Abdulhafed A. Al-Ameri[1], Abdulwareth A. Almoneafy[2*] and Abdel-Rhman Z. Gaafar[3]

Genetic Diversity Evaluation of Date Palm (*Phoenix dactylifera* L.) Cultivars in Saudi Arabia Using ISSR Molecular Markers.

Abstract:

The date palm (*Phoenix dactylifera* L.) is a fundamental crop in arid and semi-arid habitats, especially in the Kingdom of Saudi Arabia, where its genetic improvement and preservation are strategically significant. This study examined the genetic diversity of 12 cultivars collected from three major agricultural regions—Al-Ahsa, Al-Kharj, and Al-Qassim—using inter-simple sequence repeat (ISSR) markers recognized for their high polymorphism resolution and reproducibility. The three primers (ISSR-60, ISSR-64, and ISSR-81) generated different and reproducible amplification profiles, indicating significant inter-cultivar variation. Genetic similarity coefficients varied from 0.640 to 0.913, with the greatest affinity identified between Naboot Seif and Wannana and, the

[1] Department of Biology Sciences, Faculty of Education and Science at Rada'a, Albaydha University.

[2] Department of Biology Sciences, Faculty of Education and Science at Rada'a, Albaydha University.

[*] Corresponding author:, std2008@gmail.com

[3] Department, of Biology Sciences, Faculty of Education and Science at Rada'a, Albaydha University .

least between Hoshishi and both Sabaka and Rothana. Three significant genetic clusters were identified using the UPGMA, which reflected both regional origin and underlying genetic linkages. These findings highlight the usefulness of ISSR markers in detecting genomic polymorphisms and resolving cultivar-level genetic structure, providing vital insights for germplasm management, conservation measures, and the improvement of targeted breeding programs in date palm.

Keywords: *Phoenix dactylifera* L., Genetic diversity, ISSR, DNA markers, Polymorphism

Introduction:

Date palm (*Phoenix dactylifera* L.) is one of the most significant agricultural products in arid and semi-arid countries because of its substantial economic, nutritional, and cultural significance—especially in the Kingdom of Saudi Arabia (Al-Mssallem et al., 2024). With a growing interest in developing local cultivars and maintaining genetic resources, there is a greater demand for precise tools to quantify the genetic diversity of date palm cultivars. Molecular markers have emerged as potent instruments in this area, enabling reliable techniques to detect genetic variation and define relationships across crops, independent of environmental factors that frequently muddle morphological assessments (Idrees and Irshad 2014). In this paradigm, inter-simple sequence repeat (ISSR) markers have gained significance due to their high polymorphism and repeatability, placing them as attractive options for describing Saudi date palm cultivars (Sabir et al., 2014). Numerous studies have used a variety of molecular markers, including SSR, RAPD, and AFLP, to assess the genetic diversity of date palm (Rahman et al. 2022; Yaish et al. 2015; Zango et al. 2017). For example, Zhao et al. (2012) demonstrated that ISSR markers were particularly successful in cultivar differentiation and created hundreds of primers from EST sequences. Salomon-Torres et al. (2017) investigated over a thousand date palm varieties from 12 different nations using 255 SSR markers.

Inter simple sequence repeat (ISSR) markers have proven to be effective tools for assessing genetic diversity and elucidating genetic relationships among date palm cultivars (*Phoenix dactylifera* L.) to their high sensitivity in detecting polymorphisms across the genome with both efficiency and reliability (Zehdi et al., 2004; Toor et al. 2005). Numerous previous studies have found significant

genetic variation among cultivars, generally reflecting differences in geographic origin, propagation methods, and agricultural history. For example, Zehdi et al. (2004) discovered clear genetic heterogeneity among Tunisian cultivars, whereas Ahmed and Al-Qaradawi (2010) proved the accuracy of ISSR markers in differentiating Qatari cultivars. In addition, RAPD and ISSR markers have been efficient in the characterization of Iraqi date palm cultivars (Kareem et al. 2018). Meanwhile, Sabir et al. (2014) observed that AFLP and ISSR markers exhibited high discriminatory power among Saudi cultivars. Taken together, these findings highlight the importance of ISSR markers as promising techniques for defining date palm cultivars due to their high polymorphism and reproducibility.

Cluster analysis using the UPGMA algorithm to categorize cultivars based on genetic similarity coefficients is a widely accepted approach in molecular investigations. In this respect, Abdulla and Gamal (2010) divided Saudi date palm cultivars into discrete genetic groupings using RAPD markers, whereas Elmeer and Mattat (2015) divided Egyptian cultivars into homogeneous clusters using ISSR markers. These outcomes highlight the importance of molecular markers in cultivar characterization and in the development of genetic conservation methods.

The purpose of this study was to analyze the genetic diversity of date palms by applying three ISSR primers to 12 cultivars collected from three major geographic regions in the Kingdom of Saudi Arabia. The study aimed to assess the potential application of ISSR markers in the Saudi context and their role in supporting breeding programs and genetic conservation efforts. This analysis will allow us to evaluate the genetic variance and similarity between cultivars, providing useful information for understanding their genetic associations.

Materials and Methods:

Plant material and collection of samples:

Healthy leaf samples from each of the 12 date palm (*Phoenix dactylifera* L.) varieties were collected in three agricultural locations in Saudi Arabia: Qassim, Al-Ahsa, and Al-Kharj. The samples were stored at -80°C until DNA. Detailed information regarding the collected cultivars is summarized in Table 1.

Genomic DNA extraction:

Genomic DNA was isolated from young leaf tissues using the modified CTAB procedure described by Aboul-Maaty and Oraby (2019). Agarose gel electrophoresis and spectrophotometry (Thermo Scientific NanoDrop 2000) were used to determine the quality and concentration of DNA.

ISSR Marker Amplification:

Three ISSR primers were chosen based on previous research for their good repeatability and polymorphism detection effectiveness (Sabir et al., 2014). Details of the primers are presented in Table 2. The PCR reaction was conducted in a 25 µl volume, comprising 2 µl (40 ng) of template DNA, 1 µl (20 pmol) of forward primer, 1 µl (20 pmol) of reverse primer, 12.5 µl of Master Mix, and 8.5 µl of deionized water. The PCR amplification protocol was implemented as follows: initial denaturation for 5 minutes at 95º C, followed by 40 cycles of 1 minute at 94º C, the annealing step was conducted differently for each primer utilized in this investigation, in accordance with their respective annealing temperatures specified in Table 2, extension at 72º C for 1:30 min, and final extension at 72º C for 7 min. PCR amplification was carried out on a

Vertini 96-well thermal cycler from Applied Biosystems, Fisher Scientific, United States.

Table 1. List of date palm cultivars collected from three locations in Saudi Arabia

No.	Cultivars	Abbreviation	Sampling Location
1.	Khalas	Kh	Al-Hassa
2.	Sery	Se	Al-Hassa
3.	Ruzeiz	Ru	Al-Hassa
4.	Hoshishi	Ho	Al-Hassa
5.	Rothana	Ro	Al-Kharj
6.	Naboot Seif	Ns	Al-Kharj
7.	Nabtat Ali	Na	Al-Kharj
8.	Dukhaini	Du	Al-Kharj
9.	Barhi	Ba	Al-Qassim
10.	Wannana	Wa	Al-Qassim
11.	Deglet Noor	Dn	Al-Qassim
12.	Sabaka	Sa	Al-Qassim

Gel electrophoresis and visualization:

The amplified products were separated on 1.5% agarose gels stained with ethidium bromide and photographed under ultraviolet light. The banding patterns were documented using the gel documentation system (Bio-Rad Gel Doc XR+).

Table 2. List of ISSR primers used to evaluate the genetic diversity of 12 date palm cultivars

No.	Primer	Primer sequence (5′–3′)	Annealing temperature (°C)
1.	ISSR-60	5’-GGGTGGGGTGGGGTG-3’	47
2.	ISSR-64	5’-GAGAGAGAGAGAGAGACTT-3’	54
3.	Issr-81	5’-CTCTCTCTCTCTCTCTAC-3’	50

Data analysis

To create a binary data matrix, clear and reproducible bands were manually graded as present (1) or absent (0). Genetic similarity among cultivars was visualized and presented by Jaccard's coefficient and cluster analysis, utilizing a clustered heatmap generated by the R statistical program (R Studio 2025.09.0).

Results and Discussion:

Three inter simple sequence repeat (ISSR) primers were used to amplify genomic DNA fragments from 12 different date palm cultivars. Figures 1, 2, and 3 show the resulting amplification profiles.

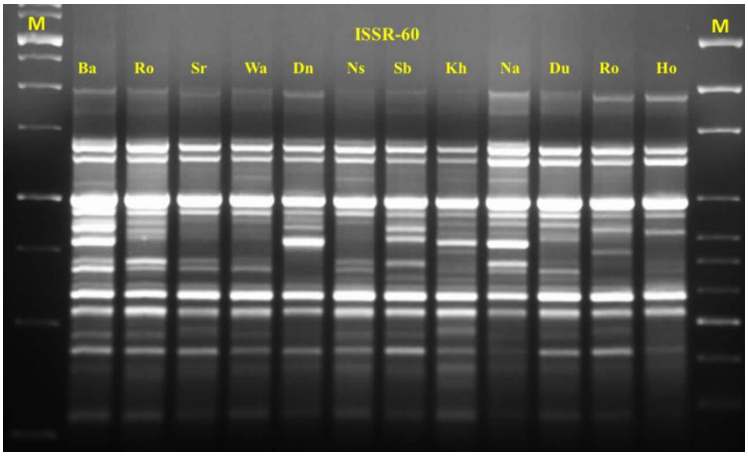

Fig 1. The ISSR fingerprint was generated with the ISSR-60 primer using the genomic DNA of *the Phoenix dactylifera* cultivars. M: DNA ladder 100 bp; Lane-Ba-Ho: 12 date palm cultivars.

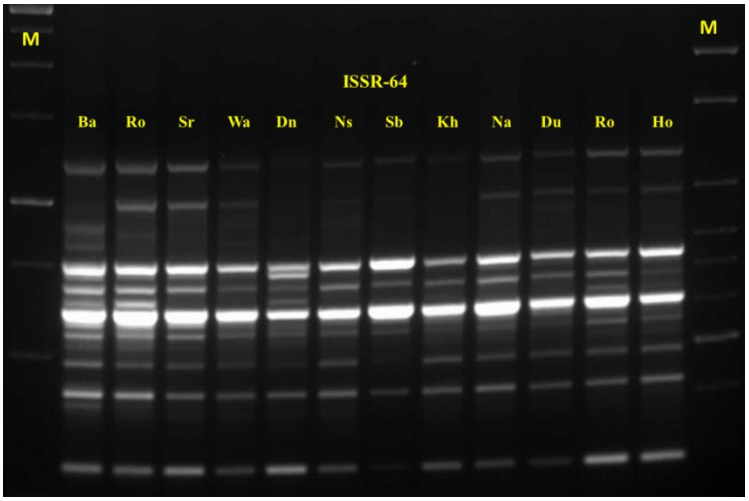

Fig 2. The ISSR fingerprint was created using the ISSR-64 primer and genomic DNA from the *Phoenix dactylifera* cultivars. M: DNA ladder 100 bp; Lane-Ba-Ho: 12 date palm cultivars).

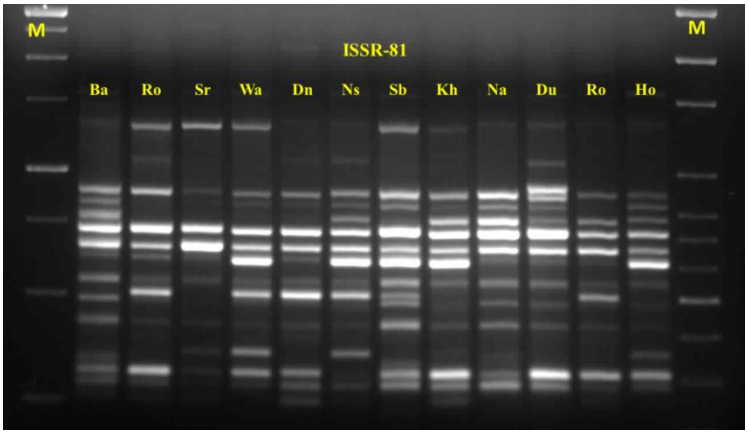

Fig 3. The ISSR fingerprint was created using the ISSR-81 primer and genomic DNA from the *Phoenix dactylifera* cultivars. M: DNA ladder 100 bp; Lane-Ba-Ho: 12 date palm cultivars.

The genetic similarity coefficients between the 12 *Phoenix dactylifera* cultivars ranged from 0.640 to 0.913 (Figure 4), indicating significant genetic heterogeneity. Naboot Seif and Wannana had the highest similarity (0.913), indicating a close genetic kinship.

In contrast, Hoshishi has the least similarity to Sabaka and Ruthana (0.640), indicating significant genetic diversity.

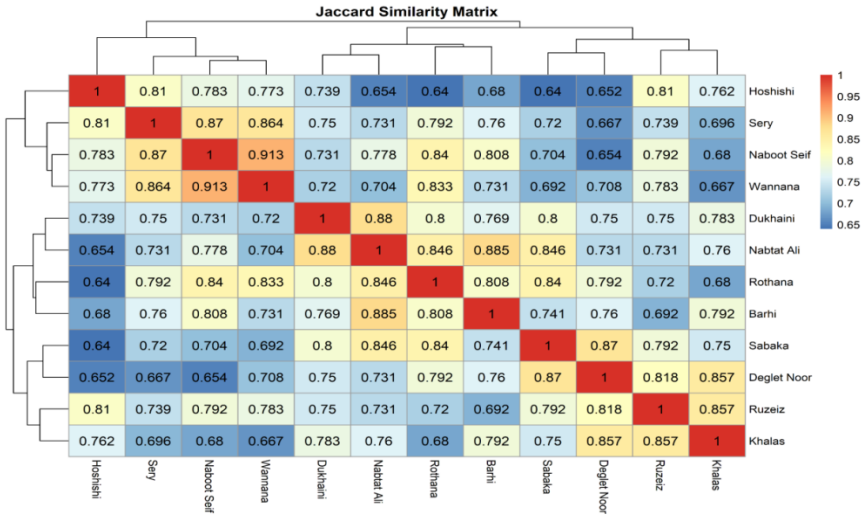

Fig 4. Clustered heatmap employing Jaccard's coefficient and UPGMA analysis, depicting the genetic relationships among the 12 date palm cultivars.

Figure 4 shows that the UPGMA dendrogram divided the cultivars into three primary clusters. The first cluster comprised Hoshishi, Naboot Seif, Wannana, and Sery, all of whom shared a high level of internal resemblance. The second cluster included Ruzeiz, Khalas, Sabaka, and Deglet Noor. The third cluster included Dukhaini, Nabtat Ali, Barhi, and Ruthana, all of whom were genetically connected to varying degrees.

The three clusters revealed by the UPGMA dendrogram in this study support previous research that used cluster analysis to visualize genetic relationships, demonstrating its efficacy in germplasm management and guiding breeding strategies (Ahmed et al., 2011; Al-Faifi et al., 2016). These findings indicate the use of ISSR markers in describing genetic variation among date palm cultivars, thereby aiding conservation efforts and selection procedures in breeding programs.

Similarly, Hoshishi's low resemblance to Sabaka and Ruthana (0.640) demonstrates genetic heterogeneity among the examined germplasm, which is consistent with Zehdi et al. (2004) and Elshibli & Korpelainen (2008).

This finding is congruent with that of Al-Faifi et al. (2016), who discovered genetic affinity between specific Saudi cultivars, most likely due to common ancestry or vegetative propagation.

In conclusion, this study confirms the efficacy of ISSR markers in detecting polymorphism and resolving genetic links among date palm cultivars. The observed diversity provides a solid platform for conservation planning and cultivar selection in breeding efforts.

References:

Abdulla M, Gamal O (2010) Investigation on molecular phylogeny of some date (*Phoenix dactylifra* L.) cultivars by protein, RAPD and ISSR markers in Saudi Arabia. J Plant Sci. 2010; 57: 69-80. *Australian Journal of Crop Science* 4(1): 23-28.

Aboul-Maaty, N.A.F, Oraby, H.A.S., 2019. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bulletin of the National Research Center 43(1): 1-10.

Ahmed, T. A., & Al-Qaradawi, A. Y. (2010). Genetic diversity of date palm genotypes in Qatar as determined by SSR and ISSR Markers. 279-286: *International Society for Horticultural Science (ISHS)*, Leuven, Belgium.

Al-Mssallem, M. Q.; Al-Khayri, J. M.; Alghamdi, B. A.; Alotaibi, N. M.; Alotaibi, M. O.; Al-Qthanin, R. N.; Al-Shalan, H. Z. (2024). The role of date palm in food and nutritional security in Saudi Arabia In J. M. Al-Khayri, M. Q. Al-Mssallem, & A. M. Al-Babili (Eds.), *Food and Nutrition Security in the Kingdom of Saudi Arabia* (Vol. 2, pp. 337–358).

Atia, M. A. M., Sakr, M. M., & Adawy, S. S. (2017). Assessing date palm genetic diversity using different molecular markers. In *Date Palm Biotechnology Protocols Volume II: Germplasm Conservation and Molecular Breeding*, 125-142 (Eds J. M. Al-Khayri, S. M. Jain and D. V. Johnson). New York, NY: Springer New York.

Elmeer, K., & Mattat, I. (2015). Genetic diversity of Qatari date palm using SSR markers *Genetics and Molecular Research* 14(1): 1624-1635.

Idrees, M., & Irshad, M. (2014). Molecular markers in plants for analysis of genetic diversity: a review. Plant Biol. *European academic research* 2(1): 1513-1540.

Kareem, M. A. H., Al-Saadi, A. H., & Naji, H. F. (2018). Genetic diversity of Iraqi Date Palm (Phoenix dactylifera L.) by using RAPD technique. *Journal of the University of Babylon for Pure and Applied Sciences* 26(1): 114-131.

Rahman, H., Vikram, P., Hammami, Z., & Singh, R. K. (2022). Recent advances in date palm genomics: A comprehensive review. Frontiers in Genetics 13:959266.

Sabir, J. S. M., Abo-Aba, S., Bafeel, S., Zari, T. A., Edris, S., Shokry, A. M., Atef, A., Gadalla, N. O. Ramadan, A. M., Al-Kordy, M. A., El-Domyati, F. M., Jansen, R. K., & Bahieldin, A. (2014). Characterization of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers. *Comptes Rendus Biologies* 337(1): 6-18.

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

Toor, A. K., Sharma, R., Singh, G., & Mohapatra, T. (2005). Genetic variation in date palm (*Phoenix dactylifera* L.) cultivars assessed using RAPD markers *Indian Journal of Genetics and Plant Breeding* 65(04): 335-336.

Yaish, M. W., Sunkar, R., Zheng, Y., Ji, B., Al-Yahyai, R., and Farooq, S. A. (2015) A genome-wide identification of the miRNAome in response to salinity stress in date palm (*Phoenix dactylifera* L.). Frontiers in Plant Science 6: 946.

Zango, O., Cherif, E., Chabrillange, N., Zehdi-Azouzi, S., Gros-Balthazard, M., Naqvi, S. A., Lemansour, A., Rey, H., Bakasso, Y., and Aberlenc, F. (2017) Genetic diversity of southeastern Nigerien date palms reveals a secondary structure within Western populations. Tree Genetics & Genomes 13(4): 75.

Zehdi, S., Trifi, M., Billotte, N., Marrakchi, M., & Pintaud, J. C. (2004). Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism *Hereditas*, *141*(2), 278–287.

Zhao, Y., Williams, R., Prakash, C. S., & He, G. (2012). Identification and characterization of gene-based SSR markers in date palm (*Phoenix dactylifera* L.). BMC Plant Biology 12(1): 237.

دراسة التنوع الوراثي لعدد من اصناف نخيل التمر (Phoenix dactylifera L.) في المملكة العربية السعودية باستخدام معلمات ISSR الجزيئية

عبدالحافظ عبدالله العامري[1]، عبدالوارث عبدالقادر المنيفي[2*] وعبدالرحمن حمزة[3]

المخلص:

يُعد نخيل التمر (*Phoenix dactylifera* L) محصولًا أساسيًا في البيئات الجافة وشبه الجافة، وخاصة في المملكة العربية السعودية، حيث يُمثل تحسينه الوراثي والحفاظ عليه أهمية إستراتيجية. تتناول هذه الدراسة تقدير التباين الوراثي لاثني عشر صنفًا جُمعت من ثلاث مناطق زراعية رئيسية هي: الأحساء، الخرج، والقصيم، وذلك باستخدام مؤشرات التكرارات البسيطة المتداخلة (ISSR) المعروفة بقدرتها العالية على كشف التعدد الشكلي و نتائجها عالية الموثوقية. ولقد أظهرت ثلاثة بادئات (ISSR-60، ISSR-64، ISSR-81) المستخدمة في هذه الدراسة نواتج PCR متميزة، مما يشير إلى وجود تباين وراثي واضح بين الأصناف المدروسة. كما أظهرت النتائج أن معاملات التشابه الوراثي تراوحت بين 0.640 و0.913، حيث سُجّل أعلى تقارب بين صنفي "نبوت سيف" و"ونانة"، وأدنى تقارب بين صنف "هوشيشي" وكل من "سباكا" و"روثانا". كما تم تحديد ثلاث مجموعات وراثية رئيسية باستخدام أسلوب المجموعات الزوجية غير الموزونة بالمتوسط الحسابي (UPGMA)، والتي عكست هذه كلاً من الأصول الجغرافية والروابط الوراثية الأساسية للاصناف المدروسة. تؤكد هذه النتائج على أهمية مؤشرات ISSR في الكشف عن التعددات الجينومية وتمييز البني الوراثية للاصناف المدروسة، مما يوفر رؤى واضحة لسبل إدارة

[1] قسم علوم الحياة، كلية التربية والعلوم برداع، جامعة البيضاء، محافظة البيضاء، الجمهورية اليمنية.

[2] قسم علوم الحياة، كلية التربية والعلوم برداع، جامعة البيضاء، محافظة البيضاء، الجمهورية اليمنية.

[*] مسؤول المراسلات على البريد الاليكتروني std2008@gmail.com

[3] قسم علم النبات والأحياء الدقيقة، كلية العلوم، جامعة الملك سعود، الرياض، المملكة العربية السعودية.