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Molecular phylogeny of date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting

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Abstract Genetic diversity among 13 different cultivars of date palm (*Phoenix dactylifera* L.) of Saudi Arabia was studied using random amplified polymorphic DNA (RAPD) markers. The screening of 140 RAPD primers allowed selection of 37 primers which revealed polymorphism, and the results were reproducible. All 13 genotypes were distinguishable by their unique banding patterns produced by 37 selected primers. Cluster analysis by the unweighted paired group method of arithmetic mean (UPGMA) showed two main clusters. Cluster A consisted of five cultivars (Shehel, Om-Kobar, Ajwa, Om-Hammam and Bareem) with 0.59–0.89 Nei and Li's coefficient in the similarity matrix. Cluster B consisted of seven cultivars (Rabeeha, Shishi, Nabtet Saif, Sugai, Sukkary Asfar, Sukkary Hamra and Nabtet Sultan) with a 0.66–0.85 Nei and Li's similarity range. Om-Hammam and Bareem were the two most closely related cultivars among the 13 cultivars with the highest value in the similarity matrix for Nei and Li's coefficient (0.89). Ajwa was closely related with Om-Hammam and Bareem with the second highest value in the similarity matrix (0.86). Sukkary Hamra and Nabtet Sultan were also closely related, with the third highest value in the similarity matrix (0.85). The cultivar Barny did not belong to any of the cluster groups. It was 34% genetically similar to the rest of the 12 cultivars. The average similarity among the 13 cultivars was more than 50%. As expected, most of the cultivars have a narrow genetic base. The results of the analysis can be used for the selection of possible parents to generate a mapping population. The variation detected among the closely related genotypes indicates the efficiency of RAPD markers over the morphological and isozyme markers for the identification and construction of genetic linkage maps.

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Introduction

Date palm (*Phoenix dactylifera* L., $2n = 36$) is a fruit tree mainly cultivated in arid regions in the Middle East, where it has been domesticated for at least 5,000 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 5,000, out of which about 450 are found only in Saudi Arabia (Bashah 1996). It is a dioecious, perennial, monocot plant, and its heterozygous form makes its progeny strongly heterogeneous (Munier 1981). True-to-type multiplication is mainly vegetative, which has made it possible to clone individuals with economically important traits. Clonal propagation of elite cultivars with known performance is highly desired in Saudi Arabia. In the Kingdom this has traditionally been made possible by vegetative propagation from the offshoots of the mother trees. The limitation to this propagation is the minimal production of offshoots, some of which will die when separated from the mother plants. Over the years many varieties have been transplanted to areas other than the area of their origin, and there may have been adapted with different names. As a result a variety may have different names in different plantation areas of the kingdom, or two genomically different varieties may have the same name (Torres and Tisserat 1980). This also reduces the genetic diversity of the cultivars, making them vulnerable to biotic and abiotic stresses.

The morphological markers used to describe varieties are mainly those of the fruit, but these are greatly affected by the environment and are also complex. In general, the identification and evaluation of genetic diversity between the cultivars on the basis of morphological markers is difficult. The identification of trees is usually not possible

until the onset of fruiting, which takes 3 to 5 years. Further, characterization of the varieties requires a large set of phenotypic data that are difficult to access statistically and are variable due to environmental effects (Sedra et al. 1993, 1996, 1998). Biochemical markers (isozymes and proteins) have proved to be effective in varietal identification (Bendiab et al. 1993; Bennaceur et al. 1991; Fakir et al. 1992). However, they give limited information and are an indirect approach for detecting genomic variation.

Random amplified polymorphic DNA (RAPD) is a powerful technique, which can be used to identify and determine specific genomes or to estimate the phylogenetic relationship among the individual genomes of date palm. RAPDs can also be used as genetic markers to generate a linkage map to facilitate the identification of molecular markers linked to economically important traits. RAPD is based on the polymerase chain reaction (PCR) (Williams et al. 1990). In addition to the technical simplicity and speed of RAPD methodology (Gepts 1993), its level of genetic resolution is comparable with restriction fragment length polymorphism (RFLP) for determining genetic relationships among genotypes of *Brassica species* (Dos Santos et al. 1994; Hallden et al. 1994). The polymorphic DNA amplified by using random 10-mer olegonucleotide primers (OPERON Model) can generate many useful genetic markers for the analysis of genetic diversity and to study the phylogenetic relationship (Clark and Lanigan 1993). To our knowledge, no detailed research has been conducted to analyze phylogenetic relationships among date palm cultivars native to Saudi Arabia using RAPD markers. Therefore, the objectives of the present communication were: (1) to screen and select primers for the generation of RAPD markers in date palm and (2) to analyze the genetic diversity among the 13 different cultivars of date palm using RAPD markers.

Materials and methods

Plant material

The plant material consisted of 13 commercial cultivars originating from various plantation areas of the Kingdom of Saudi Arabia and selected for their fruit quality (dates). The cultivars used were 1. Ajwa, 2. Barney, 3. Bareem, 4. Nabtet Saif, 5. Nabtet Sultan, 6. Om-Hammam, 7. Om-Kobar, 8. Rabeeha, 9. Shehel, 10. Shishi, 11. Sugai, 12. Sukkary Asfar, 13. Sukkary Hamra.

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 using a pestle and mortar and then by following the steps of the protocol of Dellaporta et al. (1983), with some modifications, pure and highly concentrated DNA was extracted. Using a fluorometer (Hoefer DyNA Quant 200; Pharmacia Biotech, Piscatawaym, N.J.) we determined the quantity and quality of the DNA. The stock DNA samples were diluted with sterile TE buffer to make a working solution of 10 ηg μl^{-1} for use in PCR analysis.

Polymerase chain reaction and primers

A total of 37 random 10-mer RAPD primers (OPERON Technologies, Alameda, Calif.) of the A-to G-series were used for PCR amplification of the templates. The primers were dissolved in sterilized distilled water at a concentration of 10 ηg μl^{-1}.

Amplification reactions were performed in volumes of 25 μl containing 1 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech) per reaction. PCR amplification was performed in a Perkin Elmer/Cetus Thermal Cycler 480 (Faster City, Calif.). The following PCR program was used: (1) one cycle for 3 min at 94 °C; (2) 45 cycles at 94 °C for 30 s, 36 °C for 1 min and 72 °C for

Table 1 RAPD primers (Operon, Model) with total number of amplified fragments and polymorphic bands for each primer among 13 genotypes of date palm

Number	Random primers	Total amplified fragments	Polymorphic fragments	Number	Random primers	Total amplified fragment	Polymorphic fragments
1	OPA01	7	2	21	OPD05	9	4
2	OPA07	17	11	22	OPD08	8	4
3	OPA04	12	3	23	OPD11	6	2
4	OPA10	14	9	24	OPD12	5	3
5	OPA15	13	6	25	OPD15	9	6
6	OPB08	10	4	26	OPD20	9	4
7	OPB11	5	3	27	OPE01	6	2
8	OPB13	9	5	28	OPE02	7	3
9	OPB14	8	6	29	OPE11	7	2
10	OPB15	11	3	30	OPE16	6	3
11	OPB16	8	6	31	OPE18	8	3
12	OPB19	8	4	32	OPE19	7	3
13	OPB20	10	7	33	OPF04	10	4
14	OPC01	7	2	34	OPF05	9	6
15	OPC02	9	3	35	OPF06	8	3
16	OPC04	11	4	36	OPF07	7	3
17	OPC05	8	3	37	OPF09	7	4
18	OPC10	10	4				
19	OPC18	7	2				
20	OPC19	10	3				

2 min; (3) one cycle for 10 min at 72 °C, followed by a soaking at 4 °C. The RAPD products were separated by electrophoresis according to their molecular weight on 1.4% (w/w) agarose gels submerged in 1× TBE buffer and then stained with ethidium bromide (10 μg ml^{-1}) solution for 20 min. The DNAs were visualized on a UV transilluminator and documented by using the Gel Documentation System of Bio-Rad. (Hercules, Calif.). The length of the amplified RAPD fragments was estimated by running Kilo Base DNA Marker (Amersham Pharmacia Biotech) on in the gel as standard size marker.

Analysis of amplification profiles

Amplification profiles of the 13 different date palm samples were compared with each other using the Diversity Data Base software package (Bio-Rad). The data of the selected primers were applied to estimate the similarity on the basis of the number of shared amplification products (Nei 1978; Nei and Li 1979). Cluster analysis by the unweighted pair group method of arithmetic means (UPGMA) was also performed with the help of the diversity Data Base software package (Bio Rad).

Results and discussion

The aim of the present study was to produce RAPD markers for the identification of date palm varieties of the Kingdom of Saudi Arabia. Since this was a pioneering work on date palm, we tested many protocols for the extraction of the DNA and ultimately obtained the best results with the method of Dellaporta et al. (1983) with some modifications. In this protocol proteinase K treatment was used to inactivate the tissue nucleases. Fresh young sprouting leaves with a white to pale-yellow color yielded DNA of a good quality and high quantity. The extracts were colorless and could be looped out easily, while the DNA extracted from the old, green and fully expanded leaves was not clear but greenish in color due to the presence of impurities that were difficult to remove. The average yields from 300 to 5,000 mg of the leaves were 10 to 30 μg ml^{-1} DNA. A single thick band for each variety on the gel indicated a good quality and quantity of the DNA.

Clear amplified polymorphic DNA products were obtained from the screening of 140 RAPD primers on seven genotypes that allowed selection of 37 primers, and the results were reproducible (Askari et al. 2002). All 13 genotypes in this study revealed a unique profile with the 37 primers and thus can be used for the DNA fingerprinting. Generally, a larger chromosome size and more repetitive sequences provided greater chances for the primers to find homology and to give more and differently sized amplified fragments. Different primers produced a different level of polymorphism among the 13 cultivars (Fig. 1). The number of polymorphic bands per primer varied between 2 and 17, with a mean of four major bands per primer (Table 1). To ensure the reproducibility and reliability of the RAPD markers we repeated the PCR reactions twice with each primer. The primers that showed weak or no patterns were discarded.

The pair-wise genetic distance estimates of the 13 genotypes in this study were analyzed and are given in

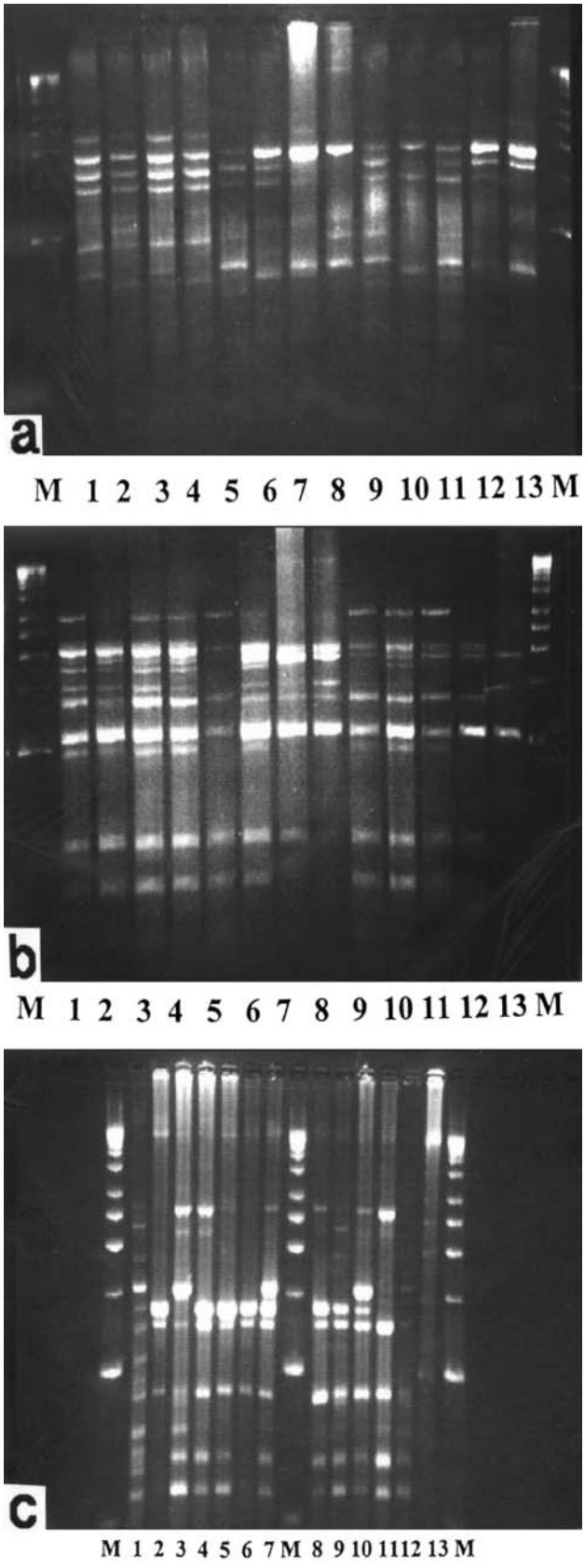

Fig. 1 RAPD profiles of 13 date palm cultivars using OPC10, (**a**), OPD15 (**b**) and OPF05 (**c**) primers. *Lanes*: *M* Molecular weight marker *1* Barney, *2* Shehel, *3* Om-Kobar, *4* Ajwa, *5* Om-Hammam, *6* Sukkary Hamra, *7* Sukkary Asfar, *8* Bareem, *9* Nabtet Saif, *10* Rabeeha, *11* Shishi, *12* Sugai, *13* Nabtet Sultan

Table 2 Similarity matrix for Nei and Li's coefficients of 13 date palm genotypes obtained from RAPD markers

		1	2	3	4	5	6	7	8	9	10	11	12	13
Ajwa	1	100.0												
Bareem	2	85.1	100.0											
Barney	3	40.7	34.5	100.0										
Nabtet Saif	4	62.2	67.4	32.8	100.0									
Nabtet Sultan	5	65.2	68.1	34.9	72.3	100.0								
Om-Hammam	6	86.0	89.1	37.5	73.7	72.7	100.0							
Om-Kobar	7	78.2	79.1	31.0	65.2	61.5	76.1	100.0						
Rabeeha	8	47.5	53.2	35.3	68.3	69.0	61.2	53.2	100.0					
Shehel	9	50.0	62.0	18.6	62.2	57.9	62.3	59.2	65.6	100.0				
Shishi	10	50.0	48.0	34.0	69.2	72.5	56.8	50.7	61.8	56.7	100.0			
Sugai	11	72.7	71.1	37.5	63.3	76.5	68.3	63.2	60.9	55.7	61.5	100.0		
Sukkary Asfar	12	69.0	72.3	32.7	72.1	79.5	74.2	65.1	71.1	58.8	66.7	76.7	100.0	
Sukkary Hamra	13	63.2	66.0	33.3	74.2	84.8	72.0	57.4	66.7	55.7	69.9	69.0	79.1	100.0

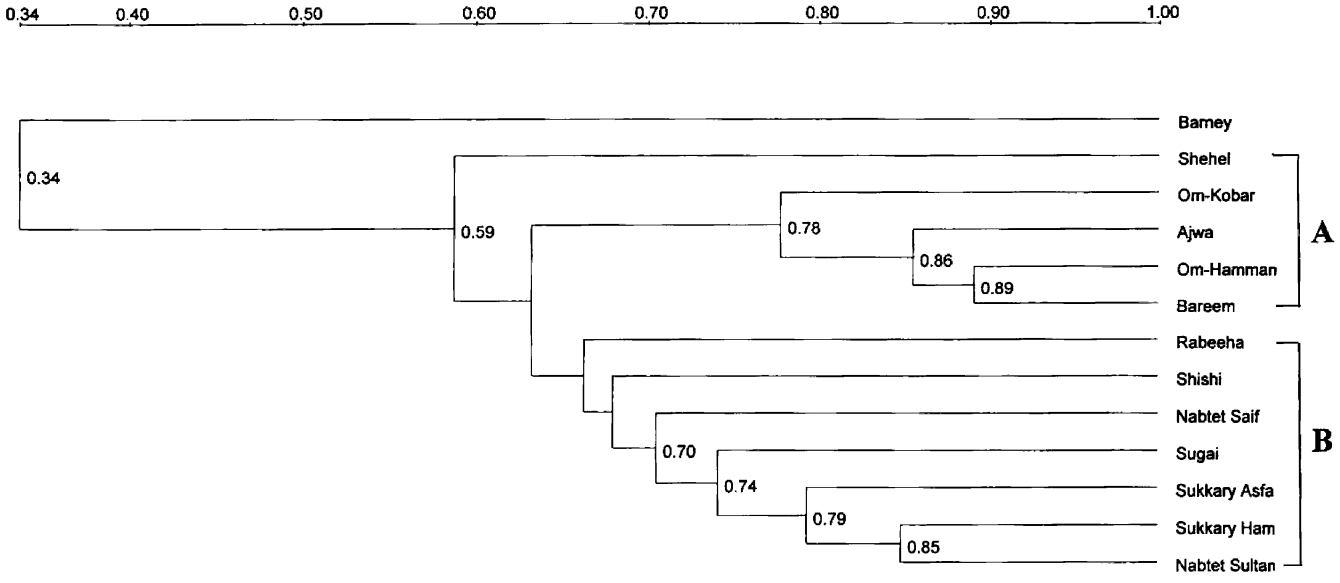

Fig. 2 A dendrogram of phylogenetic relationships among 13 cultivars of date palm based on Nei and Li's similarity coefficient obtained from 37 RAPD primers

Table 2. The similarity matrix is based on Nei and Li's (1979) similarity coefficient. The genetic distance (Nei and Li's similarity) ranged from 0.891 to 0.186. Maximum similarity was observed between Om-Hammam and Bareem (0.89). Barny in general showed a minimum degree of similarity with all the cultivars ranging from 0.375 to 0.186.

Cluster analysis by the unweighted paired group method of arithmetic mean (UPGMA) showed two clusters (Fig. 2). Cluster A consisted of five cultivars (Shehel, Om-Kobar, Ajwa, Om-Hammam and Bareem) with 0.59–0.89 Nei and Li's coefficient in the similarity matrix. Cluster B consisted of seven cultivars (Rabeeha, Shishi, Nabtet Saif, Sugai, Sukkary Asfar, Sukkary Hamra and Nabtet Sultan) with a 0.66–0.85 Nei and Li's similarity range. Om-Hammam and Bareem were the two most closely related cultivars among the 13 cultivars, with the highest value in the similarity matrix (0.89). Ajwa was closely related with Om-Hammam and Bareem with the second highest value in the similarity matrix (0.86). Sukkary Hamra and Nabtet Sultan were also closely related with a third highest value in the similarity matrix (0.85). Cultivar Barny did not belong to any of the cluster groups, it was 34% genetically similar to the rest of the 12 cultivars. In the present communication the average similarity among the 13 cultivars was more than 50%. The result suggests that RAPD analysis could be used for an efficient identification and DNA fingerprinting of the date palm varieties grown in Saudi Arabia. This will help in the collection and cataloguing of the germplasm in the form of a germplasm bank.

RAPD appears to be effective for the identification of date palm varieties, although polymorphism is low in comparison with other cultivated species (Farooq et al. 1994a, b; Khan et al. 2000; Koller et al. 1993; Yang and Quiros 1993). RAPD markers should be of high value for date palm germplasm characterization and genetic maintenance. It is necessary to increase the number of varieties

and the number of primers to assess precisely the phylogenetic relationship in a population. 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 to the maintenance of the germplasm in Saudi Arabia. The present RAPD data so far generated by different primers suggest narrow genetic diversity. Exchange of the varieties between the different plantation areas, clonal propagation of ecotypes and development of new recombinants by seedling selection and limited 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.

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