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MOLECULAR CHARACTERIZATION OF THE DATE PALM (*PHOENIX DACTYLIFERA* L.)

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SUMMARY

This study focused on four cultivars of date palms (*Phoenix dactylifera* L.) planted in DhiQar Governorate (Date Palm Genetic Bank Project): Al-Shawithi, Al-Barhi, Al-Khudrawi, and Jamal Al-Din. The study's aim was to determine the genetic fingerprint of each cultivar and probe the genetic variation and relatedness among them. The use of the PCR-ISSR technique, which relies on analyzing DNA genetic material variation, was successful. The results showed the Al-Barhi and Al-Shawithi cultivars exhibited genetic diversity using the ISSR 4 primer, resulting in 16 genetic patterns. The ISSR 7 primer produced the most genetic patterns, reaching 23 patterns. When using cluster analysis for the cultivars, the Al-Shawithi cultivar's isolation was evident from the rest of the cultivars. The results also indicated a genetic proximity between the Al-Khudrawi and Jamal Al-Din cultivars, while the Al-Barhi cultivar deviated somewhat from these two cultivars. This was prominent through the depiction of genetic ratio records.

Keywords: Date palm (*P. dactylifera* L.), genetic variation, ISSR primers, genetic fingerprint

Key findings: The date palm (*P. dactylifera* L.) cultivars Al-Barhi and Al-Shawithi exhibited genetic diversity using the ISSR 4 primer, resulting in 16 genetic patterns.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the most renowned trees known to humankind since ancient times. Its cultivation began around 7000 years ago in the Mesopotamian region, with a belief that it originated in the Arabian Gulf and Southern Iraq regions (Wrigley, 1995). The date palm is a perennial fruit tree with a diploid chromosomal number (2n = 36), dioecious reproductive system, and monocotyledonous with a single cotyledon. It belongs to the Arecaceae family (Barrow, 1998).

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In Iraq, the number of date palm cultivars has estimates of more than 600. Despite the diagnosis and classification of many of these cultivars in Iraq, there still exist unidentified ones, prompting numerous researchers to document these cultivars. However, those involved in date palm cultivation or research face challenges in categorizing these cultivars. This can be due to the same variety having different names in multiple regions or the same name being used for several closely related or similar cultivars for visible traits (Ahmed *et al*., 2013).

Studies indicate that morphological, enzymatic, and protein characteristics of date palm cultivars can be so similar that they become unreliable indicators of appearance due to their susceptibility to various factors, such as the external environment. As a result, attention has turned to their genetic content and the exploration of their components to reveal the distinguishing boundaries among these cultivars (Abdulwahid, 2011). The rapid and significant advancements in the field of molecular biology and the continuous successes achieved by genetic engineering have provided numerous appropriate solutions for employing molecular analysis of genetic material.

This has led to developing new methods for determining the genetic fingerprint of cultivars using DNA markers, as characterized by their higher accuracy, efficiency, and time and effort savings (Mathew *et al*., 2014; Kheirallah *et al*., 2017). Several techniques have been applicable, including inter simple sequence repeat (ISSR) technique, which amplifies DNA fragments to generate amplified pieces with precise sequences. This makes the ISSR technique exceptional in identifying genetic diversity (Khanam *et al*., 2012).

Past studies indicate a significant deficiency in various analytical research that addresses date palm cultivars in general, including those with cultivation in the southern region. Based on this context, the presented study aims to identify the distinctive genetic fingerprint of four date palm cultivars grown in the southern region: Shawithi, Jamal Al-Din, Barhi, and Al-Khudrawi. Additionally, the study seeks to perform the following: molecular characterization utilizing the PCR-ISSR technology to identify the genetic traits of the date palm; genetic diversity analysis investigating genetic variation among different date palm cultivars to understand their phylogenetic relationships; crop improvement to identify desirable traits in cultivars to enhance breeding programs and agricultural productivity; and genetic resource conservation in supporting efforts to preserve genetic diversity, particularly under climate change and increased demand.

MATERIALS AND METHODS

Collection of plant samples and date palm cultivars

The experiment took place at the Date Palm Genetic Bank Station in DhiQar Governorate. Three cultivars of date palm (*P. dactylifera* L.), Shawithi, Jamal Al-Din, and Al-Khudrawi were choice samples for the study. Freshly formed, white, immature leaves near the top of the palm were chosen specimens. Leaves were the options for being free from disease and other damage. Approximately five to 10 leaflets came from each sample, continued in their wrapping in aluminum foil before placing them in sealed nylon bags to prevent sample desiccation. The labeling of bags with relevant information included the date of sample collection and the name of the agricultural variety. This information was notable on specific cards designed for sample collection. The samples proceeded to transfer in an icebox to the laboratory and remained in a refrigerator until further use.

Preparation of plant samples

The leaflets' thorough washing several times with sterile distilled water removed dust and debris. Afterward, their gentle wiping with sterile cotton soaked in 70% ethanol ensured sterilization. Then, the cutting of leaflets into small pieces (approximately 1 cm^2) used clean and sterile, sharp scissors. These pieces reached placement in a ceramic mortar, adding

liquid nitrogen carefully and cautiously to avoid the potential harm caused by direct contact with skin or exposed body parts. The samples, finely ground to a white powder, utilized a sterile mortar and pestle.

The obtained powder's storage in 10-milliliter sample vials incurred labeling with the corresponding information as mentioned earlier. The meticulous washing of grinding tools with a 10% chlorine solution occurred after each round of grinding for each variety. The samples then remained in a freezer, prepared for the subsequent extraction process.

DNA extraction

DNA extraction ensued from two grams of fresh leaf tissue from the cultivars under study using a CTAB (cetyl trimethyl ammonium bromide) buffer solution, following the protocol outlined by Huang *et al*. (2013). Table 1 illustrates the proportions of the buffer solution.

Application of the inter simple sequence repeats (ISSR) technique

This study utilized nine primers obtained by importing them from Bioneer Company. Table 2 illustrates the nucleotide sequence and annealing temperature for the primers used in the study. The nine primers employed each had its corresponding nucleotide sequence and annealing temperature for the amplification process. These primers came from Bioneer Company for use in the ISSR technique as part of this study.

PCR Reactions

The PCR reaction process used the prepared Green Master Mix from Promega, with a final volume of 50 µl. The components of the reaction mixture included 0.625 µM of dNTPs, 0.2 µM of each forward and reverse primer (F, R), 1x concentration of 2 mM $MgCl_2$, 200 ng of DNA template, and 2x concentration of U Taq polymerase. The reaction proceeded in a

Table 1. The solution with percentage.

CTAB Buffer	2%
Tris – HCl	10mM – pH=8
EDTA	2 mM
NaCl	8.18 g
Distilled water	100 ml
PVP	2%
2-mercaptoethanol	(0.2% v/v)

The CTAB buffer solution of 20 ml was used for extracting 200 micrograms of DNA.

Table 2. Nucleotide sequences and binding temperature of the linked primers.

Primers	Nucleotide sequence 3-5	docking temperature
ISSR 1	GACACAGCAG	51
ISSR 2	CTGCATCGTG	55.3
ISSR 3	CACACACACA	41
ISSR 4	CAGCAGCAGCAGG	43
ISSR 5	AGCAGCAGAGC	42
ISSR 6	CTCACCGTCC	40
ISSR 7	CCAGGAGGAC	41
ISSR 8	TGTCTGGGTG	42
ISSR 9	GACACAGT	38

Table 3. PCR Conditions Program.

Stage	Temperature (°C)	Time	Number of Cycles
Initial Denaturation	94	4 min	1 cycle
Denaturation	94	1 min	
Annealing	60	1 min	
Extension	72	1 min	
Repeat Steps			35 cycle
Final Extension	72	6 min	1 cycle

thermocycler device. The thermocycler programming appears in Table 3, and following the PCR reaction was performing the electrical separation using agarose gel electrophoresis, with the method described by Bornet and Branchard (2001).

Statistical analysis

The results of the amplification process, collected in a table, relied on the presence or absence of DNA bands in the studied samples. The number 1 indicates the presence of only a clear DNA band, and the number 0 indicates the absence of the band. The tables, as organized for each primer separately, had the genetic kinship tree drawn using the unweighted pair group method with arithmetic (UPGMA). Averaging used past statistical programs (Nei and Lei, 1979).

The percentage of primer polymorphism calculation was through the following equation:

Percentage (%) of primer polymorphism for each primer = (the number of divergent bands in the primer/the total number of primer bands) × 100

The percentage of the discriminatory ability computation of each primer employed the following equation:

The discriminatory ability of each primer (%) = (the number of different packets for the initiator/the number of different packets for all primers) × 100

As for the percentage of the efficiency of each primer, its calculation was according to the following equation:

The efficiency of each primer (%) = (the total number of primer bands/the total number of all primer bands) × 100 (Grundmann *et al*., 1995).

RESULTS AND DISCUSSION

The results of gel electrophoresis for the genomic DNA migration of date palm cultivars under study revealed that DNA bands on the sucrose gel were distinct and well-defined. This indicates the success of the extraction process, which yielded a quantity and quality of DNA suitable for use in the PCR program. This DNA can be effective in the PCR program to determine genetic relatedness and identify the genetic fingerprint of the cultivars using the ISSR technique. The DNA quantity for each variety is available in Figure 1, where a small amount of DNA template not exceeding 100 nanograms is essential (Arif *et al.,* 2010; Hussien, 2015).

The amplification results and part of the band scoring process using the pre-designed software package "Photocapt" for the ISSR primer 7 are evident (Figure 2). This has a representation of '1' for the presence of the band and '0' for its absence. As for the molecular weight, it was applicable to extract PAC values (Hammer *et al.,* 2001).

Cluster analysis

The dendrogram provides a clear visualization of the genetic relationships among the studied date palm cultivars (Figure 3). Its structure into two main sections, A and B, highlighted distinct genetic clusters. Section A has further divisions of A1 and A2, with A1 exclusively representing the Jamal Al-Din variety and A2

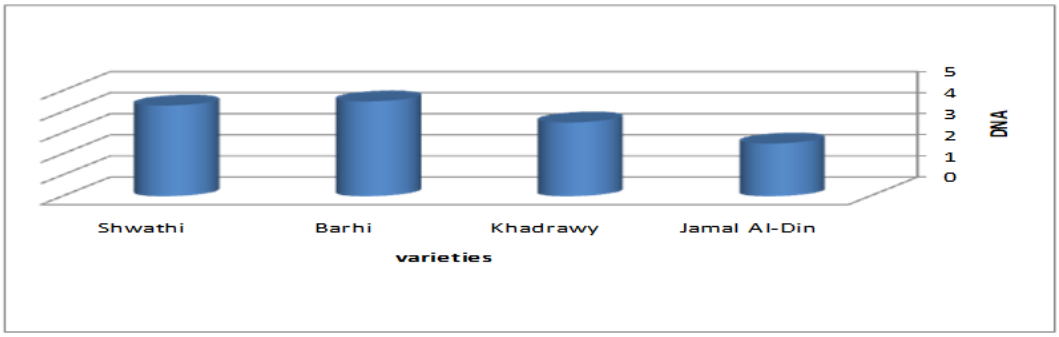

Figure 1. Quantitative DNA extracted from four cultivars of date palm.

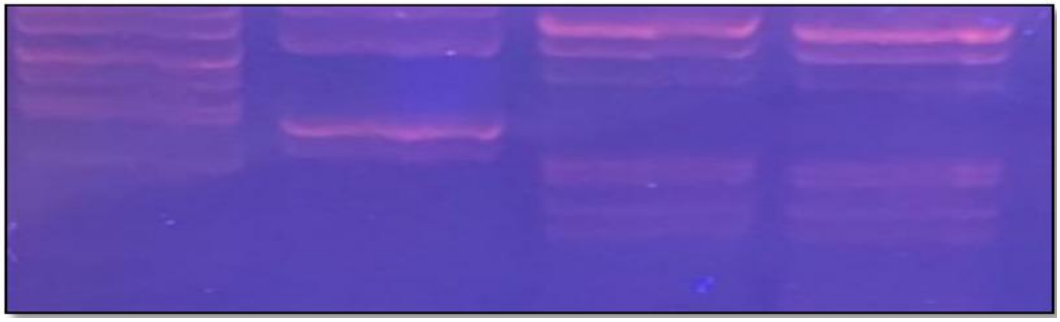

Figure 2. Amplification result of ISSR 7 primer for four palm cultivars.

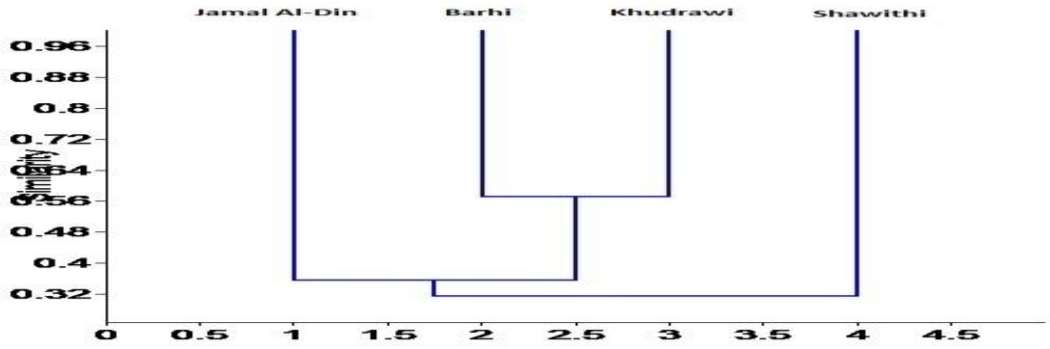

Figure 3. The dendritic cluster diagram of four palm cultivars under study.

grouping the Khudrawi and Barhi cultivars. It suggests a closer genetic similarity between the latter two. Conversely, Section B represents the Shwathi variety, indicating its genetic distinctiveness from the cultivars in Section A. The observed genetic distance, particularly the lowest similarity between the Shwathi and Khudrawi cultivars, reflects the highest genetic divergence in the study. These findings align with previous research, such as Ahmed *et al*. (2013) and Chaudhary *et al.* (2015), which documented significant genetic variability among date palm cultivars using similar clustering techniques. This divergence is likely ascribable to differences in their genetic makeup, potentially influenced by factors such as geographical origin, breeding history, and environmental adaptation.

Polymorphism

The results showed the primers used produced 442 bands, of which 93 bands were polymorphic, and the percentage of bands with polymorphism was 27.18% (Table 4). This indicates the presence of genetic divergence between the date palm cultivars used in the study. The table details that primer ISSR 1 gave the most bands (58 bands) compared with the rest of the primers used. Meanwhile, we find that primer ISSR 9 gave the fewest bands (38 bands) during the migration of samples on the polyacrylamide gel.

The difference in the number of bands resulting from each pair of primers used depends on the extent of compatibility of the primer's binding to the plant genome, as well as the components of each primer's nitrogenous bases. The difference in the sequence of the bases of the primers used leads to a difference in their binding sites with the genome of the plant under study. This further leads to a variation in the numbers of bands resulting from prefixes used (Vos *et al*., 1995). The primers used were successful in providing polymorphism among the resulting packages, as evidenced by the highest percentage of polymorphism of 32.14% in the primers (ISSR 5), while the primer ISSR 2 did not yield more than 22.22% of the polymorphism. The reason for the variation in percentages is that the plant genome's base sequences differ, which affects the primers' sites of attachment. This variation arises from rearrangement, linkage, genetic crossing, etc., which explains why the different bands either clearly appear on the gel or do not appear in certain locations (Clark and Lanigan, 1993).

The findings signified that each primer's efficiency percentage was 12.89% for primer ISSR 4 and 8.59% for primer ISSR 9. The maximum discriminatory ability emerged to be 13.33% with primer ISSR 1 and the lowest to be 7.5% with primer ISSR 3. In the future, common packages obtained from this primer, ISSR 1, could require analysis to create more specialized primers for genotype separation.

Genetic affinity among the studied genotypes

Genetic distance calculation among studied genotypes followed the equation of Kheirallah *et al*. (2011). The results showed the extent of similarity and difference between the selected cultivars (Table 5). It was apparent that the highest percentage of similarity corresponded to the lowest genetic dimension (0.57143) between the two cultivars Barhi and Khadrawi, followed by the distance (0.44444) between the two cultivars Barhi and Shwithi. The results also revealed that the lowest genetic similarity

Table 4. The number of bands, polymorphism, efficiency, and discriminatory ability of the primers.

Primer codes	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)	Primer efficiency (%)	Primer discriminatory ability (%)
ISSR 1	58	16	27.58%	13.12%	13.33%
ISSR 2	54	12	22.22%	12.21%	10%
ISSR 3	35	9	25.71%	7.91%	7.5%
ISSR 4	57	13	22.80%	12.89%	10.83%
ISSR 5	56	18	32.14%	12.66%	15%
ISSR 6	48	14	29.16%	10.85%	11.66%
ISSR 7	45	12	26.66%	10.18%	10%
ISSR 8	51	15	29.41%	11.53%	12.5%
ISSR 9	38	11	28.94%	8.59%	9.16%
total	442	120	-	-	-
Mean	49.11	13.33	27.18%	11.10%	11.10%

Table 5. Inter-variety similarity indices and pair-wise genetic distances in different date palm cultivars.

Cultivars	Khadrawi	Shwithi	Jamal Al-Din	Barhi
Khadrawi	1	0.2	0.375	0.57143
Shwithi	0.2	1	0.3	0.44444
Jamal Al-Din	0.375	0.3	1	0.33333
Barhi	0.57143	0.44444	0.33333	1

corresponded to the highest genetic dimension, which was between the Shwithi and Khadrawi cultivars, which reached 0.2; following them were the Jamal Al-Din and Shwithi cultivars, which reached 0.3.

The outcomes presented in the study indicate that cultivars showing the smallest genetic distance (such as Barhi and Khadrawi) are genetically close, suggesting similarity in their genetic makeup. This aligns with the findings of several researchers, including the study by Kheirallah (2007) and Maiuf and Al-Mayahi (2023). They highlighted the importance of these genetic distance calculations in improving plant breeding strategies by selecting cultivars based on shared genes among genetically close cultivars. Conversely, significant differences, such as those observed between Shwithi and Khadrawi, can be analogous to the findings of Mohammed *et al*. (2016). They emphasized the magnitude of genetic diversity in date palm cultivars and the impact of local environmental variations, which may contribute to the development of distinct genetic traits. These studies suggest the high genetic distances could reflect considerable variations in environmental tolerance or responses to climatic factors.

Considering these results, it is crucial to acknowledge how recent research underscores the role of genetic diversity in enhancing the ability of cultivars to resist diseases and adapt to changing environmental conditions. As illustrated by the study of Kareem *et al*. (2018) and Saboori *et al*. (2020), these explored the applications of genetic distance in the development of plant cultivars resistant to climate change.

CONCLUSIONS

High genetic diversity was evident within date palm genotypes using ISSR markers. Given the great genetic variety, there is a good chance that genotypes will continue further selection to maximize fruit quality during pollination. For conservation and improvement initiatives, these findings provide crucial information about the genetic linkages among date palms.

REFERENCES

Abdulwahid AQ (2011). Study of the genetic imprint of two cultivars of date palm stallions, *Phoenix dactylifera* L. and the effect of their pollen on the physical and chemical characteristics of the fruits of the Halawi cultivar. Ph.D. thesis, College of Agriculture, Basrah University, Iraq. p. 233.

Ahmed TA, Al-Hadidi SH, Al-Qaradawi AY, Radwan O (2013). Determination of inter- and intraspecific genetic variations among Qatari date palm cultivars using inter simple sequence repeat (ISSR) markers. *Afr. J. Biotechnol*. 12(19): 2540–2546.

Arif IA, Khan HA, Shobrak M, Al Homaidan AA, Al-Farhan AH, Al-Sadoon M (2010). Measuring the genetic diversity of Arabian Oryx using microsatellite markers: Implication for captive breeding. *Genet. Syst*. 85: pp. 141–145.

Barrow S (1998). A monograph of *Phoenix dactylifera* L. (palmae: coryphoideae) *Kew Bull*. 53: pp. 513–575.

Bornet B, Branchard M (2001). Nonanchored inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. *Plant Molecular Bio. Rep*. 19(3): 209–215.

Chaudhary DS, Kajla A, Poonia B, Brar S, Duhan JS (2015). Molecular assessment of genetic

stability using ISSR and RAPD markers in commercial banana cultivar cv. Robusta. *Indian J. Biotechnol*. 14 (3): 420–424.

Clark AG, Lanigen CMS (1993). Prospects for estimating nucleotide divergence with RAPDs. *Mol. Evol*. 10: 1096–1111.

Grundmann H, Schneider C, Hartung D, Daschner FD, Pitt TL (1995). Discriminatory power of three DNA-based typing techniques for Pseudomonas aeruginosa*. Clin. Microbiol.* 33(3): 528–534.

Hammer O, Harper DA, Ryan PD (2001). PAST: Paleontological statistics software package for education and data analysis **.***Palaeontologia electronica*. 4(1): 1–9.

Huang QX, Wang XC, Kong H, Guo YL, Guo AP (2013). An efficient DNA isolation method for tropical plants, *Afr. J. Biotechnol*. 12(19): 2727–2732.

Hussien MA (2015). Determination of sex-specific DNA markers date palm (*Phoenix dactylifera* l.) grown in Egypt utilizing nuclear microsatellite markers. *J. Agric. Chem. Biotechn. Mansoura Univ*. 6(7): 237–246.

Kareem MAH, Al-Saadi AH, Naji HF (2018). Genetic diversity of Iraqi date palm (*Phoenix dactylifera* L.) by using RAPD technique. *J. Univ. Babylon***.** 26(1):114–131.

Khanam S, Sham A, Bennetzen JL, Aly MA (2012). Analysis of molecular marker-based characterization and genetic variation in date palm (*Phoenix dactylifera* L.). *Australian J. of Crop Sci*. 6(8): 1221–1236.

Kheirallah H, Bader S, Baum M, Hamwieh A (2011). Assessment of genetic diversity for some Iraqi date palms (*Phoenix dactylifera* L.) using amplified fragment length polymorphisms (AFLP) markers. *Afr. J. Biotechnol*. 10(47): 9570–9576.

Kheirallah HS, Al-Ani MR, Al-Rawi TK (2017). Identification of the sex of date palm seedlings using some molecular DNA markers. *Iraqi Agric. J*. 48(5): 1197–1205.

Kheirallah HSEM (2007). Micropropagation of two date palm cultivars *Phoenix dactylifera* L. using inflorescence and genetic stability study using AFLP markers. Ph.D. Thesis. Department of Horticulture. University of Baghdad. Iraq. pp. 174.

Maiuf MH, Al-Mayahi FH (2023). Morphological study of olive cultivars *Olea europaea* L. cultivated in Central and Southern of Iraq. *IOP Conf. Ser.: Earth Environ. Sci*. 1262 042064DOI 10.1088/1755-1315/1262/4/042064.

Mathew LS, Spannagl M, Al-Malki A (2014). A first genetic map of date palm (*Phoenix dactylifera* L.) reveals long-range genome structure conservation in the palms. *BMC Genomics* 15: 285.

Mohammed E, Anna Z, Tomas B, Moneim F, Claid M, Larisa G (2016). Elucidating the genetic diversity of farmer cultivars of female date palms (*Phoenix dactylifera* L.) from Sudan by microsatellite markers. *Genet. Resour. Crop Evol.* 63(6):975–986.

Nei M, Lei WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*. 76: 5269–5273.

Saboori S, Noormohammadi Z, Sheidai M, Marashi S (2020). SCoT molecular tags and genetic fingerprint of dates palm (*Phoenix dactylifera* L). *Genet. Resour. Crop Evol*. 67(1): 73–82.

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Zabeau M (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic. Acids Res*. 23(21): 4407– 4414.

Wrigley G (1995). Date palm (*Phoenix dactylifera* L.). The Evolution of Crop Plants. Longman, Essex: pp. 403.