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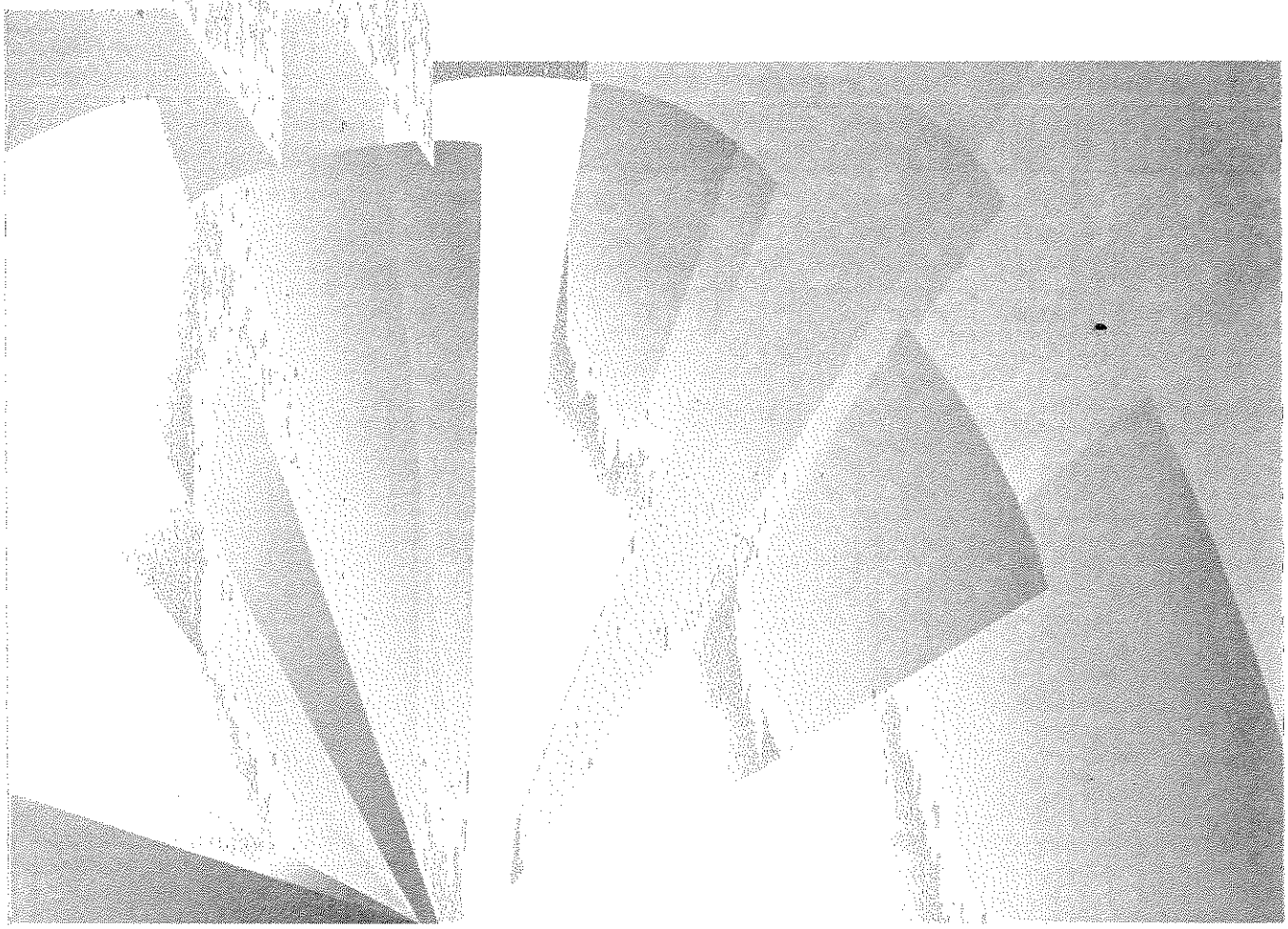

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Genetic Fingerprint of Some KSA Date Palm Cultivars Using Modern Biotechnological Techniques

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Abstract: In the present research six cultivars of date palm (Sukkari, Sifri, Sullag, Khalas, Makfazi and Maktoum) growing in the west region of KSA were investigated for genetic diversity using RAPD technique, total protein, protein patterns and amino acids analysis. Total genomic DNA extracted from the young leaves of six different cultivars. Ten decamer oligonucleotides were used as primers under optimized PCR conditions. Polymorphisms were recorded by noting the presence or absence of an amplification product from the total genomic DNA. A total of 303 DNA fragment were produced from all experiments with an average of 5.05 bands per primer and cultivar. The total number of monomorphic fragments was 222/330. While, the total number of polymorphic fragment was 81/303 fragments. In addition to amino acids analysis and protein banding were determined.

Key words: Date palm, finger print, PCR, molecular markers

INTRODUCTION

Morphological characters have traditionally provided signatures of varietal genotypes and purity. However, molecular characters that more quickly and accurately reveal genetic differences without the obscurance of environment provide significant advantage in genetic analysis, germplasm characterization and improvement programmes.

In recent years, the development of molecular markers has expanded the range of DNA polymorphism assays to be used in different areas including genome mapping, fingerprinting and for investigating genetic relatedness in the different species and germplasm collections. Genomic fingerprinting using molecular markers might have many important applications including commercial variety protection, assessment of seed purity and the verification of labell: ng and identity of plants in production and marketing (Henry, 1998; Ismail, 2004).

Date palm (*Phoenix dactylifera* L.) is a tree crop of economic importance in Saudia Arabia. It presents a source of income to desert inhabitants. In addition to the dates high nutritive value, it provides protection to under-scops from the harshness of the climate and reduces the damage from sand storms and wind erosion. The date palm is cultivated for food, fuel, shelter and fiber. It is a dioecious perennial monocotyledon diploid 2n = 36.

RAPD marker provide the geneticist with a new tool to explore the genetics of sexually reproducing organisms, with applications in gene mapping, population genetics and marker-assisted selection in plant and animal breeding. In the same time, the amplification with RAPD primers is extremely sensitive to single base changes in the primer-target site. This feature suggest that RAPDs should be highly useful for phylogenetic analysis among closely related individuals, but less useful for analysis of genetically divers individuals (Williams *et al*., 1993). Also in 1998 RAPD analysis has been employed using arbitrary 10-mer oligonucleotide primers in order to investigate the genetic differences of somatic embryogenesis-derived regenerants of oil palm. Of the 387 primers used, 259(67%) were successfully used to amplify oil palm DNA genomic fragments with consistently reproducible banding.

A random genomic library of Tunisiam date palm varieties has been constructed by Sakka *et al*. (2000) from total cellular DNA, previously amplified according to RAPD procedure, Sharifani and Jakson (2000) employed random 10-mer primers to identify 16 pear species, cultivars and few progenies using RAPD technique. Nine of twenty random decamer primers produced useful band markers. The results indicated that pear cultivars and species can be identified by using few of the primers.

Progenies were screened using these nine primers were useful to discriminate among the progenies the other primers in this respect did not produce distinct marker bands or polymorphism pattern.

El-Tarras *et al*. (2001a) employed random 10-mer (RAPD) primers to identify 4 cultivars of olive, new

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cultivars were imported from Greek (kalamata) and from France (Picholine) which are hard to propagate by cutting besides the two easy-to-root cultivars moslati and coratina. The amplification products obtained from PCR RAPD were highly polymorphic in the analysis olive cultivars.

El-Tarras *et al.* (2001b) utilized the RAPD Methodology for the genetic fingerprinting of five new strawberry cultivars. The genetic variability based on protein profile using polyacrylamide gel electophorsis and RAPD technique. Using 11 random primers 204 polymorphic DNA fragments with a high potential to differentiate strawberry genotypes could be produced. Genomic DNA was extracted from young leaves of date plam, these cultivars were zaghlovl and Samani (soft fruit), Al-Amri, Al-Aglani and Al-Sewei (Semi dri fruit), Sakkoty, Bertmoda, Ganddeala, Malikabi and Shameia (dri fruit). The pre-screening of 35 primers allowed selection of 13 primers which revealed polymorphism and gave reproducible results. A total of 1845 bands from all experiments could be enough for the identification and the evaluation of genetic similarities (El-Tarras *et al.*, 2002a and b).

In 2004, two types of molecular markers, i.e., RAPD and ISSR to assess the genetic polymorphism and to develop a finger print for five important date palm cultivars (Malikaby, Sakkoty, Shameia, Gunddeala and Bertmoda) from upper Egypt (Aswan). The genetic similarity based on the data from RAPD analysis was estimated according to Dice coefficient producing a similarity matrix that was used to develop a dendrogram showing the genetic relationship this information would be useful for future date palm germplasm collection preservation and in date palm improvement programes.

MATERIALS AND METHODS

This research was carried out in Makkah region and included 6 date palm accessions collected from Taif governtan. These accessions represent date palm.

Cultivars: Sukkari, Sifri, Sullaj, Khalas, Makfazi and Maktoum.

Extraction and purification of genomic DNA: A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Aitchitt *et al.* (1993) was adopted for obtaining good quality total DNA.

Fresh leaves were collected from Sukkari, Sifri, Sullaj, Khalas, Makfazi and Maktoum.

Approximately 2 g of leaves were ground using mortar and pastle in the presence of liquid transferred to buffer and 150 mg pvp (polyvinyl pyroliodone) and 45 mL B-Mercaptoethanol (0.3%) were added and the mixture was mixed by inversion and incubated at 65°C for 1 h.

Table 1: Sequence of the ten decamer arbitrary primers assayed in RAPD-PCR

Primer	Sequences	G+C%
OPA-10	5`-GTGATCGCAG-3`	60
OPB-03	5`-TCGGCGATAG-3`	60
OPB-03	5`-CATCCCCCTG -3`	70
OPB-07	5`-GGTGACGCAG-3`	70
OPB-10	5`-CTGCTGGGAC-3`	70
OPB-13	5`-TTCCCCCGCT-3`	70
OPB-14	5`-TCCGCTCTGG-3`	70
OPB-18	5`-CCACAGCAGT-3`	70
OPB-05	5`-TCCCATGCTG-3`	60
OPZ-08	5`-GGGTGGGTAA-3`	60

Twenty milliliter of chloroform: isoamyl alcohol (24:1) was added and the content were mixed by inversion to form an emulsion, then the tubes were centrifuged at 6000 rpm (NaCl).

Half-volume of 5M NaCl and two volumes of cold absolute ethanol were added to the supernatant and mixed well. The tubes were incubated at -20°C overnight, then centrifuged at 8000 rpm for 15 min.

To remove RNA contamination, 4 μL (10 mg mL^{-1}) RNase A were added to the DNA solution and incubated at 37°C for 2 h.

To remove protein, 4 μL (1 mg mL^{-1}) proteinase were added and incubating at 37°C for 2 h, then 300 μL of tris-saturating phenol-chloroform were added and mixed by inversion.

Tubes were centrifuged at 14000 rpm for 15 min. in new tubes and 150 μL of TE buffer was added to the phenol phase, mixed, spun for 10 min, then the upper layer containing the DNA was removed.

DNA was precipitated overnight at -20°C using 0.1 volume 3M sodium acetate and two volumes of absolute ethanol, the samples were centrifuged at 14000 rpm at 4°C for 15 min, the DNA was washed with 70% ethanol, briefly air-dried and re-dissolved in TE buffer.

RAPD-PCR reaction: RAPD analysis was carried out using 10 oligonucleotide primer (10-mer) (Table 1) in the detection of polymorphism among the 6 date plam cultivars. RAPD-PCR was carried out according to the procedure doing by Williams *et al.* (1990). The amplification reaction was carried out in 25 μL reaction volume containing PCR buffer, $MgCl_2$, dNTP, primer, Taq DNA polymerase and template DNA. PCR amplification was performed for 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

Gel electrophoresis and visualization of DNA bands: The amplification products were resolved by elctrophoresis

in 1.5% agarose gel containing ethidium bromide (0.5 μg mL^{-1}) in 1 X TBE buffer at 100 volts for 2 h. A garose gel were examined on ultraviolet transilluminator filler, photography was made by using Polaroid film type 57 (ASa 3000).

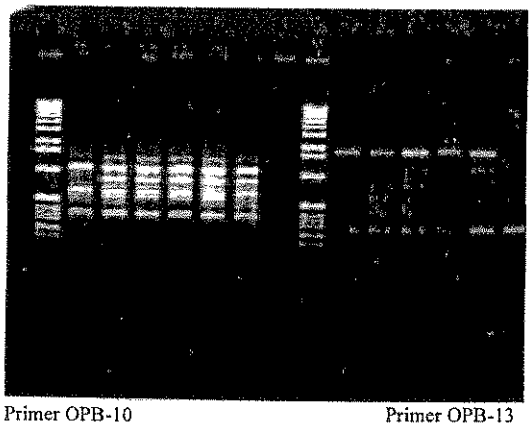
RESULTS AND DISCUSSION

Molecular markers are efficient tools for cultivar identification and estimation of relatedness through DNA fingerprinting. RAPD markers where developed by Williams *et al.* (1990). RAPD technique using single arbitrary 10-mer oligonucleotides primers to amplify discrete fragments of DNA using Polymerase Chain Reaction (PCR). This technique has been used extensively in many different applications and in different plant species because of its simplicity (El-Tarras *et al.*, 2001a, b; 2002a, b).

RAPD-PCR reactions were performed with six date palm cultivars and ten different 10-mer primers, which were pre selected for their performance with date palm DNA. Table (2 and 3) record the number of amplified fragments scored for each cultivar. The amplified products were highly polymorphic among the six different date palm cultivars. A total of 303 fragments from all analysis were enough for the identification and the evaluation of genetic similarities and designing the phylogenetic tree for these six different date palm cultivars (Fig. 1-6).

The total number of bands as shown in Fig. 1-5 varied from 50 bands (OPB-18) to 6 bands (OPZ-05). The total of monomorphic amplicons where 222/303 and the total of polymorphic amplicons were 81/303.

The total number of amplified fragment fro Sukkari, Sifri, Sullaj, Khalas, Makfazi and Maktoum were 43, 54, 53, 49, 54 and 50, respectively, represented by 14.2, 17.8, 17.5, 16.2, 17.8 and 16.5%, respectively. In this research, the total number of fragments produced by the ten primers was 303 fragments with an average of 30.3 fragments/primer (Table 2). While, the total number of monomorphic fragments was 222 with an average of 22.2 fragments /primer. While, the total number of polymorphic fragments was 81 fragments with an average of 8.1 fragments per primer. A maximum number of polymorphic fragments were amplified by OPB-14 (25/81), While, the minimum number of polymorphic fragments where amplified by OPB-18 (2/81), in the same time, the no polymorphic fragments were produced by OPZ-05 and OPZ-08 in all cultivars under investigation.



Primer OPB-10 Primer OPB-13

Fig. 1: RAPD's product of primers OPB-10 and OPB-13 on six date palm cultivars

Table 2: Total number of amplicons including monomorphic and polymorphic bands

Primer	Total No. of amplicons	Monomorphic amplicons	Polymorphic amplicons
OPA-10	47.0	36.0	11.0
OPA-12	33.0	30.0	3.0
OPB-03	27.0	12.0	15.0
OPB-07	28.0	24.0	4.0
OPB-10	46.0	36.0	10.0
OPB-13	23.0	12.0	11.0
OPB-14	37.0	12.0	25.0
OPB-18	50.0	48.0	2.0
OPZ-05	6.0	6.0	0.0
OPZ-08	6.0	6.0	0.0
Total	303.0	222.0	81.0
Average	30.3	22.2	8.1

Table 3: Percentage of similarities among the six cultivars under investigation, using the amplicons of ten different primers

	Types							
Primer	Sukkari	Sifri	Sullaj	Khalas	Makfazi	Maktoum	Total	%
OPA-10	8.0	8.0	7.0	8.0	8.0	8.0	47	15.5
OPA-12	5.0	5.0	6.0	6.0	5.0	6.0	33	10.9
OPB-03	3.0	4.0	7.0	4.0	6.0	3.0	27	8.9
OPB-07	5.0	5.0	4.0	5.0	4.0	5.0	28	9.2
OPB-10	8.0	8.0	7.0	7.0	8.0	8.0	46	15.2
OPB-13	2.0	5.0	5.0	3.0	4.0	4.0	23	7.6
OPB-14	2.0	8.0	7.0	6.0	8.0	6.0	37	12.2
OPB-18	8.0	9.0	8.0	8.0	9.0	8.0	50	16.5
OPZ-05	1.0	1.0	1.0	1.0	1.0	1.0	6	2.0
OPZ-08	1.0	1.0	1.0	1.0	1.0	1.0	6	2.0
Total	43.0	54.0	53.0	49.0	54.0	50.0	303	100.0
%	14.2	17.8	17.5	16.2	17.8	16.5	100	

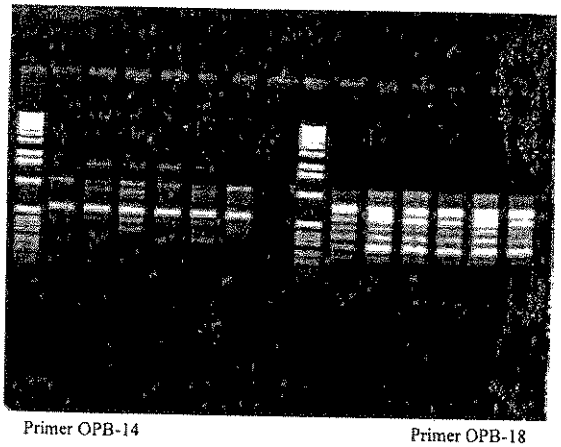


Fig. 2: RAPD's product of primers OPB-14 and OPB-18 on six date palm cultivars

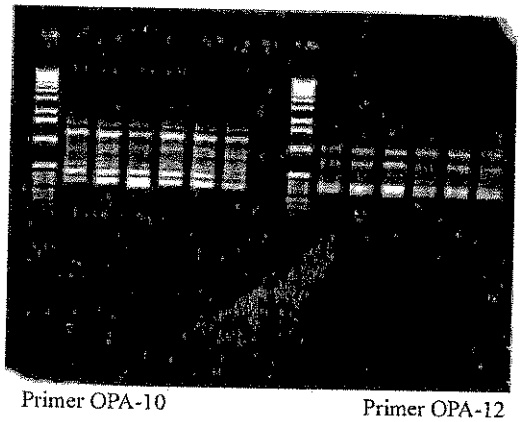


Fig. 3: RAPD's product of primers OPA-10 and OPA-12 on six date palm cultivars

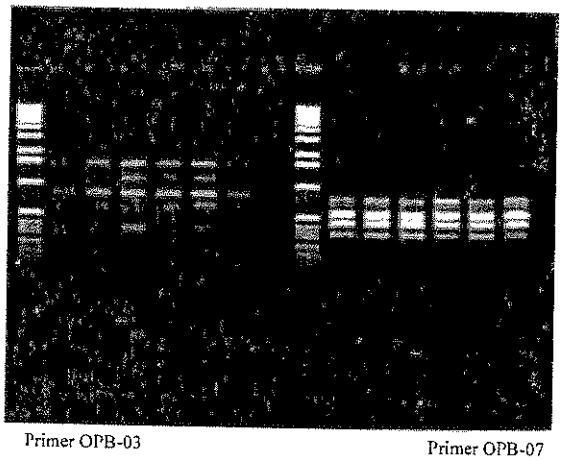


Fig. 4: RAPD's product of primers OPB-03 and OPB-07 on six date palm cultivars

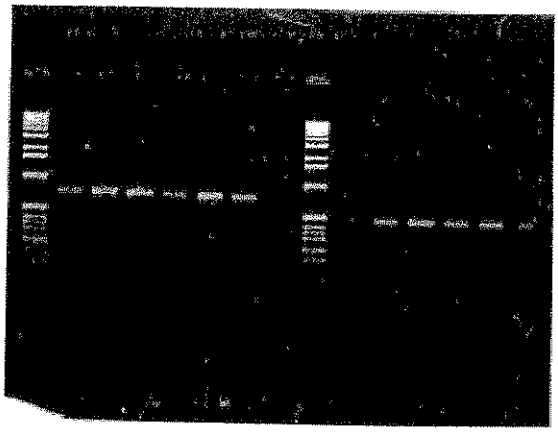


Fig. 5: RAPD's product of primers OPZ-05 and OPB-08 on six date palm cultivars

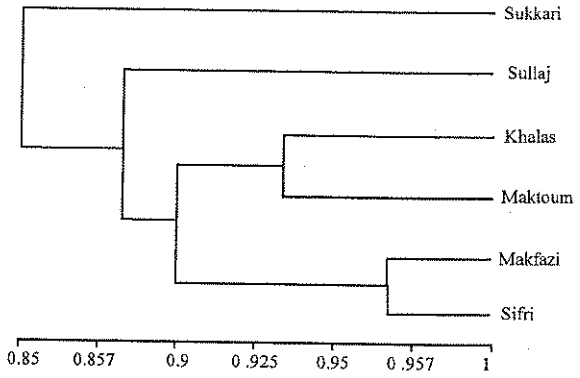


Fig. 6: Genetic relationship analysis among six date palm cultivars

A maximum number of fragments were amplified from Sifri (54/303) and Makfazi (54/303) while, the minimum number of fragments were produced from Sukkari (43/303).

To analyze the genetic distances between and within the different date palm cultivars in the present study a dendrogram was constructed displaying the genetic similarity among the genotypes. For this purpose a matrix of relative genetic similarities according to Rohlf (1990) was computed from 303 fragments. This similarity matrix was then used to construct the dendrogram. As shown in Table 3, the estimated genetic similarities ranged from 96.3 to 85% revealing high level of genetic similarity (96.3%) between Sifri and Makfazi, this was followed by (92.3%) between Khalas and Maktoum. In the same time, the genetic similarity between Sifri, Makfazi and Khalas and Maktoum was 90%, while the lowest genetic similarity (85%) was observed between Sukkari and other five cultivars. The genetic relationship among cultivars have several important application for crop improvement. In

plant breeding programes, this information could be useful in insisting the selections of parents for crossing and estimating the genetic distance and also for identifying the heterotic group. Therefore, in the present study the genetic dissimilarity matrices were employed to generate a dendrogram using Nei and Lis coefficient method (Nei and Li, 1979).

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