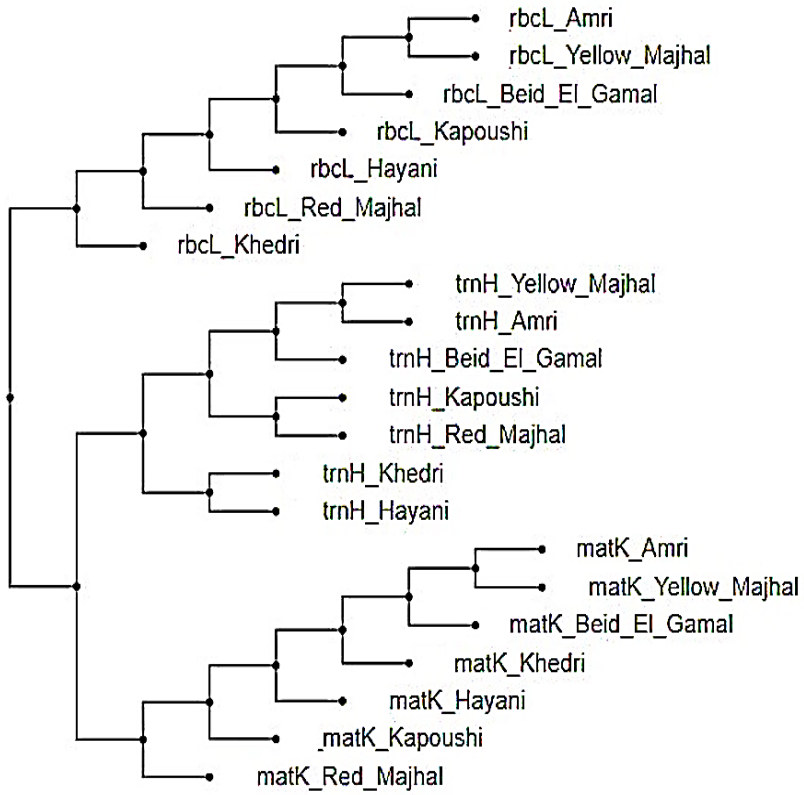

Figure-10. A consensus phylogenetic tree constructed based on the rbcL, matK and trnH DNA barcoding region for seven date palm cultivars using the https://mafft.cbrc.jp/alignment/server/index.html.

Morphological and genetic diversity analysis

The morphological and molecular characteristics revealed clear patterns of variation among the seven date palm cultivars. ANOVA of 38 morphological traits indicated significant differences ($P \leq 0.05$) among the cultivars, a finding that was further corroborated by the polymorphisms observed in molecular markers.

Amri and Yellow Majhal cultivars exhibited the highest levels of vegetative morphological and genetic similarity, with a genetic similarity coefficient of 0.93. These cultivars shared distinctive vegetative features, including large trunk diameter (170.33 cm and 169.33 cm, respectively) and a high number of pinnae (177.66 and 170.00, respectively). This close relationship was confirmed by both IRAP and SCoT marker analyses, particularly with IRAP-2204 and IRAP-2200 primers, which showed a polymorphism rate of 46%. Khedri and Kapoushi cultivars formed a separate cluster based on both morphological and molecular data. These cultivars showed similar leaf characteristics (leaf length 351.33 cm and 390.66 cm, respectively) and

clustered together in the rbcL gene phylogenetic analysis. Their genetic similarity coefficient (0.88) corroborated their morphological grouping

Fruit trait analysis revealed three distinct groups that aligned with molecular findings: First, Amri-Yellow Majhal group had the highest fruit weight (27.16 g and 26.51 g, respectively) and shared specific SCoT marker bands (SCoT-09 profile) that were clustered together in matK gene analysis (Genetic similarity coefficient 0.93). Second, Kapoushi-Khedri group has medium fruit weight (16.53 g and 22.10 g, respectively), also similar IRAP banding patterns grouped in trnH phylogenetic analysis (Genetic similarity coefficient 0.87). Third, Hayani-Red Majhal group has lower fruit weight (10.24 g and 9.80 g, respectively) and also shared molecular markers in SCoT analysis, as well as similar clustering in rbcL gene analysis (Genetic similarity coefficient 0.85).

Principal Component Analysis (PCA) of morphological traits explained 60.70% of the total variation (PC1: 31.79%, PC2: 28.91%), which corresponded well with the molecular marker clustering. IRAP analysis generated 97 amplicons with 36% polymorphism, supporting the morphological grouping of cultivars. Eight primers showed polymorphism levels from 15% to 46% and PIC values between 0.13 and 0.34. Meanwhile, SCoT analysis yielded 89 amplicons with 45% polymorphism, confirming cultivar relationships identified morphologically. Ten primers revealed polymorphism ranging from 0% to 82%, with PIC values from 0 to 0.37.

The three chloroplast genes provided complementary information to morphological groupings, rbcL analysis, and separated cultivars into distinct clusters. Supported morphological grouping of Amri and Yellow Majhal showed a correlation with fruit characteristics. The matK analysis has the highest resolution in distinguishing closely related cultivars and confirms genetic relationships indicated by morphological traits, particularly effective in resolving the Amri-Yellow Majhal relationship. The trnH analysis divided cultivars into two main clusters, supported morphological trait-based groupings, and provided additional resolution for closely related cultivars.

The hierarchical morphological and molecular data analysis revealed four main clusters with genetic similarity coefficients from 0.83 to 0.93. Cluster 1 included Amri and Yellow Majhal (highest similarity), Cluster 2 had Kapoushi and Khedri, Cluster 3 consisted of Hayani and Red Majhal, and Cluster 4 featured Beid El Gamal (most distinct). Heatmap analysis confirmed these relationships, showing a strong correlation between morphological traits and molecular marker patterns. Variance decomposition indicated that within-class variation was 7.40% and between-class variation was 92.60%, supporting the strength of the classification.

This integrated analysis demonstrates strong concordance between morphological traits and molecular markers, providing a comprehensive understanding of genetic relationships among the studied date palm cultivars. Meanwhile, combining approaches offers more reliable identification of cultivars than using either method alone.

Discussion

For sustainable date palm production, it is necessary to verify cultivars' identities. This process ensures the reliable selection of cultivars that are better suited to climate change and market demands while also avoiding unknown cultivars that may have suboptimal quality. In this study, seven date palm cultivars were found to exhibit highly significant variations ($P \leq 0.05$) in their tree, leaf, fruit, and seed morphological characteristics when assessed under the conditions of North Sinai, Egypt. Many studies on date palms have highlighted the potential of vegetative and fruit morphological traits as tools for differentiating between cultivars (El Kadri et al., 2019; Rizk and EL-Sharabasy, 2019). In Pakistan, Ahmad et al. (2023) successfully explored the diversity among 50 date palm genotypes using 16 morphological traits. Likewise, Eissa et al. (2009) distinguished nine Egyptian date palm cultivars based on 76 morphological traits. Mostafa et al. (2007) also contributed to this field by utilizing 44 traits to examine the diversity among six Egyptian date palm cultivars.

The PCA biplot results revealed strong correlations among specific morphological traits of date palm cultivars. Amri and Yellow Majhal shared 7 traits, while Hayani and Red Majhal had 8 common traits. Kapoushi and Khedri also showed similarity in 7 traits, and Beid El Gamal was distinguished by five traits. This finding is consistent with Simozrag et al. (2016), who found that 48 date palm cultivars were differentiated mainly by fruit and seed widths, while 34 cultivars shared similarities in fruit and seed lengths. Similarly, Elboghdady et al. (2023) noted that

traits like leaf length and spathe length were associated with Hayani, while traits such as spine number and thickness were correlated with Meghal. Hierarchical clustering with heat map grouped seven studied date palm cultivars into three clusters based on vegetative traits and four clusters based on fruit traits. This suggests that fruit characteristics are more effective for distinguishing cultivars than leaf traits, particularly under the conditions in North Sinai, Egypt. This finding is consistent with El-Sharabasy and Rizk (2005); Eissa et al. (2009), Lemine et al. (2014), and Ahmad et al. (2023), who highlighted the importance of fruit traits for differentiating closely related date palm cultivars. On the contrary, Hammadi et al. (2009) and Elboghdady et al. (2023) noted the significance of vegetative traits in identifying relationships among date palm cultivars, particularly during the non-fruiting period.

A hierarchical cluster analysis of seven date palm cultivars based on morphological traits identified four distinct clusters. The five classified date palm cultivars exhibited significant dissimilarity from one another, clustering into three different clusters, except Kapoushi, which was grouped with Khedri in cluster four. In contrast, the two unknown cultivars were categorized alongside the classified cultivars: Yellow Majhal grouped with Amri and Red Majhal grouped with Hayani, as they shared many morphological traits. These relationship similarities may be attributed to one of two reasons. First, Yellow Majhal trees might be propagated using seeds from Amri fruits, while Red Majhal trees could be propagated from seeds of Hayani fruits. This process may have led to some genetic variations (Elsafy et al., 2015). Second, we believe that the offshoots of the Amri and Hayani date palm trees have been influenced by environmental factors since their cultivation, resulting in morphological changes. This assumption is supported by notable similarities in leaf characteristics, such as trunk diameter (VP2) and leaf length (VP3), along with fruit attributes, including fruit volume (FP4), fruit shape (FP5), and fruit color (FP9). These findings suggest that local selection pressures and cultivation practices have significantly influenced the diversity of date palms in the region, as supported by the findings of Ennouri et al. (2018) and Ahmad et al. (2023). Furthermore, due to the sandy soil, limited rainfall, and high temperatures prevalent in North Sinai, the morphological characteristics of date palms are strongly affected by environmental factors across different growing regions, according to Al-Mssallem et al. (2013). Environmental factors played a decisive role in the expression of the physical characteristics of date palms at the three study sites, especially in the vegetative characteristics. While fruit characteristics remained relatively constant, aspects such as leaf length, number of leaflets, and the presence of spines showed significant differences. This suggests that the different traits respond to their environment with varying degrees of flexibility. The hierarchical clustering of cultivars into four distinct groups aligns with their geographical distribution and historical cultivation patterns in North Sinai. This pattern suggests that local selection pressures and cultivation practices have played significant roles in shaping date palm diversity in the region, supporting findings by Ennouri et al. (2018) and Ahmad et al. (2023).

The complementary patterns of polymorphism between IRAP (36%) and SCoT (45%) marker systems enhanced overall discrimination capacity, compensating for the high genetic similarity. Certain primers, such as IRAP-2204, IRAP-2200, and SCoT-09, showed exceptional resolving power even between closely related cultivars. This suggests that strategic primer selection can overcome limitations imposed by high genetic similarity, supporting Al-Qurainy et al.'s (2015) findings on the differential effectiveness of SCoT primers for date palm cultivar discrimination. The varying resolving power of different primer combinations indicates that high genetic similarity does not uniformly affect all genomic regions. As demonstrated by Zhao et al. (2012) in other crop species, genetic diversity in date palms appears concentrated in specific genomic regions that can be effectively targeted with appropriate marker systems. Our results show that despite high overall similarity, sufficient polymorphism exists in targeted regions to enable effective cultivar discrimination.

Morphological analysis showed phenotypic similarities due to genetic and environmental factors, while molecular markers revealed genetic relationships unaffected by the environment. DNA barcoding added evolutionary context by showing maternal lineage connections. This approach was especially useful for identifying the taxonomic status of unknown Majhal cultivars, confirming the close relationship between Yellow Majhal and Amri despite their different local names. Morphological traits were practical for field classification, but molecular markers uncovered genetic relationships not visible from phenotypes. DNA barcoding provided standardized genetic markers for comparison across studies and

regions, making the research globally relevant. Experts also said that this multifaceted approach is the best way to characterize plant genetic resources (Enan and Ahamed, 2014; Gros-Balthazard et al., 2020; Ahmad et al., 2023).

DNA barcoding showed different levels of effectiveness in distinguishing between plant varieties, with the matK gene being particularly good at telling apart closely related types. This matches what Enan and Ahamed found in 2014 with date palms. The matK gene could even tell the difference between Amri and Yellow Majhal cultivars, which are very similar genetically, showing it's useful for detailed distinctions, as noted by Parks et al. (2009). While physical traits showed how plants looked and IRAP/SCoT markers revealed genetic links, barcoding uncovered maternal lineage connections that the other methods couldn't. The rbcL gene did a good job of grouping cultivars into main lineages that matched major physical types, but wasn't very detailed within those groups. On the other hand, the trnH gene showed a lot of variation but wasn't always reliable in showing true relationships, sometimes disagreeing with findings from physical traits and other genetic studies. According to DNA barcoding results as a complementary tool, the effectiveness of chloroplast genes (rbcL, matK, and trnH) in supporting morphological and molecular marker-based groupings demonstrates their value in date palm systematics. This supports the idea from Jeanson et al. (2011) that using multiple methods gives a fuller picture of plant evolution than just one.

This study provides several new insights for previous palm research. It shows how the combination of IRAP and SCoT markers with DNA barcoding can effectively identify cultivars, a method not previously applied to date palms in North Sinai. In addition, the genetic links between classified cultivars and unknown cultivars have been clarified, answering long-standing questions about their classification. In addition, stable morphological markers have been identified that allow consistent differentiation between cultivars under different environmental conditions, providing practical tools for use in the field. Finally, the detailed characterization of these cultivars provides a solid basis for future breeding programs aimed at improving the resilience of this date palm to climate change.

Conclusion

This study demonstrates the value of integrating morphological, molecular, and DNA barcoding approaches for accurate date palm characterization. The complementary nature of these methods provides a more complete understanding of genetic relationships among cultivars than any single approach. Environmental factors significantly influence morphological traits, especially vegetative characteristics, highlighting the need to consider growing conditions when using these traits for identification. Despite the high genetic similarity among cultivars, careful selection of molecular markers allowed for effective discrimination, showing that the right marker choice can overcome narrow genetic diversity. DNA barcoding complemented traditional methods by providing an evolutionary context and standardized genetic references, with matK showing superior resolution for closely related cultivars. The identification of previously unknown Majhal cultivars is a significant contribution to date palm germplasm management in the region, offering clear evidence of their genetic relationships to established cultivars. The practical recommendations for breeding and conservation strategies presented in this study provide a roadmap for the sustainable use of these genetic resources in the face of changing environmental conditions.

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Contribution of Authors

Awad MA & Nagaty MA: Conceptualization, designed research methodology, data collection, analyses, interpretation and writing the original draft

Awad MA, Nagaty MA, Yahya NA & Ibrahim SD: Investigations and literature review

Awad MA: Data curation and editing

Yahya NA & Ibrahim SD: Prepared plant samples, performed molecular analyses, data analyses, interpretation and wrote the manuscript

All authors read and approved the final draft of the manuscript.

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