# DNA fingerprinting of some Iraqi date palm (*Phoenix dactylifera* L.) cultivars

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## ABSTRACT

**Date palm is the most important fruit tree in Iraq. DNA markers are a powerful tool to provide information on the relatedness of various clones or varieties that are difficult to distinguish morphologically. Five Iraqi date palm cultivars (Maktoum, Khidrawi Mandli, Osta Emran, Teberzal and Breem) were assessed using 23 RAPD markers. The results revealed that % of polymorphism ranged from 0 (primer OPC4,OPC14 and OPR7) to 85.7% (primer OPA5 ). One unique single band was shown in Breem variety obtained from primer OPE13, OPH7 and OPS12 in 800, 320 and 750 bp respectively, in Khidrawi Mandli variety from primer OPA8 and OPA11 at 320 and 500 respectively, in Teberzal variety from primer OPA17and OPH9 at 250 and 1100 bp respectively and in Osta Emran variety from primer OPA20 at 250 and 550bp, from primer OPC15 at 320 bp from primer OPF2 at 700 bp and from primer OPF8 at 400 and 580bp. Our results provide evidence of ability of RAPD markers to detect a genetic diversity among the tested date cultivars and this methodology can be extended to other cultivars.**

## INTRODUCTION

A variety of morphological characters of date fruits like shape, size, weight, color, texture, *etc*., have earlier been employed for the identification of date fruits, however discrimination among closely related cultivars by using fruit morphology traits are often unreliable and extremely difficult because of the influence of environmental conditions (Elhoumaizi *et al.,* 2002) and can be observed only in mature trees. Although biochemical studies including protein markers, isozyme analyses and activity analyses have been used to characterize date palms (Baaziz and Saaidi, 1988; Baaziz, 1988; Bendiab *et al.,* 1998), protein markers have been largely replaced by DNA-based approaches, mainly due to the fact that protein markers are limited in number and are dependence of their expression on environmental conditions (influenced by different environments as well as the developmental stage of the plant), and often-limited amount of detectable polymorphism (Winter and Kahl, 1995; Kunert *et al.,* 2001).

DNA markers are a powerful tool to provide information on the relatedness of various clones or varieties that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs. Several marker systems have been used widely and efficiently to analyze the genetic diversity within and among date palm cultivars like randomly amplified polymorphic DNA, RAPD (Al-Khalifah *et al.,* 2012), Amplified fragment length polymorphic AFLP (Jubrail *et al.,* 2005), inter simple sequence repeats –ISSR ( Ahmed and Al-Qaradawi, 2010), microsatellites –SSR (Elshibli and Korpelainen, 2008) and restriction fragment length polymorphisms (RFLP) markers. Each marker type has specific advantages and disadvantages and their applications vary depending on the nature and objective of the investigation and the properties of the species. For genetic diversity studies, the RAPD technique shows some important advantages in date palm such as easier and faster way and simplest test technically (Abdulla and Gamal, 2010). Hence, several studies implied RAPD for the molecular characterization of date palm of Egypt (Soliman *et al.,* 2003 and Eissa *et al* .,2009, Sakr *et al.,* 2012), Iraq (Ali *et al.,* 2007), Syria (Haider

*et al.,* 2012), Tunisia (Trifi *et al.,* 2000), Moroco (Sedra *et al.,* 1998), Saudi Arabia (Abdulla and Gamal 2010).

There are up to 5,000 date palm cultivars all around the world (Jaradat and Zaid 2004). Based on botanical descriptions, there are more than 600 cultivars in Iraq and the most important commercial varieties which representing 85% of the number of palm trees are Zahdi, Sayer, Helawi and Khidrawi and the rest (15%) including the most important and rare are Barhi, Breem, khistawi, Maktoum, Ashrasi, Al Cabcab, Deri, Teberzal, Hasawi, Ashger and Um Al Dehin.

Despite the large number of Iraqi varieties, little research has been undertaken on Iraqi date palm varieties depends on molecular markers. Among the marker system tested, employ PCR-RAPD markers for the early detection of genetic variations in *in vitro* culture-derived plants for Maktoum and Barhi varieties (Ali *et al.,* 2007; Bader *et al.,* 2007) and AFLP and SSR markers (Jubrail *et al.,* 2005 and Hamwieh *et al.,* 2010) for testing the genetic relatedness, this shows the lack of research on the DNA fingerprint of the Iraqi varieties. The objective of this study, therefore, was to assess PCR-RAPD markers which could be used in cultivar identification

# MATERIAL AND METHODS

Date palm samples were collected from the Date Palm Experimental Station at Al Zufaranya, Ministry of Agriculture, Iraq during 2013 season. The samples are five female cultivars (Maktoum, Khidrawi Mandli, Osta Emran, Teberzal and Breem). The experiment was conducted in Agricultural Research Directorate, Ministry of Science & Technology, Baghdad- Iraq. Leaf tissue was ground to a fine powder, then 600 µl of CTAB extraction buffer (2% CTAB, 0.7M NaCl, 0.1M Tris-HCl pH 8, 20 mM EDTA and 1% β-mercaptoethanol) were added, mixed well and incubated at 60°C in a water bath. After 30 min of incubation with gentle swirling, the resulting cell lyses were extracted with 400 µl of chloroform / isoamyl alcohol (24:1, v/v). The cell lysate was then centrifuged at 13000 rpm for 15 min. The aqueous phase was transferred into another tube and precipitation occurred with the addition of 600 µl of isopropanol. The precipitate was then collected by centrifugation at 13000 rpm for 15 min. Pellets were washed with 70% ethanol, dried and dissolved overnight at 4°C in 50 µl of TE buffer (10mM Tris – HCl pH 8.0, 1mM EDTA). DNA concentration was read with Nano-Drop spectrophotometer (Bio-Rad, USA). A total of 23 random decamer primers (OPERON Model) manufactured by Bioneer-Korea were used. PCR was performed using an AccuPower©PCR Premix (Bioneer, Korea), containing 250 µM of each deoxyribonucleoside triphosphate, 30 mM of KCl, 10 mM of Tris- HCl (pH 9.0), 1.5 mM of MgCl2, and 1 Unit of Top DNA polymerase. 100 ng of genomic DNA and 100 ng of RAPD primer were then add to a PCR Premix tube. Amplification was performed in Thermocycler (FlexCycler, Germany) using program for: I cycle at 94°C for 4 min, 40 cycles as follows: 94°C for 45 sec, 36°C for 1 min, 72°C for 2 min, the last cycle at 72 °C for 10 min. Amplification products were loaded on 1% agarose gels and stained with ethidium bromide (0.5 mg/ml). The DNA banding patterns were visualized on an UV transilluminator and documented by using Gel Documentation System, E-Graph (AE-9000, Japan). Fragment length was estimated by comparison with standard size markers (100 bp DNA Ladder Size range (bp): 100 - 2000, Bioneer-Korea ). Fragments (bands) were recorded numerically as (1) when present or (0) when absent. Fragments with the same mobility were considered as identical, irrespective of fragment intensity. Bands pattern data were analyzed using the SPSS 12 program to calculate similarity coefficient values according to Jaccard (1908).

# REDULT AND DISCUTION

DNA of five Iraqi date palm cultivars was isolated from the leaf and amplified by PCR using 23 random oligonucleotide primers. Amplification products were separated by agarose gel electrophoresis to reveal band polymorphism. The result showed (Table1) that all primers produced clear reproducible bands and yielded 793 bands. The number of bands from each primer varied from 17 to 69, the primer OPA8 produced 69 fragments whereas, primer OPE13 produced 17 bands, primers showed polymorphic lines that ranged from 0 (primer OPC4,OPC14 and OPR7) to 85.7% (primer OPA5 ). RAPD variation has also been reported in many studies. For example, Haider *et al*. (2012) reported that % of polymorphism for syrian date palm varieties ranged from 0 to 92% while Saker *et al*. (2012) found that % of polymorphism of Egyptian date palm varieties ranged from 4.25 to 9.52.

In profiles generated, the sizes of the fragments ranged from 100 to 2000 bp. On the other hand, one unique single band was shown in Breem variety obtained from primer OPE13, OPH7 and OPS12 (Fig.1) in 800, 320 and 750 bp respectively, in Khidrawi Mandli variety obtained from primer OPA8 and OPA11 (Fig. 2) at 320 and 500 respectively, in Teberzal variety obtained from primer OPA17(Fig.3) and OPH9 at 250 and 1100 bp respectively and in Osta Emran variety from primer OPA20 at 250 and 550bp, from primer OPC15 at 320 bp (Fig.3), from primer OPF2 at 700 bp and from primer OPF8 at 400 and 580bp. Unique single bands thus can be used for the DNA fingerprinting, this confirms findings of Al-Khalifah and Askari (2003).

Band pattern data analyzed using the SPSS 12 program to calculate similarity coefficient values according to Jaccard (1908). A similarity matrix between Iraqi date palm cultivars showed an average similarity coefficient range from 0.631 to 0.785 (Table2). The highest similarity coefficient value was observed between Breem and Kadrawi Mandily which seem to be the nearest two varieties and can be closely

regrouped. The similarity matrices were used in the cluster analyses which were employed to generate dendrograms. The dendrogram shown in Figure 4, illustrates the divergence between the studied Iraqi date palm cultivars and suggests their tree branching which provide evidence of divergence among all tested genotypes since they were grouped in clusters. This confirms findings of many studies (Al-Khalifah *et al.,* 2012; Haider *et al.,* 2012; Sakr *et al.,* 2012, Sedra *et al.,* 1998), which found that RAPD markers are a powerful tool to provide information on the relatedness of various date palm varieties that are difficult to distinguish morphologically, therefore molecular marker might be the easier criteria to distinguished date palm cultivars

# CONCLUSION

RAPD markers have been used to assess the molecular characterization and relationships of Iraqi date palm cultivars. Our results provide evidence of ability of RAPD markers to detect a genetic diversity among the tested date cultivars. The methodology followed in this study also can be extended to other cultivars

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## Tables

**Table1:** RAPD-PCR amplification products of five date palm cultivars using 23random primers

| Primer | Total number of band | Number of polymorphic band | Polymorphism (%) |
|---|---|---|---|
| OPA5 | 35 | 30 | 85.7 |
| OPA8 | 69 | 22 | 31.9 |
| OPA11 | 31 | 11 | 35.5 |
| OPA15 | 27 | 2 | 7.4 |
| OPA17 | 67 | 17 | 25.4 |
| OPA20 | 47 | 27 | 57.4 |
| OPB5 | 28 | 8 | 28.6 |
| OPB10 | 21 | 9 | 42.9 |
| OPC4 | 45 | 0 | 0.0 |
| OPC15 | 55 | 0 | 0.0 |
| OPD2 | 33 | 8 | 24.2 |
| OPE8 | 40 | 15 | 37.5 |
| OPE13 | 19 | 4 | 21.1 |
| OPF2 | 20 | 5 | 25.0 |
| OPF8 | 24 | 9 | 37.5 |
| OPF12 | 37 | 2 | 5.4 |
| OPH7 | 17 | 12 | 70.6 |
| OPH9 | 37 | 27 | 73.0 |
| OPH15 | 35 | 15 | 42.9 |
| OPO16 | 37 | 12 | 32.4 |
| OPR7 | 35 | 0 | 0.0 |

| Primer | Total number of band | Number of polymorphic band | Polymorphism (%) |
|---|---|---|---|
| OPS12 | 35 | 15 | 42.9 |
| OPZ11 | 23 | 3 | 13.0 |

**Table2:** Similarity matrix of 5 date palm varieties obtained from RAPD markers

| Varieties | Osta Emran | Teberzal | Maktom | Kadrawi Mandily |
|---|---|---|---|---|
| Breem | 0.674 | 0.698 | 0.784 | 0.785 |
| Kadrawi Mandily | 0.631 | 0.701 | 0.797 | |
| Maktom | 0.696 | 0.729 | | |
| Teberzal | 0.702 | | | |

## Figures

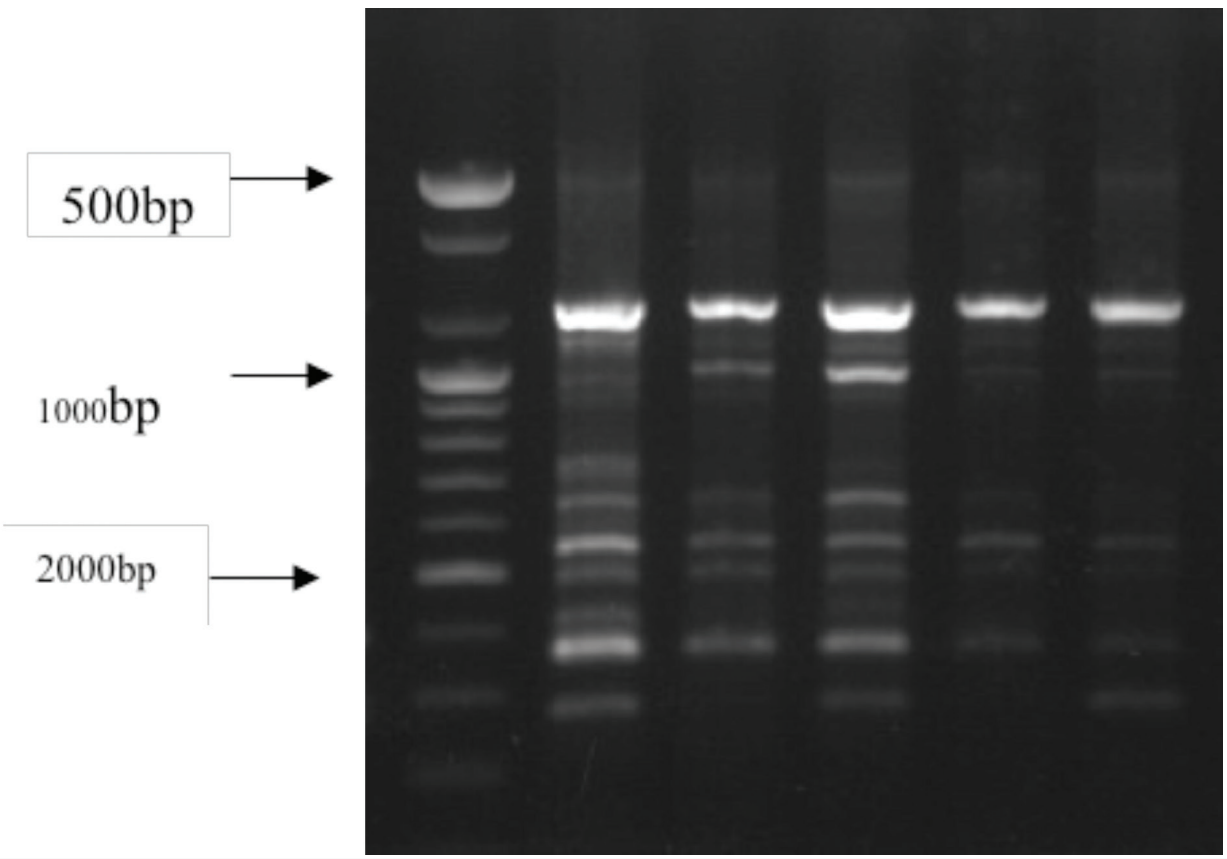


Figure 1. Agarose gel electrophoresis of RAPD fragments generated by primer OPS12 of different date palm female cultivars, Molecular marker (bp) (lane 1); Breem cv. (lane 2); Khidrawi Mandli cv. (lane 3); Maktoum cv. (lane 4); Teberzal cv. (lane 5); Osta Emran cv. (lane 6).

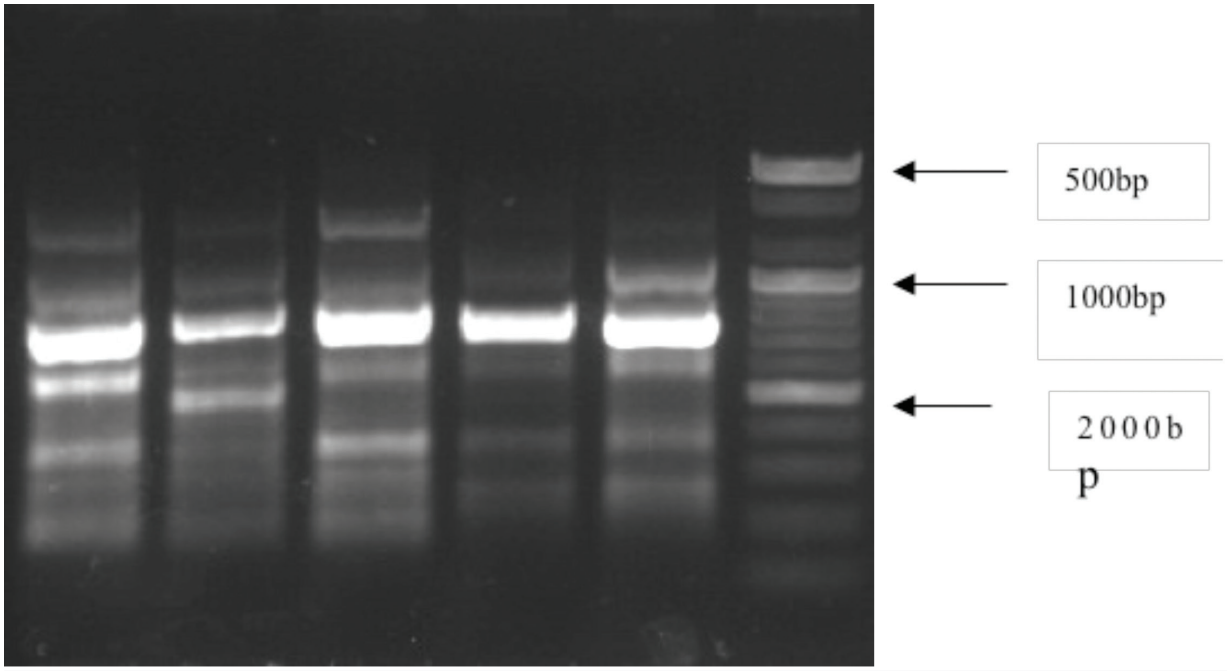


Figure 2. Agarose gel electrophoresis of RAPD fragments generated by primer OPA11 of different date palm female cultivars, Breem cv. (lane 1); Khidrawi Mandli cv. (lane 2); Maktoum cv. (lane 3); Teberzal cv. (lane 4); Osta Emran cv. (lane 5); Molecular marker (bp) (lane 6).

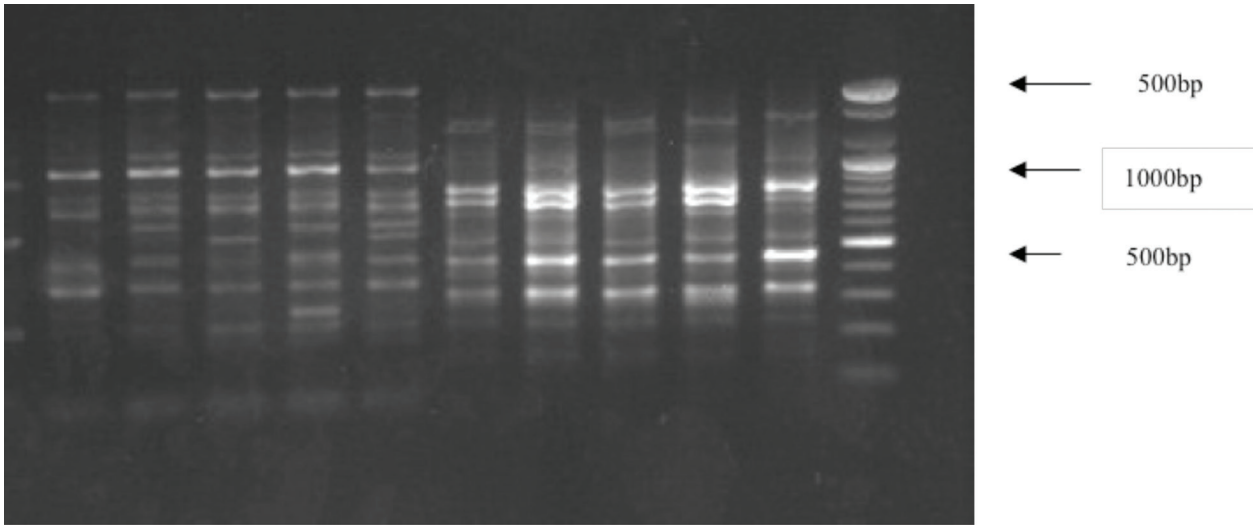


Figure 3. Agarose gel electrophoresis of RAPD fragments generated by primers OPA17 (lane 1-5) and OPC15 (lane 6-10 of different date palm female cultivars, Breem cv. (lane 1); Khidrawi Mandli cv. (lane 2); Maktoum cv. (lane 3); Teberzal cv. (lane 4); Osta Emran cv. (lane 5); Breem cv. (lane 6); Khidrawi Mandli cv. (lane 7); Maktoum cv. (lane 8); Teberzal cv. (lane 9); Osta Emran cv. (lane 10);Molecular marker (bp) (lane 11).

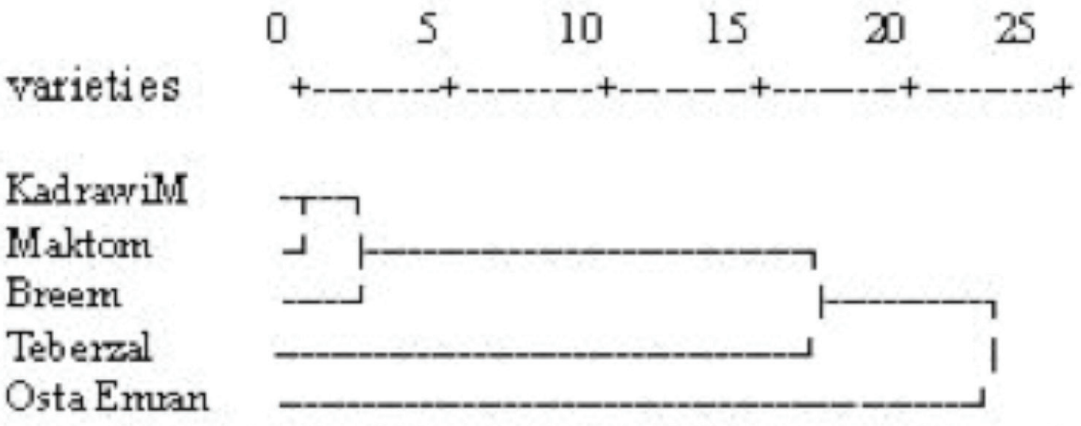


Figure 4: Dendrogram of 5 Iraq date palm varieties based on jaccard genetic similarity