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## STUDY OF GENETIC DIVERSITY OF FOURTEEN IRAQI DATE PALM CULTIVER ECOTYPES USING ISSR MARKERS

Nahlah Hammody Hussein<sup>1</sup>

[Nahlah@Coagri.Uobaghdad](mailto:Nahlah@Coagri.Uobaghdad)

Iqbal Harbi Mohammed<sup>2</sup>

[iqbalharbi5@gmail.com](mailto:iqbalharbi5@gmail.com)

Muhanad Qassim khalaf<sup>3</sup>

[Admiraq15@gmail.com](mailto:Admiraq15@gmail.com)

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### ABSTRACT

This study investigated the genetic diversity of fourteen Iraqi date palm cultivars. Fresh leaf samples were collected from Stations belong Directorate of Horticulture - Ministry of Agriculture. Genomic DNA was isolated using a commercial DNeasy Plant System Kit.

A total of five Inter Simple Sequence Repeat (ISSR) single primers were evaluated for DNA amplification. Initial screening confirmed that all five primers successfully produced clear, amplifiable bands. Consequently, each primer was used individually to genotype the complete set of fourteen samples.

A total of 505 bands were generated across all samples, averaging 101 bands per primer. The number of amplified bands varied significantly among the cultivars. The highest number of bands was produced by primer DPISR5 (120 bands), followed by DPISR4 (114 bands), DPISR2 (108 bands), and DPISR1 (102 bands).

In contrast, primer DPISR3 yielded the lowest number, producing only 61 bands. Genetic markers were scored based on the presence or absence of corresponding bands across the cultivars.

Finally, a similarity matrix was constructed from the data and subjected to cluster analysis to establish genetic relationships.

**Keywords:** Date palm, Inter Simple Sequence Repeat, cluster analysis

### INTRODUCTION

*Phoenix dactylifera* L., commonly known as the date palm, is an iconic plant species that has played a vital role in the cultural, economic, and culinary heritage of various regions worldwide for centuries [18]. It belongs to the Arecaceae family

<sup>1</sup> College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

<sup>2</sup> Directorate of Horticulture, Ministry of Agriculture, Baghdad, Iraq.

<sup>3</sup> Office of the Advisor, Ministry of Agriculture, Baghdad, Iraq.

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(Angiosperms, Monocotyledons), which comprises approximately 200 genera and over 5,000 species distributed globally. The specific epithet *dactylifera* means "finger-bearing," referring to the shape of the fruit clusters. The term is a combination of the Greek word *dactylus* (finger) and the Latin word *ferous* (bearing) [5].

According to FAO [15] ecological and socioeconomic significance of the nutrient-dense date palm as a commercial crop and its indigenous production in many parts of the world (4.50) Date palm fruits possess notable nutritional and therapeutic properties, exhibiting potent antioxidant, antibacterial, antifungal, and anti-proliferative activities. However, their improvement is constrained by slow growth rates, dioecious reproductive nature, and the delayed expression of adult traits until full their maturity [1, 12, 28].

It is a dioecious and monocotyledon, characterized by having 18 pairs of diploid chromosomes in its genome ( $2n = 2x = 36$ ), ranking second economically among monocotyledons with an approximately estimated size of 670 Mb [2].

The identification of date palm genotypes depends mainly on morphological, biochemical, and molecular markers [14, 26]. The genetic studies explored the high diversity of the date palm genome, including stress resistance and sugar metabolism-related genes; hence, date palms have a considerable role in evolution. DNA-based molecular marker development has targeted genetic diversity, sex determination, cultivar identification, phylogenetic study, and the latest various innovative and potential marker techniques [6, 4]. The data from the genome sequencing holds great promise for developing new markers across various crop plant species [13]. The DNA markers derived from genetic variations within the DNA have significantly established new findings in different biological fields, including taxonomy, phylogenetic relationships, and genetics [11].

Date palm genetic diversity and molecular characterisation have been investigated using a variety of molecular markers. Simple sequence repeat (SSR) markers. Random amplified polymorphic DNA (RAPDs) chloroplast DNA sequences circular plasmid-like DNAs for molecular characterization, enzymatic polymorphism, amplified fragments length polymorphism (AFLP) [16], inter-simple sequence repeats (ISSR) [21, 34], and random amplified microsatellite polymorphism (RAMPO) [32] are among them. Moreover, ISSR molecular markers are reliable and inexpensive [19, 22].

Individuals can be identified by their unique DNA by genetic fingerprinting, also known as DNA typing. Numerous molecular markers are used to identify date palm cultivars, as well as to comprehend and examine the genetic diversity and interactions between date palm variants. Given the advancement of DNA-based methods, this review was carried out to highlight the significance of using the Inter Simple Sequence Repeats (ISSR), which is regarded as one of the practical molecular methods for assessing genetic diversity and relationships among various date palm cultivars and has a strong capacity to differentiate Of the cultivars s one of the most rapid and reliable methods, it provides high levels of DNA polymorphism and has produced notable outcomes in the assessment of genetic distance and proximity between date palm cultivars, the identification of novel cultivars and seed strains, and the study of genetic diversity. The ISSR technique was likewise effectively employed to distinguish DNA between male and female date palms, yielding reliable results for sex identification. ISSR is a useful tool for identifying molecular polymorphisms in palm, demonstrating its discriminatory power [8]. The assessment of genetic diversity and the susceptibility

of palm plants to genetic erosion are essential steps in date germplasm conservation. landraces of palms [10]. This study focused on the assessed genetic diversity among 14 of the most known Iraqi date palm cultivars using ISSR markers.

## MATERIALS AND METHODS

### Plant materials

There are fourteen distinct reference We gathered Iraqi date palm varieties from the Ministry of Agriculture's Al-Zaafarana Date Palm Station in Baghdad. Table 1, Total genomic DNA was extracted from young and healthy leaves according to the procedure mentioned by Benito *et al.* [19] with minor modifications. After purification, the resultant DNA was quantified using 1% agarose gel electrophoresis [9].

Table 1: Name and gender (all female) of 14 Iraqi date palm cultivars.

| N  | Name of Dates        |
|----|----------------------|
| 1  | Barhi                |
| 2  | Maktum               |
| 3  | Khadrawi             |
| 4  | Sukkari              |
| 5  | Kuntar               |
| 6  | Meer Haj             |
| 7  | Pink                 |
| 8  | Deari                |
| 9  | Fingers of the Bride |
| 10 | Prim                 |
| 11 | Shwethi Ahmer        |
| 12 | Barban               |
| 13 | Awena Ayoub          |
| 14 | Umm El Dahan         |

### PCR amplification and ISSR assay

The amplification reactions for each date palm sample were performed utilizing five distinct primer (Table 2). 25 µl was the reaction volume, and the following procedures were followed to carry out the reaction: the master reaction mixture made from Bioneer, DNA template at a concentration of 2 µl, and primer at a concentration of 1.5µl. Table 3 [25].

Table 2: List of ISSR primers used in this study

| No. | Name   | Sequence              |
|-----|--------|-----------------------|
| 1   | DPISR1 | AGGAGGAGGAGGAGGAGG    |
| 2   | DPISR2 | AGAGAGAGAGAGAGAGAGAGG |
| 3   | DPISR3 | AGAGAGAGAGAGAGAGAGAGC |
| 4   | DPISR4 | AGAGAGAGAGAGAGAGAGAGT |
| 5   | DPISR5 | CTCTCTCTCTCTCTCTCTA   |

**Table 3: program used in ISSR markers**

|                           |                                                       |              |
|---------------------------|-------------------------------------------------------|--------------|
| Initial denaturation      | Temp.: 94°C                                           | Time: 5 min  |
| No. of cycles = 40 cycles |                                                       |              |
| Denaturation              | Temp.: 94°C                                           | Time: 1 min  |
| Annealing                 | Temp.: 50-56°C<br>depending on GC% for<br>each primer | Time: 1 min  |
| Extension                 | Temp.: 72°C                                           | Time: 2 min  |
| Final extension           | Temp.: 72°C                                           | Time: 10 min |

### Data analysis

The Euclidean distances matrix was produced following the determination of the genetic similarity values (Similarity index) between these varieties using the Jaccard Biodiversity Handbook, genetic diversity, and the Polymorphsim Information Content (PIC) genetic diversity index for phenotypic indicators using the Power Marker 3.7 program [20].

Utilizing the unweighted pair group method with arithmetic average (UPGMA), a dendrogram of the taxa under investigation based on the Euclidean dimensions between these individuals is created, [25, 33] to illustrate how the taxa are distributed into major and secondary groups or clusters based on the distance between them. The data were examined using the biostatistical tool PAST version 1.62 in a family tree diagram in accordance with the previously created characterisation tables [30, 17].

## RESULTS AND DISCUSSION

All samples had DNA extracted with concentrations ranging from 75 to 98 ng/μl and purity levels between 1.8 and 1.94. By following the usual methodology used in many research and genetic investigations, this concentration and purity of DNA solutions were suggested as appropriate for additional genetic analysis [30]. The current study satisfies one of the ISSR technique's most crucial requirements, which is the detection of high levels of polymorphism (Figure 1).

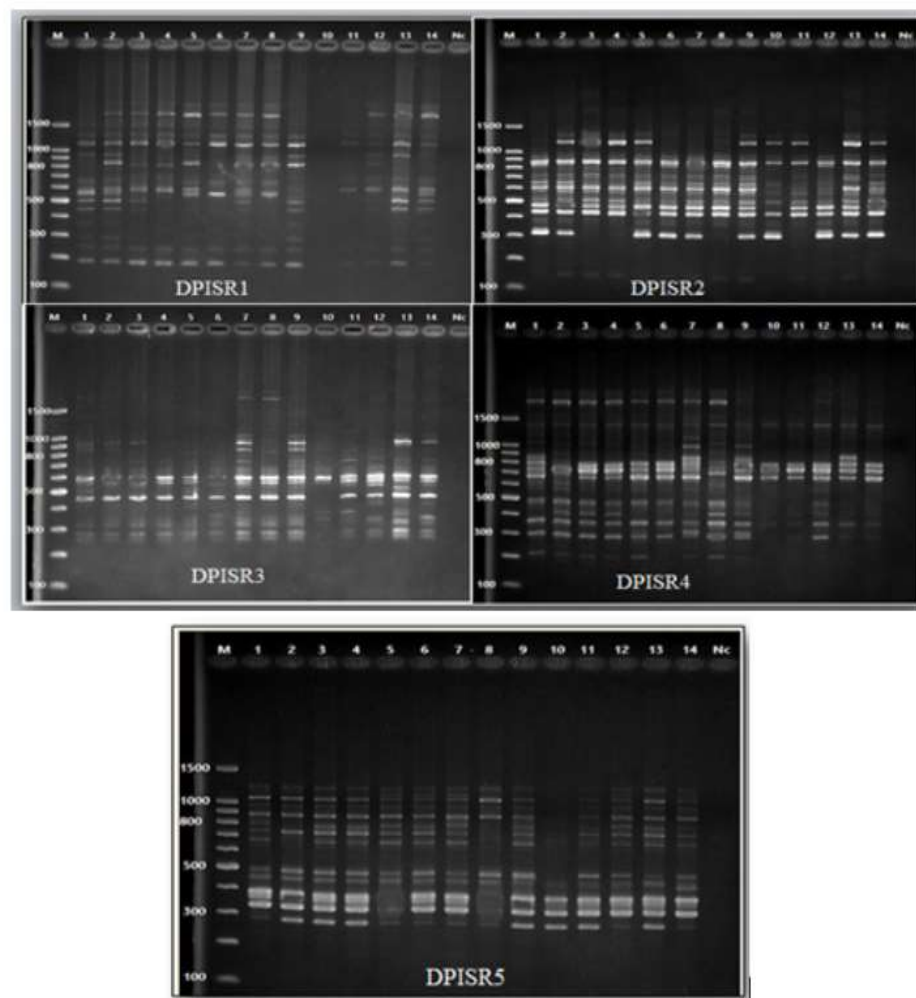


Figure 1: ISSR profiles of the 14 date palm genotypes amplified with Fife ISSR primers, M: molecular weight marker

The genomic DNA of the 14 samples was utilized to amplify DNA fragments utilizing a total of 5 ISSR single primers. Using date palm genomic DNA, the first screening was conducted to see if these primers could amplify distinct bands. In the initial screening, each of the five ISSR primers generated distinct bands. Following that, each primer was utilized independently to genotype the complete set of fourteen date palm samples. For the 14 Date palm cultivars, a total of 505 bands were produced using the five ISSR primers.

The third table 101 bands were produced by each primer on average. Cultivars differed in the amount of amplified bands. For the 14 cultivars, Primers 1, 4, 5, and 4 produced the most bands, whereas Primers 3 produced the fewest, with only 61 bands. The inter-specific variation across the cultivars under study was significantly greater than the variation within each individual cultivar as measured by the number of polymorphic fragments. The cultivars Pink and Khadrawi had the highest mean number of bands and both uncommon and common bands were seen in the cultivars under study.

Table 4: Monomorphic and polymorphic percentage for Date palm varieties using 5 ISSR.

| Marker | PIC      | No. of bands product | No. of Alleles | Mono morphic | %      | Polym orphic | %      |
|--------|----------|----------------------|----------------|--------------|--------|--------------|--------|
| DPISR1 | 0.698061 | 102                  | 14             | 0            | 0.00   | 102          | 100.00 |
| DPISR2 | 0.720058 | 108                  | 11             | 1            | 0.93   | 107          | 99.07  |
| DPISR3 | 0.379184 | 61                   | 12             | 0            | 0.00   | 61           | 100.00 |
| DPISR4 | 0.754011 | 114                  | 16             | 1            | 0.93   | 113          | 99.07  |
| DPISR5 | 0.790213 | 120                  | 11             | 0            | 0 0.00 | 120          | 100.00 |
|        |          | 505                  | 64             | 2            |        | 503          | 99.625 |

The difference in loci band productivity due to the different primer combinations' capacities to match with compatible sequences throughout the genome and detect population polymorphism is the reason behind the polymorphic bands found by loci DPISR2 and DPISR4 across all varieties. [29]. As shown in Table 3, these results showed a significant degree of polymorphism in the date palm germplasm, with an average of 99.62%. Based on this, we may deduce that local farming practices are most likely the cause of the genetic differences among date palms.

To measure informativeness of ISSR primers PIC was calculated. the Mean of PIC was 0.6683054 for five primers. The highest PIC was observed in DPISR5 (0.790213) represent highly differentiation marker and suitable for mapping, and the lowest was in DPISR3 (0.379184). will be classified as informative marker. The dendrogram derived from an UPGMA cluster analysis of the ISSR markers are explained in Fig. 1 The results have been revealed in the Figure 3. There are relationships with fourteen genotypes, divided into two main clusters, the first includes the Prim and Shwethi Ahmer varieties, and second cluster encompasses the remaining varieties, with a similarity level of 40% observed between the two primary clusters. Furthermore, the second cluster is subdivided into additional subgroups., the first includes the Barhi cultivar, and the second the rest of the cultivars, which in turn are divided into several secondary groups, and the two closest classes in these groups are my class. Khadrawi and Sukkari the result depend on ISSR marker were consent with.

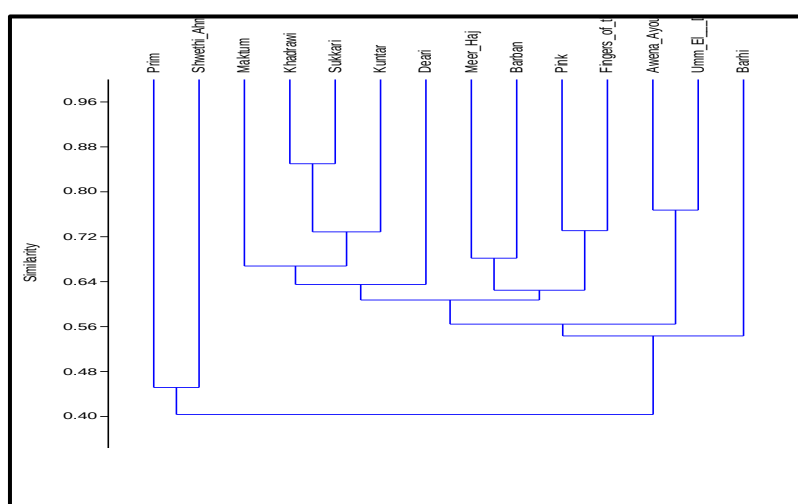


Figure 2: UPGMA dendrogram of Genetic relationships among fourteen date palm cultivars estimated by ISSR primers. With Jaccard's similarity index.

Table 5: genetic distance among date palm cultivars

|                      | Barhi | Maktum | Khadrawi | Sukkari | Kuntar | Meer Haj | Pink  | Deari | Fingers of the Bride | Prim  | Shwethi Ahmer | Barban | Awena Ayoub | Umm El Dahan |
|----------------------|-------|--------|----------|---------|--------|----------|-------|-------|----------------------|-------|---------------|--------|-------------|--------------|
| Barhi                | 0.000 |        |          |         |        |          |       |       |                      |       |               |        |             |              |
| Maktum               | 0.373 | 0.000  |          |         |        |          |       |       |                      |       |               |        |             |              |
| Khadrawi             | 0.442 | 0.296  | 0.000    |         |        |          |       |       |                      |       |               |        |             |              |
| Sukkari              | 0.500 | 0.327  | 0.150    | 0.000   |        |          |       |       |                      |       |               |        |             |              |
| Kuntar               | 0.479 | 0.375  | 0.334    | 0.210   | 0.000  |          |       |       |                      |       |               |        |             |              |
| Meer Haj             | 0.429 | 0.459  | 0.432    | 0.341   | 0.280  | 0.000    |       |       |                      |       |               |        |             |              |
| Pink                 | 0.440 | 0.437  | 0.392    | 0.366   | 0.347  | 0.292    | 0.000 |       |                      |       |               |        |             |              |
| Deari                | 0.500 | 0.396  | 0.420    | 0.312   | 0.362  | 0.414    | 0.366 | 0.000 |                      |       |               |        |             |              |
| Fingers of the Bride | 0.490 | 0.453  | 0.659    | 0.380   | 0.393  | 0.471    | 0.270 | 0.412 | 0.000                |       |               |        |             |              |
| Prim                 | 0.685 | 0.652  | 0.524    | 0.610   | 0.652  | 0.650    | 0.680 | 0.675 | 0.623                | 0.000 |               |        |             |              |
| Shwethi Ahmer        | 0.610 | 0.646  | 0.438    | 0.472   | 0.587  | 0.645    | 0.569 | 0.578 | 0.500                | 0.544 | 0.000         |        |             |              |
| Barban               | 0.479 | 0.471  | 0.383    | 0.327   | 0.305  | 0.319    | 0.347 | 0.396 | 0.393                | 0.620 | 0.489         | 0.000  |             |              |
| Awena Ayoub          | 0.499 | 0.481  | 0.435    | 0.375   | 0.345  | 0.470    | 0.474 | 0.471 | 0.491                | 0.660 | 0.534         | 0.451  | 0.000       | 0.233        |
| Umm El Dahan         | 0.405 | 0.405  | 0.435    | 0.392   | 0.405  | 0.423    | 0.463 | 0.426 | 0.481                | 0.645 | 0.450         | 0.370  | 0.233       | 0.000        |

Genetic distance among date palm species: Table 4 data of SSR markers scanned from 14 species with reproducible primers were used to genetic distance and similarity value coefficient. A maximum genetic distance value was observed between Prim and Barhi with genetic similarity reached 32%. low genetic distance with high genetic similarity reached 85%, observed between Khadrawi and Sukkari.

Fruit from the date palm (*Phoenix dactylifera* L.) grows well in tropical and subtropical environments that are dry or semi-dry. The progress of any genetic preservation is determined by an understanding of the quantity and distribution of genetic variation in the genetic pool [23, 24]. Both the expansion of the genetic base for date palm breeding and the conservation of genetic resources depend on assessing the genetic diversity and population structure of date palm germplasm. DNA in plants

ISSR method takes advantage of the ubiquitously distributed SSRs in the eukaryotic genomes. Because of those abundant and rapidly evolving SSR regions, ISSR amplification has the potential of revealing larger numbers of polymorphic fragments per primer than any other marker system used such. As the PCR reaction amplify the sequence between two SSRs, the PCR products generated reveal multilocus profiles which could be revealed on agarose or polyacrylamide gels [27].

ISSR markers revealed genetic variability within 14 date palms of Iraq. These findings can be used in future breeding program of date palm in the country.

### CONCLUSION

The molecular characteristics and phylogenetic relationships of Iraqi date palm cultivars have been evaluated in this study using ISSR markers. Our findings demonstrate the genetic variety among the Iraqi date cultivars under study as well as the capacity of ISSR markers to identify genetic variation in date palm. Despite the agronomic differences across date-palm ecotypes, we can deduce that they are all connected. Iraqi date palm cultivars could potentially be reclassified based on genetic similarity analyses and dendrogram clustering, with two cultivars (Prim and Shwethi Ahmer) being removed from the group because of their differences from the others. Since Khadrawi and Sukkari were closed cultivars, they may be regarded as having a common ancestor. A few cultivars were arranged in pairs that were similar to one another. In conclusion, the date palm genotypes can be identified using ISSR markers because of their high reproducibility and high discrimination efficiency. We were able to quantify the levels of genetic diversity in the population of date palms collected from various regions using the ISSR marker in this study.

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## دراسة التنوع الوراثي لأصناف نخيل التمر العراقية باستخدام

### تقنية التكرارات الترادفية الداخلية البسيطة (ISSR)

اقبال حربي محمد<sup>2</sup>

[iqbalharbi5@gmail.com](mailto:iqbalharbi5@gmail.com)

نهلة حمودي حسين<sup>1</sup>

[Nahlah@Coagri.Uobahdad](mailto:Nahlah@Coagri.Uobahdad)

مهند قاسم خلف<sup>3</sup>

[Admiraq15@gmail.com](mailto:Admiraq15@gmail.com)

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### الملخص

دراسة التنوع الوراثي لأصناف نخيل التمر العراقية، تم جمع أربعة عشر عينة من نخيل التمر من محطات دائرة لبستنة - وزارة الزراعة. استخلص الحامض النووي من الأوراق الطازجة باستخدام كت استخلاص تجاري. تم استخدام 5 بادئات فردية من نوع التكرارات الترادفية الداخلية البسيطة (ISSR) لتضخيم الحمض النووي باستخدام الحمض النووي الجينومي للعينات الـ 14. تم تقييم قدرة البادئات على تضخيم من خلال الفحص الأولي باستخدام الحمض النووي الجينومي لنخيل التمر. أنتجت جميع بادئات ISSR الخمسة باندات واضحة بنجاح في الفحص الأول. بعد ذلك، تم استخدام كل بادئ على حدة لتحديد النمط الجيني للمجموعة الكاملة المكونة من 14 عينة من نخيل التمر. تم إنشاء ما مجموعه 505 حزمة لعينات نخيل التمر الـ 14. في المتوسط، أنتج كل بادئ 101 حزمة. يختلف عدد الحزم المضخمة من صنف إلى آخر. تم الحصول على أعلى عدد من الحزم باستخدام البادئات DPISR5 (120 حزمة)، والبادئات DPISR4 (114 حزمة)، بينما أنتجت البادئات DPISR2 و DPISR1 حزمة 108، 102 على التوالي. تم الحصول على أقل عدد من الحزم باستخدام البادئات DPISR3 حيث أنتجت 61 حزمة فقط. تم تسجيل العلامات لوجود وغياب الحزمة المقابل بين الأصناف المختلفة. خضعت البيانات لتحليل عنقودي. تم إنشاء مصفوفة تشابه واستخدمت قيم التشابه لتحليل العنقود.

الكلمات الدالة: نخيل التمر، تقنية التكرارات الترادفية الداخلية البسيطة (ISSR)، التنوع الجيني

<sup>1</sup> جامعة بغداد، كلية العلوم الهندسة الزراعية، بغداد، العراق.

<sup>2</sup> وزارة الزراعة، دائرة البستنة، بغداد، العراق.

<sup>3</sup> وزارة الزراعة، مكتب المستشار، بغداد، العراق.

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