Tikrit Journal for Agricultural Sciences
ISSN:1813-1646 (Print); 2664-0597 (Online)
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq
DOI: https://doi.org/10.25130/tjas.25.4.10

TJAS
Tikrit Journal for Agricultural Sciences

Phylogenetic Barcoding of Genetic Diversity in Iraqi Date Palm Cultivars Using the Plastid Non-Coding Region psbA-trnH

Mohammed Kh. Omer, and Reham F. Faris
Department of Biology, Collage of Science, Tikrit University,Tikrit, Iraq

*****Correspondence: **mohammed.k.omer@tu.edu.iq**

KEY WORDS:
psbA-trnH ;Non-Coding DNA ; Phoenix dactylifera ;Plastid DNA ; Genetic diversity

Received: 17/02/2025
Revision: 05/08/2025
Proofreading: 29/10/2025
Accepted: 22/09/2025
Available online: 31/12/2025

ABSTRACT

Understanding and evaluating genetic diversity among *Phoenix dactylifera* cultivars is essential for identifying genetic variation, preserving biodiversity, and guiding crop improvement initiatives. In this study, specific primers were designed to amplify the non-coding plastid intergenic spacer **psbA-trnH** across ten Iraqi date palm cultivars. The resulting sequences were analyzed to detect nucleotide polymorphisms and assess genetic distances between the cultivars. The minimum genetic distance (0.00342) was observed between 'Khastawi' and 'Qarnfuli', while the maximum distance (0.02703) occurred between 'Tabarzul' and 'Dayri'. A total of 17 polymorphic sites were detected, revealing moderate sequence variation. Additionally, the haplotype diversity variance was calculated at 0.002, nucleotide diversity (Pi) was 0.01054, and theta (per site) from Eta was estimated at 0.01275. These molecular diversity indices highlight the potential of the **psbA-trnH** region as a useful marker for genetic analysis in date palms. The outcomes of this research contribute valuable insights for conservation planning, germplasm management, and future breeding programs targeting local Iraqi cultivars.

الترميز التسلسلي للتنوع الوراثي في أصناف نخيل التمر العراقية باستخدام منطقة البلاستيد غير المشفرة psbA-trnH

محمد خطاب عمر و ريهام فراس فارس
قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، العراق

الخلاصة

يُعد فهم التنوع الوراثي بين أصناف نخيل التمر أمرًا جوهريًا في التمييز بين الاختلافات الجينية للأفراد، كما يسهم في جهود الحفاظ على التنوع الحيوي وتحسين المحاصيل. في هذه الدراسة، تم تصميم بادئات نوعية (Primers) لتضخيم المنطقة غير المشفرة psbA-trnH من البلاستيد لعشرة أصناف من نخيل التمر العراقي. جرى تحليل التباينات النيوكليوتيدية لتقدير المسافات الوراثية، حيث سُجلت أقصر مسافة بين صنفي "الخستاوي" و"القرنفلي" (0.00342)، وأكبر مسافة بين "التبرزل" و"الديري" (0.02703). تم تحديد ما مجموعه 17 موقعًا متعدّد الأشكال (Polymorphic sites)، مع تباين في تنوع الأنماط الفردانية (Haplotype diversity variance) بلغ 0.002، وتنوع نيوكليوتيدي (Pi) قدره 0.01054، بينما قُدّرت قيمة θ (لكل موقع) اعتمادًا على قيمة Eta بـ 0.01275. تعكس هذه النتائج كفاءة استخدام المناطق غير المشفّرة من البلاستيدات في تقييم التنوع الوراثي، وتوفر أساسًا علميًا يمكن الاستناد إليه في برامج التربية المستقبلية واستراتيجيات الحفاظ على نخيل التمر العراقي.

الكلمات المفتاحية: منطقة psbA-trnH ،الحمض النووي غير المشفر، نخيل التمر، الحمض النووي للبلاستيدات.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), a monocotyledonous plant from the Arecaceae family (2n = 36), is one of the most economically significant fruit crops in many Arab countries (ElJuhany, 2010). It is cultivated for various reasons, including its high productivity, the rich nutritional value of its fruits, its role in preserving ecosystems threatened by desertification, and its ability to establish a microclimate suitable for agriculture in arid regions. Due to its strategic importance, the study of date palm genetic diversity has gained increasing attention in recent years. Globally, the total number of date palm trees exceeds 150 million, encompassing over 3,000 varieties and being distributed across more than 30 countries (Sedra, 2013; Al-Khayri *et al*., 2015). Understanding and evaluating the genetic diversity of date palm varieties at the DNA level is crucial for genetic improvement and conservation programs. DNA typing has proven to be the most reliable method for identifying genetic variability among plant varieties, analyzing genetic diversity, and determining phylogenetic relationships (Amom & Nongdam, 2017).

Traditionally, researchers have identified date palm genotypes using morphological traits and biochemical markers. However, these markers are often influenced by environmental factors and exhibit low levels of polymorphism, making them less reliable for genetic variation studies (Salomon-Torres, 2017). A number of techniques are used to analyze the genetic diversity of date palms, where initial studies relied on phenotypic (Elboghdady *et al*.,2023) and chemical (Al-Jibouri and Adham (1990) and Azeqour *et al*. (2002) indicators, but the impact of environmental factors on these indicators prompted researchers to adopt more accurate and reliable molecular indicators (Ibrahimi *et al*. 2023). To overcome these limitations, various DNA-based molecular markers have been applied to analyze the genetic relationships of date palm cultivars in different countries, including Egypt (Abd-Alla, 2010), Tunisia (Karim *et al*., 2015), Morocco (Bodian *et al*., 2012), and Syria (Haider *et al*., 2012).

Date palm varieties exhibit extensive genetic diversity due to both environmental and genetic factors, making the application of molecular techniques essential for the conservation of genetic resources and the enhancement of agricultural cultivars (Al-Khayri *et al*., 2015). Among these techniques, molecular markers have been widely utilized to study genetic variation. One particularly effective marker is the psbA-trnH non-coding region of plastid DNA, which has demonstrated high efficacy in molecular classification and genetic relationship studies among plants (Shaw *et al*., 2005). This region is characterized by a high mutation rate compared to coding regions, making it a powerful tool for distinguishing different plant varieties and analyzing nucleotide diversity (Kress & Erickson, 2007).

Recent global research underscores the growing utility of chloroplast DNA (cpDNA) markers for assessing genetic diversity, phylogenetic relationships, and cultivar identification in plants. In particular, the non-coding plastid spacer psbA-trnH has been repeatedly validated for distinguishing cultivars in *Phoenix dactylifera* and other angiosperms due to its high polymorphism and PCR reliability (Sabir *et al*., 2014). A comprehensive chloroplast genome survey in Tunisian date palm cultivars further revealed rich haplotype diversity and clear phylogenetic structuring that complement conventional cpDNA approaches (Hamza *et al*., 2023).Additionally, broader analyses across flowering plants confirm that cpDNA-based barcoding, especially using non coding regions like psbA-trnH, is effective for resolving closely related lineages and supporting conservation and breeding programs (Soumaya *et al*., 2023).
This study aims to analyze nucleotide variation in the psbA-trnH non-coding region between some Iraqi Date palm varieties , which plays a crucial role in understanding genetic relationships between species, assessing genetic diversity, identifying evolutionary links, and examining mutations and their impact on gene function.

MATERIAL AND METHODS

Sample collection and DNA extraction

Ten Iraqi date palm (*Phoenix dactylifera* L.) cultivars were selected based on key morphological traits, particularly fruit shape and size. The selected cultivars—chosen for their recognized economic value and strong market demand—are among the most widely cultivated and commercially successful in Iraq, as shown in Table (1). The plant leaves were thoroughly washed multiple times to ensure complete removal of dust and debris. Genomic DNA was then extracted using the extraction kit provided by Tinzyme. The presence of DNA was confirmed via agarose gel electrophoresis at a concentration of 0.8%, followed by quantification and purity assessment using a NanoDrop spectrophotometer (Thermo scientific NANO DOP2000c). The extracted DNA samples were subsequently stored for further use.

Table (1) shows the selected date palm varieties and their collection

Sample	Collection area	Code
Al-Khadrawi	Babylon	M1
Qarnafly	Baghdad	M2
Khastawi	Salah Aldain	M3
Tabarzal	Babylon	M4
Jabjab	Baghdad	M5
Medjool	Baghdad	M6
Barhi	Salah Aldain	M7
Maktoum	Karbala	M8
Deri	Karbala	M9
Barban	Baghdad	M10

DNA amplification and polymerase chain reaction PCR

The psbA-trnH region was amplified using primers specifically designed for this study through the NCBI primer design tool . The PCR reaction was conducted with the AccuPower PCR PreMix kit from Pioneer, following the provided instructions and additives. The reaction mixture consisted of 10 µl of 2X Master Mix, 1µl (at a concentration of 10 picomoles) of each primer

(Table 2), 2 µl of DNA, and the total volume was adjusted to 20 µl using DNase-free distilled water.

Table 2 shows the primer, its sequence, and its binding region.

Primer name	Sequence (5′ → 3′)	Target Region
psbA-F	ACTTTTGTGTCTTAGTGTATATGAATCGTTG	End of psbA gene
trnH-R	TCTGACCTCTCCATACTTAGATCGAGATA	Start of trnH gene

The reaction was carried out in a Veriti 96 well Thermal Cycler (Applied Biosystems) according to the following program: 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. A final extension step was performed at 72°C for 7 minutes to ensure that any remaining single-stranded DNA was extended.

DNA Sequencing and Data analysis

The samples were sent to Macrogen in South Korea for Sanger sequencing using the ABI Prism Terminator technique. The resulting sequences were compared with the reference gene JN854235.1 via the BLAST tool on the NCBI website, and further analyzed using MEGA X software. The mutations were identified and classified into substitutions, transitions, and transversions, with the frequency of each mutation type calculated. Additionally, the genetic distance between the samples was calculated using MEGA X, based on appropriate genetic distance models. Finally, a phylogenetic tree was constructed using the Maximum Likelihood approach to illustrate the evolutionary relationships among the samples. Genetic diversity was analyzed using DnaSP version 6.12.03, where the number of polymorphic sites was calculated to assess the level of variation within the samples.

Genetic distances among sequences were calculated using the Kimura 2-parameter (K2P) model implemented in MEGA version X software. This model was chosen because it accounts for transition and transversion rate differences, which is appropriate for analyzing chloroplast DNA sequences. Furthermore, the variance of haplotype diversity was measured as an indicator of genetic differences, and nucleotide diversity (Pi) was estimated to determine the degree of variation between sequences.

Additionally, Theta (per site) from Eta was calculated to estimate the nucleotide mutation rate based on the number of variable sites. These analyses provide a comprehensive approach for assessing the genetic variation among Iraqi date palm cultivars, offering deeper insight into their genetic relationships.

RESULTS AND DISSCUSION

Ten samples of Iraqi date palm varieties were selected based on morphological characteristics, specifically fruit shape. These varieties were chosen for their economic significance, high market demand, and strong sales. DNA extraction was highly efficient, yielding high-purity DNA with values ranging from 1.71 to 1.85, along with sufficient concentrations. The psbA-trnH region was successfully amplified using primers designed for this study (Table3). The obtained sequences exhibited variations in both length and nucleotide composition. The length of the psbA-trnH region ranged from 586 base pairs in the M2 variety to 596 base pairs in the M8 variety, with an average length of 593.8 base pairs.

The average nucleotide composition was as follows: Adenine (A): 29.824% , Thymine (T): 40.941% , Cytosine (C): 14.012% , Guanine (G): 15.223% , Additionally, the GC content varied from 28.91% in M7 to 29.58% in M1, with an average of 29.24% (Table4).

Table 3. The success rate of PCR amplification and DNA fragment sequencing of psbA-trnH

Locus	**N1**	**P(%)**	**N2**	**S(%)**
psbA-trnH	10	100	10	100

N1=number of sample amplified by PCR , N2= number of sample sequenced, p=PCR success ,

Table(4) illustrates the psbA-trnH region sizes and the nitrogenous base content for each sample

	T	C	A	G	Total	CG %
M1	40.5	13.95	29.92	15.63	595	29.58
M2	41.81	13.82	29.18	15.19	586	29.01
M3	41.18	14.29	29.58	14.95	595	29.24
M4	40.84	14.12	29.91	15.13	595	29.25
M5	41.01	13.95	29.58	15.46	595	29.41
M6	40.91	14.48	29.8	14.81	594	29.29
M7	40.84	13.78	30.25	15.13	595	28.91
M8	40.94	13.59	29.87	15.6	596	29.19
M9	40.81	14.17	29.84	15.18	593	29.35
M10	40.57	13.97	30.31	15.15	594	29.12
Avg.	40.941	14.012	29.824	15.223	593.8	29.24

The results of the sequence analysis revealed a significant polymorphism in nucleotide polymorphisms among date palm cultivars in the psbA-trnH region. The analysis included 10 nucleotide sequences and showed a large number of mutations, ranging from 30 mutations in cultivar M4 to 37 mutations in cultivar M6. The analysis showed that the incidence of transversion (SV) mutations was higher than the incidence of transposition (SI) mutations, resulting in an R value of less than 1 in all the studied cultivars Table (5).

Regarding transition patterns, the G → T transition was the most frequent, occurring at a rate of 0.171053, while the G → C transition was the least frequent, with a rate of 0.016447 (Table 6).

Table (5): type and number of mutations recorded in the studied samples

	C-G	G-A	A-G	T-A	A-T	T-G	G-C	G-T	C-T	SV	SI	R	Deletion	Insertion/sit	Total
M1	1	4	5	3	3	5	1	5	3	18	12	0.67	0	T/35,A/42	32
M2	1	5	4	4	4	5	0	6	3	20	12	0.60	A	G/582	34
M3	1	5	4	4	4	5	1	5	3	20	12	0.60	A,A	G/582	35
M4	1	5	4	3	3	5	1	5	3	18	12	0.67	0	0	30
M5	1	5	4	3	3	5	1	5	3	18	12	0.67	A,A	0	32
M6	1	5	4	4	4	5	1	5	3	20	12	0.60	A,A,T	G/581,G/585	37
M7	1	5	4	3	3	5	0	6	3	18	12	0.67	A	0	31
M8	1	5	4	3	3	5	0	6	3	18	12	0.67	T,A	0	32
M9	1	4	3	4	4	6	0	4	3	19	10	0.53	A,A	A/582	32
M10	1	5	4	3	3	5	0	5	3	17	12	0.71	A,T	0	31

Table (6) : Transition and transverstion rates of psbA-trnH nucleotide sequences in date palm varieties

	A	T	C	G
A	-	0.111842	0	0.157895
T	0.111842	-	0.098684	0.171053
C	0.000	0.0000	-	0.016447
G	0.131579	0.167763	0.032895	-

Note: Each entry shows the probability of substitution from one base (row) to another base (column). Only entries within a row should be compared. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics, the sum of r values is 1.

The genetic distance matrix calculated for the studied varieties revealed that the genetic distances ranged from 0.00342 to 0.02703, with an average of 0.01415. The lowest genetic distance was recorded between the M2 and M3 cultivars, reflecting a strong genetic affinity between them. This close relationship may result from a shared evolutionary background, adaptation to similar environmental conditions, or limited divergence due to restricted gene flow, while the largest genetic distance was found between the M4 and M9 varieties, The largest genetic distance observed between the M4 and M9 cultivars reflects a significant level of genetic divergence, suggesting that these two varieties have distinct evolutionary backgrounds. This divergence may be attributed to differences in their geographical origins, adaptations to varying environmental conditions, or restricted gene flow between their populations. (Table 7).

Table (7) Genetic distance between the studied varieties

	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**
M1	0.00000									
M2	0.01365	0.00000								
M3	0.01684	0.00342	0.00000							
M4	0.01176	0.01024	0.01515	0.00000						
M5	0.00673	0.00855	0.01513	0.00505	0.00000					
M6	0.02024	0.00856	0.01010	0.01686	0.01515	0.00000				
M7	0.01010	0.00855	0.01518	0.01178	0.00675	0.01689	0.00000			
M8	0.01008	0.00853	0.01849	0.01345	0.00840	0.01852	0.00673	0.00000		
M9	0.02534	0.01541	0.02024	0.02703	0.02530	0.02027	0.02200	0.02530	0.00000	
M10	0.01180	0.01027	0.01520	0.01686	0.01351	0.01692	0.01010	0.01012	0.02030	0.00000

A phylogenetic tree was constructed to analyze the evolutionary relationships among date palm cultivars using the psbA-trnH gene sequence data. The phylogenetic tree was constructed using the Neighbor-Joining method using MEGA software, while the gene sequences were aligned using the ClustalW tool. The phylogenetic tree showed that the cultivars were divided into two main groups: I and II. The first group (I) included only the cultivar M9, while the second group (II) was divided into two subgroups (II1 and II2). In the first subgroup (II1), the first branch included the cultivars M2 and M3 together in one group, while the cultivar M6 was in an independent group within the same subgroup. As for the second subgroup (II2), it included the rest of the cultivars and was divided into two subgroups: the first: included the cultivar M10. The second: included the cultivars M4, M5, and M1 in one group, and the cultivars M8 and M7 in another group. These divisions reflect the degree of genetic variation among the cultivars, as shown in the figure (1). 17 polymorphic sites were detected within the ten genetic sequences used

in the study, reflecting the presence of differences in the nitrogenous base sequences among date palm cultivars. The number of polymorphic sites reflects the level of genetic diversity among cultivars, which is an indicator of the presence of a degree of genetic variation among Iraqi date palm cultivars. The variance of haplotype diversity (VHD) was 0.002, indicating the extent of distribution of different genotypes within the sample. This low value indicates that genetic variation among cultivars is concentrated around a few haplotypes, reflecting low or limited genetic diversity despite the presence of significant differences. The value of nucleotide diversity (Pi) was 0.01054, indicating limited differences among genetic sites in these samples. This result shows a medium to low level of diversity, reflecting a relative closeness among the studied cultivars. The Theta value of 0.01275 indicates the mutation rate per gene locus, based on the number of mutations detected (Eta). This value reflects a slightly higher overall genetic variation level than the Pi value, indicating a moderate mutation rate in the studied gene region, although the distribution of these mutations may not lead to significant variation between individual gene loci (Table8). Based on these results, although some genetic variation was detected between cultivars, the studied Iraqi date palm cultivars show significant genetic closeness with limited diversity. This useful diversity can be an effective tool in assessing the genetic relationships between cultivars.

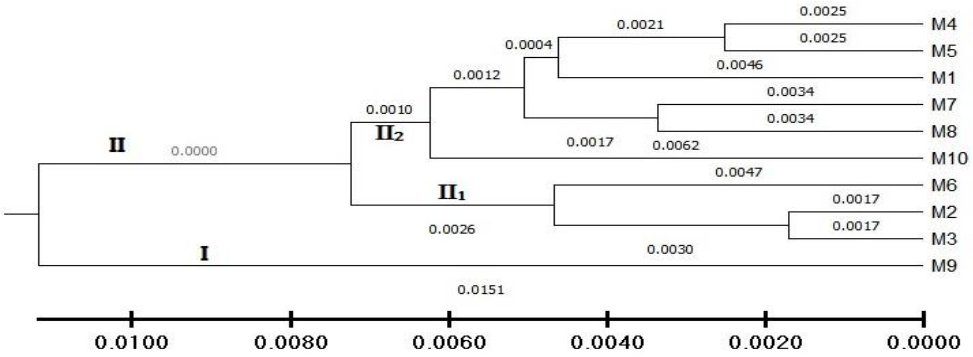

Figure (1): shows the phylogenetic tree constructed to analyze the genetic evolution among the studied date palm cultivars, based on the genetic sequence data from the psbA-trnH region.

Table 8. Summary of Statistics of psbA-trnH Fragments for Date Palm Varieties

Variable (polymorphic) sites	17
Variance of Haplotype diversity	0.00200
Nucleotide diversity, (Pi)	0.01054
Theta (per site) from Eta	0.01275

The plastid genome of date palms exhibits remarkable stability primarily due to its maternal mode of inheritance, wherein the plastid DNA (cpDNA) is exclusively transmitted from the mother plant. As a result, variations within the cpDNA arise mainly from mutations occurring in the studied regions, although such mutations tend to be minimal (Clegg *et al*., 1994). Notably, non-coding regions constitute approximately 41.57% of the total cpDNA in date palms, indicating that genetic changes occur less frequently in these regions compared to coding sequences, thereby reinforcing the overall stability of the plastid genome. Most genetic alterations and polymorphisms

are observed within these non-coding regions, which contributes to the conserved nature of the plastid DNA (Yang *et al*., 2010).

The genetic variation detected among date palm cultivars can be largely attributed to the dispersal mechanisms of pollen, seeds, and offshoots. While offshoots and pollen are widely distributed locally among farmers within villages, provinces, or countries, seeds are often transported over long distances by travelers and traders, crossing geographical boundaries (Elshibli and Korpelainen, 2008). Additional factors potentially influencing sequence variation among cultivars include geographical barriers such as mountains and lowlands, assortative mating, mutation events, genetic drift, gene flow, natural selection, long-term evolutionary history, successional stages, and anthropogenic activities, all of which shape the genetic diversity patterns in plant populations (Nybom, 2004). Environmental heterogeneity and the presence of genetically diverse cultivars further contribute to this variation (Fakir & Car Munier, 1981). Moreover, the dioecious reproductive system of date palms may play a significant role in the observed high genetic variability, as documented in Tunisian date palms (Nybom & Bartish, 2000). Understanding and characterizing this genetic variation is crucial for practical applications such as breeding programs, where identifying diverse genotypes can guide the development of cultivars with improved traits like disease resistance, drought tolerance, and enhanced productivity. Conservation efforts also benefit from such genetic insights by preserving the breadth of genetic diversity essential for long-term sustainability and adaptation.

In this study, the genetic diversity observed among Iraqi date palm cultivars, as revealed by the psbA-trnH plastid non-coding region, is consistent with global research findings on date palms. The identification of 17 polymorphic sites and a moderate nucleotide diversity (Pi = 0.01054) reflects a degree of genetic variation comparable to other regional studies. For example, Hamza *et al*. (2023) reported substantial haplotype diversity and distinct phylogenetic structuring among Tunisian date palm cultivars based on complete chloroplast genome analyses, highlighting the efficacy of plastid markers in detecting genetic differentiation. Similarly, Soumaya *et al*. (2023) demonstrated the effectiveness of cpDNA markers, including non-coding regions like psbA-trnH, for discriminating closely related Tunisian cultivars.

Nonetheless, the moderate level of genetic diversity and limited haplotype variance observed in Iraqi cultivars may indicate relatively recent domestication events or specific gene flow dynamics within the region. This observation aligns with Nybom's (2004) emphasis on the influence of geographical isolation, human agricultural practices, and gene flow in shaping genetic variation among date palms worldwide. Furthermore, the maternal inheritance and inherent stability of the plastid genome (Clegg *et al*., 1994; Yang *et al*., 2010) contribute to the conserved nature of the psbA-trnH region, making it a valuable marker for phylogenetic and barcoding studies, albeit with less variability than nuclear DNA markers.

Overall, the findings corroborate global patterns of plastid DNA variation in date palms and support the continued application of plastid non-coding regions in conservation genetics and breeding programs, especially in underexplored regions such as Iraq where genetic resources are not yet fully characterized. This study has limitations, including a relatively small sample size and the use of only one plastid marker (psbA-trnH), which may not capture the full genetic diversity of Iraqi date palms. Future research should include a larger number of samples and multiple genetic markers, including nuclear DNA, to provide a more comprehensive understanding. Employing advanced genomic tools like whole-genome sequencing could further enhance genetic insights and aid breeding and conservation efforts.

CONCLUSION

The results of this research demonstrate the presence of significant genetic diversity among the studied Iraqi date palm cultivars, as demonstrated by the sequence analysis of the non-coding plastid region psbA-trnH. This diversity was manifested by differences in the nitrogenous base sequence and variations in nucleotide positions, confirming the presence of mutations of the type translocations and transversions that contributed to the genetic divergence between samples. Genetic distance and phylogenetic tree analysis also revealed the presence of kinship relationships and differences among cultivars, enhancing the potential for using this data to determine the genetic identity of local cultivars, in palm improvement programs, and in the conservation of genetic resources. The results confirm that the psbA-trnH region is an effective molecular marker for assessing genetic diversity within date palm cultivars and open the door to further expanded studies that include a larger number of cultivars and wider geographic regions.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff of the Department of Biology, College of science ,Tikrit University for their technical and general support.

REFERENCES

Abd-Alla, M.M. (2010).Genetic stability on *Phoenix dactylifera* var. Karama produced in vitro.New York Science Journal, 3(12), pp. 1–7.

Al-Jibouri, A. A. M., & Adham, K. M. (1990). Biochemical classification of date palm male cultivars. *Journal of Horticultural Science*, *65*(6), 725-729.

Al-Khayri, J.M., Jain, S.M. and Johnson, D.V. (2015). Date Palm Genetic Resources and Utilization, Vol. 1. New York: Springer.

Amom, T. and Nongdam, P. (2017).The use of molecular marker methods in plants: a review.International Journal of Current Research and Review, 9(17), pp. 1–7.

Azeqour, M., Majourhat, K., & Baaziz, M. (2002). Morphological variations and isoenzyme polymorphism of date palm clones from in vitro culture acclimatized and established on soil in South Morocco. *Euphytica*, *123*(1), 57-66.

Bodian, A., Nachtigall, M., Frese, L., Elhoumaizi, M.A., Hasnaoui, A., Ndir, K.N. and Wehling, P. (2012).Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) cultivars from Figuig oasis (Morocco) using SSR markers.International Journal of Science and Advanced Technology, 2(3), pp. 1–9.

Clegg, M.T., Gaut, B.S., Learn, G.H. and Morton, B.R. (1994).Rates and patterns of chloroplast DNA evolution.Proceedings of the National Academy of Sciences, USA, 91, pp. 6795–6801.

El-Assar, A. M., Krueger, R. R., Devanand, P. S., & Chao, C. C. T. (2005). Genetic analysis of Egyptian date (*Phoenix dactylifera* L.) accessions using AFLP markers. *Genetic Resources and Crop Evolution*, *52*(5), 601-607.

Elboghdady AE, A.; Gomma, A.H.; Hamed, A.M.; Abdallatif, A.M. Assessment of phenotypic diversity of some date palm male genotypes growing under Egyptian conditions. Rev. Bras. Frutic. 2023, 45, e-896.",

Elshibli, S., & Korpelainen, H. (2008). Microsatellite markers reveal high genetic diversity in date palm (*Phoenix dactylifera* L.) germplasm from Sudan. *Genetica*, *134*(2), 251-260.

Fakir, S. and Car Munier, P. (1981).Origine de la culture sur palmier dattier et sa propagation en Afrique.\" Fruits, 36, pp. 437–450.

Haider, N., Nabulsi, I. and MirAli, N. (2012). Phylogenetic relationships among date palm (*Phoenix dactylifera* L.) cultivars in Syria using RAPD and ISSR markers.Journal of Plant Biology Research, 1(2), pp. 12–24.

Hamza, H., Villa, S., Torre, S., Marchesini, A., Benabderrahim, M. A., Rejili, M., & Sebastiani, F. (2023). Whole mitochondrial and chloroplast genome sequencing of Tunisian date palm cultivars: diversity and evolutionary relationships. BMC genomics, 24(1), 772.

Ibrahimi, M.; Brhadda, N.; Ziri, R.; Fokar, M.; Iraqi, D.; Gaboun, F.; Labhilili, M.; Habach, A.; Meziani, R.; Elfadile, J.; *et al*. Analysis of genetic diversity and population structure of Moroccan date palm (*Phoenix dactylifera* L.) using SSR and DAMD molecular markers. J. Genet. Eng. Biotechnol. 2023, 21, 66.

Karim, K., Ines, R., Souhayla, M. and Khayria, H. (2015). Morphological and molecular evaluation of the genetic diversity of Tunisian local date palm pollinators.Academic Journal of Biotechnology, 3(2), pp. 026–034. DOI: 10.15413/ajb.2015.0113.

Kress, W. J., & Erickson, D. L. (2007). A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. PLoS ONE, 2(6), e508.

Nybom, H. (2004).Comparison of different nuclear DNA markers for estimating intra-specific genetic diversity in plants.\" Molecular Ecology, 13, pp. 1143–1155.

Nybom, H., & Bartish, I. V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in plant ecology, evolution and systematics*, *3*(2), 93-114.

Sabir, J. S., Arasappan, D., Bahieldin, A., Abo-Aba, S., Bafeel, S., Zari, T. A., ... & Jansen, R. K. (2014). Whole mitochondrial and plastid genome SNP analysis of nine date palm cultivars reveals plastid heteroplasmy and close phylogenetic relationships among cultivars. PloS one, 9(4), e94158.

Salomon-Torres, R., Ortiz-Uribe, N., Villa-Angulo, C., Villa-Angulo, R. and Yaurima-Basaldúa, V.H. (2017). Assessment SSR markers used in analysis of genetic diversity of date palm (*Phoenix dactylifera* L.).Plant Cell Biotechnology and Molecular Biology, 18(5&6), pp. 269–280.

Sedra, M.H. (2013). Genetic diversity analysis, Moroccan cultivar genotyping and rapid screening for Bayoud disease resistance in date palm using molecular techniques.Acta Horticulturae, 994, pp. 271–285.

Shaw, J., Lickey, E.B., Schilling, E.E. and Small, R.L. (2005).Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. Systematic Botany, 30(1), pp. 116–129.

Soumaya, R. C., Sarra, C., & Khaled, C. (2023). Sequences Evolution and Population Structure of Tunisian Date Palm (*Phoenix dactylifera* L.) Revealed by Chloroplast DNA Markers. In Chloroplast Structure and Function. IntechOpen.

Yang, M., Zhang, X., Liu, G., Yin, Y., Chen, K., Yun, Q., and Yu, J. (2010). The complete chloroplast genome sequence of date palm (*Phoenix dactylifera* L.). PloS one, 5(9), e12762