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RAPD Analysis of Date Palm Cultivars of Bahrain

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ABSTRACT

The date palm (*Phoenix dactylifera* L.) is a monocotyledonous woody perennial plant that plays an important socio-economic role in Middle Eastern countries. The identification and evaluation of genetic variability between cultivars of date palm on the basis of morphological and biochemical markers are difficult, time-consuming and provide limited information. The aim of this study was to detect genetic variability in different cultivated date palm populations around the city of Manama in Bahrain. Random amplified polymorphic DNA (RAPD) was applied to study the genetic diversity in date palm plants. The leaves were colleted in the fruiting stage from ten different plants. Twenty different RAPD primers were used in the experiment and among them only three showed polymorphism and reproducible results. The plants were distinguished by their unique banding patterns produced by the three primers. Analysis of molecular variance (AMOVA) by a similarity matrix showed 52% average genetic similarity among the tested plants. Cluster analysis by the unweighted paired group method of arithmetic mean (UPGMA) detected two clusters and the phylogenetic relationships between the plants were demonstrated by a dendrogram. Our results suggest the presence of a moderate level of genetic variation within the cultivated plants of Bahrain in Manama.

Keywords: genetic similarity, *Phoenix dactylifera*, phylogenetic relationship, polymorphism

INTRODUCTION

Date palms are long-living dioecious monocots of the Arecaceae family, and one of the major cultivated crops in Bahrain (Barreveld *et al*. 1993). Moreover, date palms are essential integral components of farming systems in dry and semi-arid regions (Al-Moshileh *et al*. 2004). The date palm is considered one of the most important commercial crops in the Arab world (Nixon 1951; Bashah 1996). Great diversity exists in date fruit size, color, quality, quantity and harvesting time. Among them, only a few cultivars have immense value in maintaining and enhancing the quality of date palm cultivation, agro management and commercialization of this crop (Corniquel and Mercier 1994). Slow growth, dioecy, slow offshoot-based clonal propagation system and impossibility of evaluation of genetic diversity on the basis of seedling morphological characteristics have severely restricted the improvement program of date palm cultivation (Al-Moshileh *et al*. 2004). Improvement of date palm cultivation depends on numerous factors, including selection, identification and multiplication of the best cultivars based on molecular analysis of the available genetic diversity and development of breeding strategies in this regard. Moreover, incorporation of disease and pest resistance in improved cultivars are other important considerations in a date palm quality improvement program (Bendiab *et al*. 1993).

The correct identification of palms based on physiological and morphological markers is usually not possible until fruits are produced. Moreover, the characterization and evaluation of genetic diversity based on a large set of phenotypic data is often difficult to assess due to environmental influences (Sedre *et al*. 1998). Molecular analysis of genotypic markers offers an attractive, reliable alternative method in identifying plants at early growth stage. Molecular techniques based on DNA have been very successful in the analysis of a variety of crop plants. Techniques of genome fingerprinting include several techniques, one of which is randomly amplified polymorphic DNA (RAPD). RAPD is a powerful technique which can be used to separate, identify and determine the specific genomes or to estimate the phylogeny among the individual genomes (Clark and Lanigan 1993). Genetic diversity among thirteen cultivars of date palm of Saudi Arabia were studied using RAPD markers (Al Khalifah and Askari 2003). The DNA of four unknown male and female Egyptian date palm plants were analyzed by RAPD to study the genetic similarity between them (Soliman *et al*. 2003).

The objective of this study was to test the genetic diversity in some economically important germplasm of date palm of Bahrain collected around the city of Manama. This is the first report of RAPD analysis of Bahrain date palm cultivars.

MATERIALS AND METHODS

Plant material

Young sprouting leaves from ten different five-year old individual plants growing in the locality of Manama, Bahrain during their fruiting stage were randomly collected in July, 2005.

DNA extraction

Total genomic DNA was extracted from 0.5 g of young sprouting leaves. The leaves were first washed with distilled water, surface dried then ground into a fine powder by mortar and pestle using liquid nitrogen. DNA was isolated from the powder using the Wizard® genomic DNA Kit (Promega, Madison, USA) following their plant genomic DNA isolation protocol, and isolated DNA was dissolved in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Three independent DNA preparations were made from each leaf tissue collected from each plant. The quantity and quality of DNA sample were estimated by comparing band intensities on a 1% agarose gel (Promega, Madison, USA). The stock DNA samples were diluted with sterile TE buffer to make a working solution for RAPD analysis using PCR.

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Short Communication

Table 1 Selected RAPD primers with total number of amplified products, polymorphic bands for each primer and percentage of polymorphism among 10 date palm plants were presented.

Primer	Sequence (5′-3′)	№ of amplified products	№ of polymorphic products	Polymorphism (%)
OPA 08	GTGACGTAGG	8	4	50
OPA 09	GGGTAACGCC	6	2	33
OPA 12	TCGGCGATAG	11	7	64

Table 2 Similarity matrix for Nei and Li's coefficients of 10 date palm plants obtained from RAPD markers.

	1	2	3	4	5	6	7	8	9	10
1	100									
2	72.7	100								
3	61.5	76.9	100							
4	57.1	66.6	57.1	100						
5	43.3	36.3	53.3	56.9	100					
6	38.1	46.1	54.5	60.0	61.6	100				
7	50.0	45.3	40.7	45.3	40.7	62.6	100			
8	34.5	53.3	67.0	54.5	52.5	48.9	46.7	100		
9	23.6	38.9	27.2	34.1	32.5	20.9	22.6	19.6	100	
10	45.8	49.5	38.0	46.6	23.0	63.7	45.3	54.5	25.8	100

RAPD primers and PCR amplification

Twenty random decamer primers were obtained from Kit A and Kit B (Qiagen, Germany) for amplification of genomic DNA. PCR reactions were performed according to the protocol of Williams *et al*. (1990). Initially a pilot experiment were carried out using varying concentration of primers, template DNAs and *Taq* PCR Mastermix (mixture of dNTPs, *Taq* polymerase, $MgCl_2$, PCR buffer, obtained from Qiagen, Germany). The final amplification reaction contained 100 ng of template DNA, 50 pmol primers and *Taq* PCR Master mix. The amplification of DNA by PCR was performed in a Gene Cycler (Bio-Rad, Tokyo, Japan). The PCR cycling reaction program was: 94°C for 2 min, then 45 cycles of 94°C for 1 min, 38°C for 1.5 min, and 72°C for 2 min, and finally 72°C for 10 min The RAPD products were separated by electrophoresis on a 1.2% agarose gel in 1X TBE buffer and visualized image using the Bio-Rad Gel Documentation system after staining with ethidium bromide (10 µg ml $^{-1}$) for 10 min (Farooq *et al*. 1994).

Data analysis

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each random primer. Comparisons of plants were based on the presence or absence of unique/shared polymorphic products to generate similarity coefficient based on simple matching. Amplification profiles of ten different date palm samples were compared with each other using Diversity Data Base software package (Bio-Rad). The data obtained from selected primers was applied to estimate the similarity on the basis of number of unique or shared amplification products (Nei and Li 1979). The similarity co-efficient were then used to construct a dendrogram by Unweighted Pair-Group Method with Arithmetic means (UPGMA).

RESULTS AND DISCUSSION

Initially, twenty random primers were used to amplify DNA segments of ten date palm plants of Bahrain. In some cases, the products were non-specific, and no products or no clear bands were obtained. Clear amplified polymorphic DNA bands were obtained from ten different plants using three primers (**Table 1**). OPA 12 random primer gave a large number of DNA bands and the level of polymorphism was high. The number of polymorphic bands varied between three to seven with a mean of three major bands per primer. The size of the amplified products raged from 300-4000 bp (**Fig. 1**). To ensure the reproducibility and reliability of RAPD markers, the PCR reactions were performed three times with each primer. To ensure reproducibility and genetic persistence of RAPD marker data, the primers generating no, weak or complex pattern were discarded. RAPD analysis of three selected primers showed 30-65% polymorphism (average = 42% polymorphism) and the level of

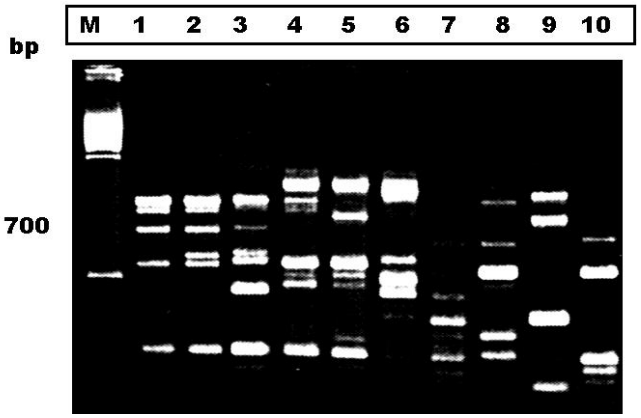

Fig. 1 RAPD profiles of ten date palm plants is presented by using OPA12 random primer. Lanes: M, Molecular weight marker; 1-10, plants sampled.

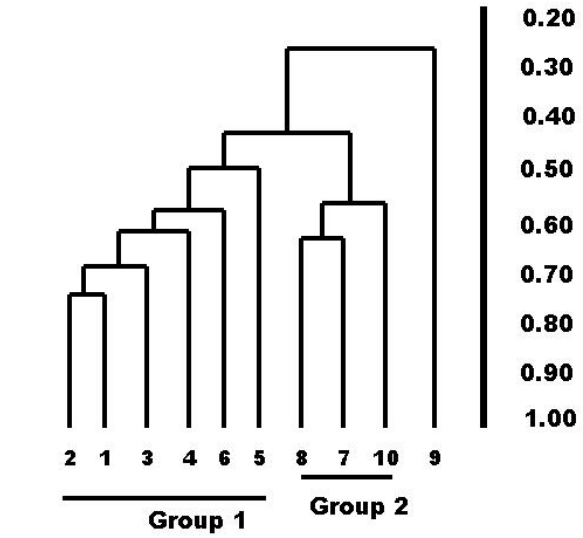

Fig. 2 Dendrogram of phylogenetic relationships among 10 date palm plants collected from Manama based on Nei and Li's similarity coefficient using 3 primers (OPA 08, OPA 09, OPA 12).

polymorphism was higher for OPA 12 (**Table 1**). The average similarity ranged between 40 to 65% among the ten date palm plants (**Figs. 1, 2**). The average similarity among 13 date palm cultivars in Saudi Arabia was more then 50%, as reported by Al-Khalifah and Askari (2003). The variation detected among the closely related genotypes indicates the efficiency of RAPD markers over the morphological

and isozymes markers for the identification and construction of genetic linkage maps (Bendiab *et al*. 1993, Askari *et al*. 2002).

Distance and similarity matrixes were calculated based on Nei and Li's co-efficient (**Table 2**). The cluster analysis by UPGMA showed two clusters (**Fig. 2**). Cluster 1 consisted of five plants (1, 2, 3, 4, 5, 6) and Cluster 2 consisted of three plants (7, 8, 10). Plant number 9 was not clustered in either group 1 or group 2. RAPD appears to be effective for the identification of date palm varieties, although the level of polymorphism is low in comparison with other cultivated species (Koller *et al*. 1993; Khan *et al*. 2000). The comparative analysis suggests that RAPD analysis could be used for the efficient identification as well as isolation of superior clones or varieties of date palm grown in Bahrain. This study is important in cataloguing and making a DNA database of date palms in Bahrain.

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