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MOLECULAR PHYLOGENY OF SEVEN DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS BY DNA FINGERPRINTING

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Abstract

Random Amplified Polymorphic DNA (RAPD) was used to detect polymorphism among the 7 different cultivars of date palm (*Phoenix dactylifera* L.) from the Kingdom of Saudi Arabia. Total genomic DNA extracted from young sprouting leaves (white to pale yellow) were amplified using random sequenced 10-mer OPERON primers. Out of 140 primers initially screened, 42 detected polymorphism among the cultivars. A total of 213 bands were generated with an average of 5.6 RAPD markers per primer. Out of a total 213 amplified fragments, 132 (61.97%) were polymorphic. Cluster analysis by the unweighted paired group method of arithmetic mean (UPGMA) showed two clusters. Cluster 'A' consisted of 3 cultivars viz., Khalas, Barhy and Deglat Noor with a 0.76-0.81 Nei and Li's similarity range. Cluster 'B' consisted of 3 cultivars viz., Omal-Khashab, Hilwa and Shagra with a 0.79-0.83 Nei and Li's similarity range. Hilwa and Shagra are more closely related as compared to all the rest of the 7 cultivars. They have the highest value in the similarity matrix for Nei and Li's coefficient (0.83). Barhy and Deglat Noor are also closely related with a second highest value in the similarity matrix (0.81). The cultivar Hilali does not belong to any of the cluster groups and is 69% genetically similar to the rest of the 6 cultivars. The average similarity among the 7 cultivars was more than 50%. Our study indicated the possibility of using RAPD markers for the identification and for determining genetic diversity among the different cultivars of date palm.

Introduction

Date palm is a fruit tree mainly cultivated in arid regions in the Middle East, where it has been domesticated for at least 500 years and is believed to have originated in Mesopotamia. It was introduced as a cultivated tree crop in India, North Africa and Spain in the early history of mankind. The number of known date palm varieties that are distributed all over the world are approximately 5000 out of which about 450 are alone found in the Kingdom of Saudi Arabia (Bashah, 1996). Date palm is a dioecious plant and its obligate outbreeding habit makes its progeny strongly heterogeneous (Munier, 1981). True-to-type multiplication is mainly vegetative and has made it possible to clone individuals with economically important traits. Clonal propagation of elite cultivars with known performance is highly desired for the date palm industry, particularly in the Kingdom of Saudi Arabia. In Saudi Arabia traditionally this has been made possible by vegetative propagation from the offshoots of the mother trees. Over the years while so many varieties have been transplanted to areas other than the area of their origin, they may have adapted a different name in different plantation areas of the kingdom or two genetically different varieties may have the same name.

The morphological markers used to describe varieties are mainly those of fruit but are greatly affected by the environment and are also complex. Usually identification and evaluation of genetic diversity between the cultivars on the basis of morphological markers is difficult. Identification of trees is usually not possible until the onset of fruits which takes three to five years. Furthermore, to characterize the varieties require a large set of phenotypic data that are difficult to access statistically and are variable due to environmental effect (Sedra *et al.*, 1993, 1996). Biochemical markers (isozymes and proteins) have proved to be effective in varietal identification (Bendiab *et al.*, 1993; Fakir *et al.*, 1992; Bennaceur *et al.*, 1991). However, they give limited information and are an indirect approach for detecting genomic variation.

RAPD, RFLP and AFLP are some of the techniques of molecular biology for the DNA fingerprinting of genomes. DNA fingerprinting is a powerful tool, which can be used in the identification and determination of specific genomes or to estimate the phylogenetic relationship among the individual genomes. It can also be used as genetic markers to generate linkage maps to facilitate the identification of molecular markers linked to economically important traits. RAPD is a technique based on the Polymerase Chain Reaction (PCR) (Williams *et al.*, 1990; Welsh, *et al.*, 1990). The polymorphic DNA amplfied by using random 10-mer oligonucleotide primers (OPERON Model) can generate many useful genetic markers for the analysis of genetic diversity and to study the phylogenetic relationships.

The objectives of the present study were: (1) to screen and select primers for the generation of RAPD markers and (2) to analyze the genetic diversity among the 7 different cultivars of date palm using RAPDs.

Materials and Methods

Plant materials: The plant material consisted of 7 commercial varieties viz., Deglat Noor, Barhy, Hilali, Khalas, Omal Khashab, Shagra and Hilwa collected from Al-Hufuf date palm center of the kingdom of Saudi Arabia and selected for their fruit quality.

Total genomic DNA extraction: Total genomic DNA was extracted from the young sprouting leaves (white to yellow in color) of each variety. The leaves were first ground into a fine powder in liquid nitrogen by using pestle and mortar and then by following the steps of the protocol (Dellaporta *et al.*, 1983) for the extraction of DNA, pure and highly concentrated DNA was extracted. Using a UV Spectrophotometer at wavelength 260 and 280 nm, the quantity and quality of the DNA were determined. The stock DNA samples were diluted with distilled water to make working solution of 10 ng/ul DNA.

Polymerase chain reaction and primers: A total of 140 random 10-mer RAPD primers (OPERON Technologies Inc., CA, USA) of A to G-series were used for PCR amplification of the template. Amplification reactions were performed in volumes of 25 μl containing 1 unit of *Taq* polymerase (Perkin Elmer Cetus) per reaction.. The RAPD reaction were run in a Perkin Elmer/Cetus Thermal Cycler 480. The following PCR program was used: (i) 94°C for 3 min: 1 Cycle; (ii) 94°C for 30 sec., 36°C for 1 min., 72°C for 2 min: 45 Cycles; (iii) 72°C for 2 min: 1 Cycle followed by a soaking at 4°C. The RAPD products were separated by electrophoresis according to their molecular weight in 1.4 % w/w agarose gels submerged in 1XTBE buffer and then stained with ethidium bromide (10 μg/mL) solution for 20 minutes. The DNA were visualized on UV

transilluminator and documented by using gel documentation system (BioRad.). The lengths of the amplified RAPD fragments were estimated by running the 1 Kilobase DNA Marker in the gel as standard size marker.

Morphological identification: Date palm trees are mainly identified by their fruit characters like shape, size, color and or fruit quality). The morphological characteristics of the fruits were also used as a confirmatory tool for the DNA fingerprinting results.

Analysis of amplification profiles: Amplification profiles of the 7 different date palm samples were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0). The data of the selected 42 primers was applied to estimate the similarity on the basis of the number of shared amplification products (Nei & Li, 1979). A dendrogram based on similarity coefficient was also made with the help of unweighted pair group of arithmetic means (UPGMA).

Results and Discussion

The aim of the present study was to develop RAPD markers for the identification of date palm varieties of the Kingdom of Saudi Arabia. Since this was a pioneering work on date palm many protocols were tested for the extraction of the DNA. Best results were observed where the method of Dellaporta *et al.*, (1983) with some modifications was used. In this protocol proteinase K treatment was used to inactivate the tissue nucleases. The fresh young sprouting leaves with white to pale yellow color yielded good quality and quantity of DNA. Genomic DNA were colorless and could be readily spooled following ethanol precipitation while the DNA extracted from the old and fully opened leaves were not clear and greenish in color due to the presence of impurities which were difficult to remove. The average yield from 300 to 5000 mg of the leaves were 10 to 30 μg/ml DNA. The concentration and purity was estimated by using UV-Spectrophotometer at 260 and 280 nm and by agarose electrophoresis. A single, clear and unsmeared band for each variety on the gel indicated good quality and quantity of the DNA.

Clear amplified polymorphic DNA products were obtained from the 42 different random primers out of a total of 140 primers used for the screening and selection. All of the 7 genotypes revealed a unique profile with 42 primers and thus can be used for the DNA fingerprinting. Different primers produced a different level of polymorphism among the different cultivars (Fig. 2a, b, c). A total of 213 bands were generated, with an average of 5.6 RAPD markers per primer. Out of a total 213 amplified fragments, 132 (61.97%) were polymorphic. The number of polymorphic bands per primer varied between 2 to 6, with a mean of 3 major bands per primer (Table 1). Out of 140 primers of Series A to G, 42 were selected for further analysis based on the intensity, size and number of the RAPD bands. To ensure reproducibility and reliability of the RAPD markers PCR reactions were repeated twice with each primer. The primers that showed weak or no patterns were discarded.

The cultivars under study were easily identified when the morphology of the fruits was compared with the results of the RAPD experiments. The morphological markers (Fig. 1) were very helpful in confirming the cultivars through DNA fingerprinting (Fig. 2).

Table 1. RAPD primers (Operon, Model) with total number of amplified fragments and polymorphic bands.

#	Random Primers	Total Amplified Fragments	Polymorphic Fragments	#	Random Primers	Total Amplified Fragment	Polymorphic Fragments
1.	OPA01	6	5	22.	OPD08	8	4
2.	OPA08	6	4	23.	OPD11	6	1
3.	OPA16	9	5	24.	OPD12	5	3
4.	OPB01	10	3	25.	OPD15	8	2
5.	OPB02	6	1	26.	OPD20	9	4
6.	OPB08	6	4	27.	OPE01	6	1
7.	OPB11	5	3	28.	OPE02	7	3
8.	OPB13	10	5	29.	OPE11	7	2
9.	OPB14	8	6	30.	OPE16	6	3
10.	OPB15	11	3	31.	OPE18	8	3
11.	OPB16	8	6	32.	OPE19	7	3
12.	OPB19	8	4	33.	OPF04	10	4
13.	OPB20	10	4	34.	OPF05	4	1
14.	OPC01	7	2	35.	OPF06	8	3
15.	OPC02	9	3	36.	OPF07	7	3
16.	OPC04	11	4	37.	OPF09	7	4
17.	OPC05	8	3	38.	OPF10	6	3
18.	OPC16	5	2	39.	OPF12	9	4
19.	OPC18	7	2	40.	OPF16	5	2
20.	OPC19	10	1	41.	OPG02	8	3
21.	OPD05	9	4	42.	OPG04	4	2

Table 2. Similarity matrix for Nei and Li's coefficients of 7 date palm genotypes obtained from RAPD markers.

Nos.	Species name	1	2	3	4	5	6	7
1.	Deglat Noor	1						
2.	Barhy	0.811	1					
3.	Hilali	0.697	0.704	1				
4.	Khalas	0.75	0.779	0.666	1			
5.	Omal-Khashab	0.711	0.752	0.679	0.743	1		
6.	Shagra	0.730	0.758	0.712	0.726	0.809	1	
7.	Hilwa	0.730	0.778	0.742	0.76	0.756	0.833	1

RAPD appears to be effective for the identification of date palm varieties, although polymorphism is low in comparison with other cultivated species (Yang & Quiros, 1993; Koller *et al.*, 1993; Farooq *et al.*, 1994 a; Farooq *et al.*, 1994 b; Khan *et al.*, 2000). RAPD-markers should be of high value for date palm germplasm characterization and genetic maintenance. Low RAPD polymorphism and the lack of evident organization observed among the date palm varieties could be due to the nature of introduction of the varieties in the country and also due to maintenance of the germplasm in Saudi Arabia. The present RAPD data so far generated by screening the 140 different primers suggests narrow genetic diversity. Exchange of the varieties between the different plantation areas and development of new recombinants by seedling selection and sexual reproduction may have been the main reason for less diversity among them. Also the selection by the farmers may represent only a small fraction of the date palm germplasm.

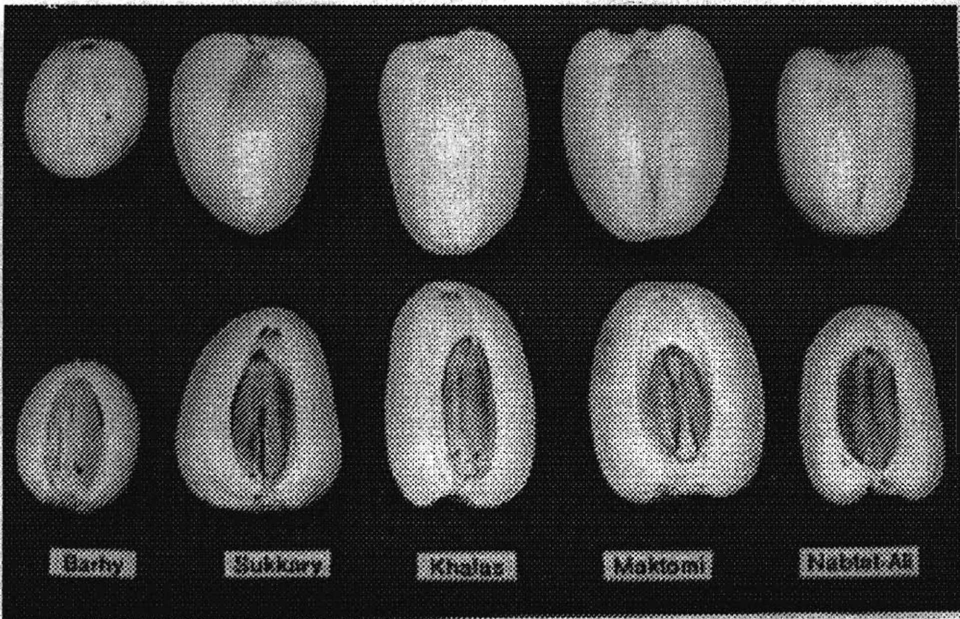

Fig. 1. a) Morphological characteristics of date palm fruits used for the identification of cultivars, **b)** Trees can be identified by their fruit

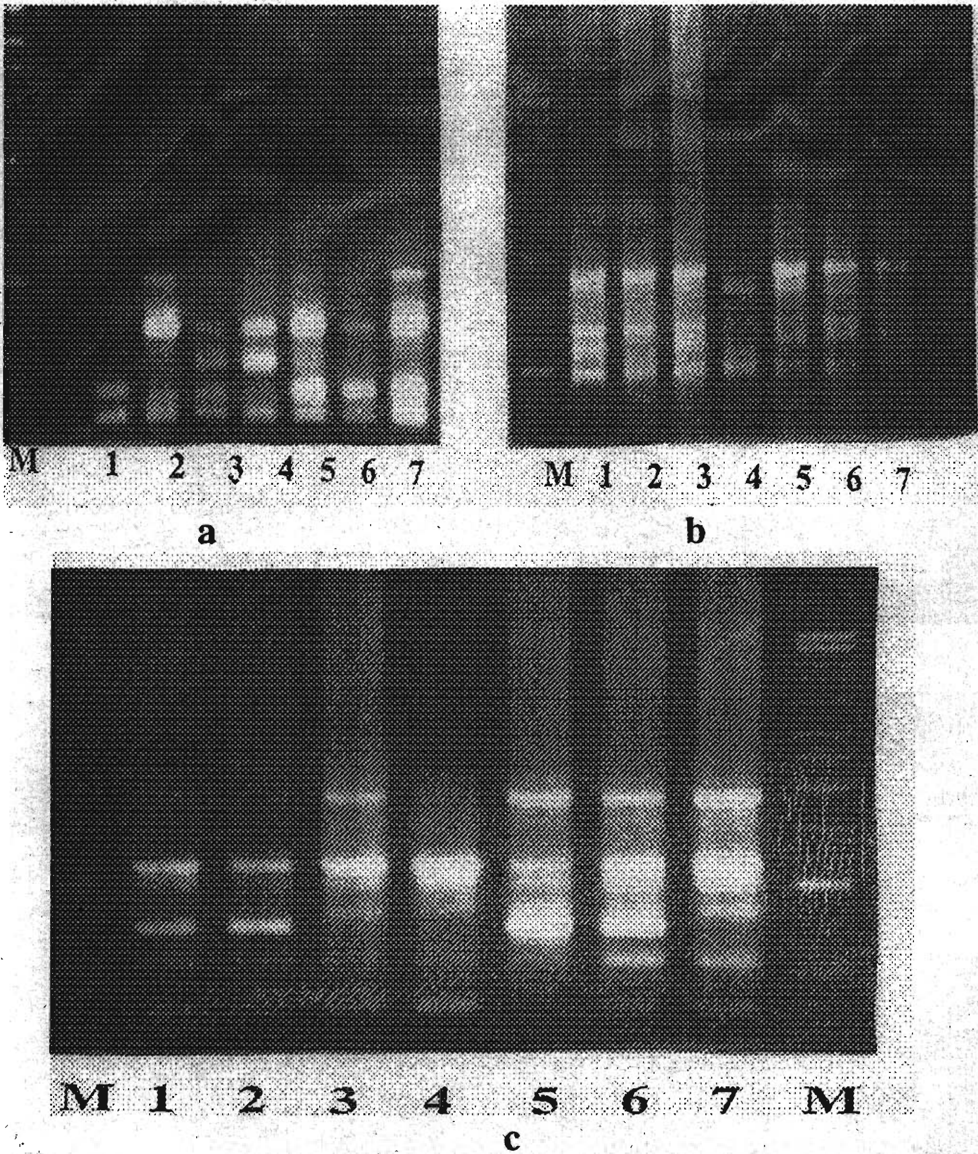

Fig. 2 a, b & c: RAPD profiles of date palm varieties using OPA 01, OPA08 and OPB16 primers respectively. Molecular weight marker (lane M), Deglet Noor (lane 1), Barhy (lane 2), Hilali (lane 3), Khalas (lane 4), Omal Khashab (lane 5), Shagra (lane 6) and Hilwa (lane 7).

The pair-wise genetic distance estimates of the 7 genotypes were analyzed and are given in Table 2. The similarity matrix is based on Nei and Li's similarity coefficient. The genetic distances (Nei and Li's similarity) ranged from 0.66 to 0.83. Maximum similarity was observed between Hilwa and Shagra (0.83). Hilali in general showed a minimum degree of similarity with all other cultivars ranging between 0.66 to 0.742.

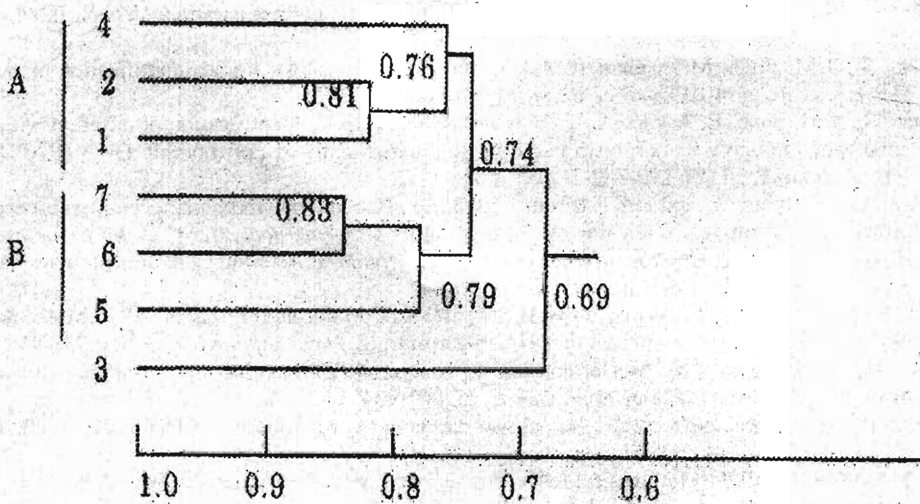

Fig. 3. A dendrogram of phylogenetic relationships among 7 cultivars of date palm based on the RAPD analysis using 37 primers.

Cluster analysis using UPGMA resulted in two cluster groups as shown in Fig. 3. In cluster 'B' out of three cultivars Hilwa and Shagra are more closely related as compared to all the rest of the 7 cultivars. They have the highest value in the similarity matrix for Nei and Li's coefficient (0.83). Omal-Khashab is 79% genomically similar to Hilwa and Shagra. In cluster 'A', out of three cultivars Barhy and Deglat Noor are more related genomically (0.81) while Khalas is 76% related to both Barhy and Deglat Noor. The cultivar Hilali does not belong to any of the cluster groups. It is 69% genetically similar to the rest of the 6 cultivars.

In the present communication the average genetic similarity of all the 7 cultivars of date palm was more than 50%. The results suggest that RAPD analysis could be used for an efficient identification and DNA fingerprinting of the date palm varieties grown in the Kingdom of Saudi Arabia and worldwide. This will help in collection and cataloguing of the germplasm in the form of a germplasm bank.

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