Molecular characterization of some Egyptian date palm germplasm using RAPD and ISSR markers

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ABSTRACT

The genetic variability and relationships among 14 date palm (Phoenix dactylifera L.) accessions representing six Egyptian cultivars were assayed using 27 RAPD and 10 ISSR primers. The level of polymorphism among the 14 accessions as revealed by RAPD and ISSR was 25.2% and 28.6%, respectively. These low levels of polymorphism reflect the narrow genetic background of these accessions. The genetic relationships among the 14 accessions were estimated in terms of similarity using Dice coefficients. The genetic similarity ranged from 96.1% to 99.5% and from 91.2% to 100% for RAPD and ISSR, respectively. The inter-cultivar relationships among the six date palm cultivars based on RAPD and ISSR revealed the highest genetic similarity between the cultivar Bertmoda and each of the cultivars Malkaby and Sakkoty. The RAPD and ISSR based dendrograms clustered the accessions belonging to each of the 3 cultivars Fraihy, Siwi and Gandila in separate groups. However, the reshuffling in the position of some of the accessions belonging to the other cultivars in the different dendrograms revealed that they share common gnenetic background. Cultivar-specific DNA markers characterized different genotypes and therefore, were used to generate unique fingerprint for each genotype. The RAPD and ISSR revealed 17 and 5 cultivar unique DNA markers, characterizing 4 and 5 cultivars, respectively. Moreover, each of the RAPD and ISSR was successful in identifying accession-specific markers characterizing five accessions.

Key words: *Date palm, DNA markers, RAPD, ISSR, Unique markers, Cluster analysis.*

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a tree crop of economic importance in Egypt. It represents a source of income to oases inhabitants, provides protection to under-crops from the harshness of the climate and reduces the damage from sand storms and wind erosion. Therefore, the date palm is cultivated for food, fuel, shelter and fiber. It is a dioecious, perennial, monocotyledon, diploid (2n = 36) with long generation time. There are 3 main types of dates based on fruit moisture content, i.e., soft, semi-dry, and dry cultivars. Slow growth, dioecy, the slow offshoot-based propagation system and the impossibility of predicting adult characteristics of the seedling have severely restricted improvement of this ancient tree crop. Therefore, determination of genetic variability and proper cultivar identification in date palm would be of major importance in

improvement programs and in germplasm characterization and conservation to control genetic erosion. Morphological characters have traditionally provided signatures of varietal genotype and purity. However, molecular characters that more quickly and accurately reveal genetic differences without the obscurance of environment provide significant advantages in genetic analysis, germplasm characterization, and improvement programs.

The RAPD technique provides genetic markers which have been used extensively in many different applications and in different plant species because of its simplicity (Cabrita *et al*., 2001; Cipriani *et al*., 1996; Goulão *et al*., 2001; Hormaza, 2001; McGregor *et al*., 2000; Salimath *et al*., 1995; Samaee *et al*., 2003; Ulanovsky *et al*., 2002). Moreover, RAPDs have been successfully used to study the genetic relationships among various date palm accessions and cultivars from Egypt, Morrocco, Tunisia, Iraq and Saudi Arabia (Adawy *et al*., 2002; Motawei *et al*., 2003; Sakka *et al*., 2000; Sedra *et al*., 1998; Soliman *et al*., 2003; Trifi *et al*., 2003).

The ISSR technique is a powerful, rapid, simple, reproducible and inexpensive way to assess genetic diversity or to identify closely related cultivars in many species, including fruit trees (González *et al*., 2002). ISSR technique permits the detection of polymorphisms in microsatellites and inter-microsatellites loci without previous knowledge of the DNA sequence. The technique involves the use of a single primer composed by a microsatellite sequence plus a short arbitrary sequence (anchor) which target a subset of 'simple sequence repeats' (SSRs) or microsatellites and amplify the region between two closely spaced and oppositely oriented SSRs (Moreno *et al*., 1998). The sequences of repeats and anchored nucleotides are randomly selected (Fang *et al*., 1997). ISSRs have been used in assessing genetic relationships among various accessions of different species (Bornet and Branchard, 2001; Fang *et al*., 1998; Lanham and Brennan, 1999; McGregor *et al*., 2000). ISSRs have been also successfully employed to identify date palm cultivars (Adawy *et al*., 2002; 2004; Ben Saleh and El-Helaly, 2003).

The main objectives of the present investigation were; 1) to investigate the level of intra- and inter-cultivar polymorphism among 14 Egyptian date palm accessions representing six cultivars, i.e., Sakkoty, Bertmoda, Malkaby, Gandila, Siwi, and Fraihy using RAPD and ISSR markers; 2) to assess the genetic relationships among these accessions; and 3) to develop cultivar-specific molecular fingerprints through identifying unique DNA markers characterizing each cultivar.

MATERIALS AND METHODS

Plant Material

The study included 14 date palm (*Phoenix dactylifera* L.) accessions collected from different locations in Egypt. These accessions represent six date palm cultivars: Sakkoty, Bertmoda, Malkaby, Gandila, and Fraihy (dry cultivars), and Siwi (semi-dry). Samples were collected from the young leaves surrounding the palm meristem of 3 to 5 palms from each location. The accessions, cultivars and the respective locations are represented in Table (1).

Extraction and Purification of Genomic DNA

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al*. (1997) was adopted for obtaining good quality total DNA. After estimating the concentration of the individual DNA samples, aliquots of the same

concentration of the DNA of the individual trees were mixed into a bulk of DNA representing each accession. Hence, the outcome was 14 bulked DNA samples representing the different accessions of date palm cultivars included in this study.

RAPD analysis

A set of thirty-seven random 10-mer primers was used in the detection of polymorphism among the 14 date palm accessions. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) at AGERI-Egypt. RAPD-PCR was carried out according to the procedure given by Williams *et al*. (1990) with minor modifications. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 25 ng templates DNA. PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts.

ISSR analysis

The technique was carried out according to Adawy *et al*. (2002; 2004). Ten oligonucleotides composed wholly of defined, short tandem repeat sequences with anchor, and representing different microsatellites (di- and tri-repeats) have been used as generic primers in PCR amplification of inter simple sequence repeat regions. PCR was performed in 25 µl reaction volume containing 1X PCR buffer, 1.75 mM $MgCl_2$, 5 mM of each dNTPs, 40 µM oligonucleotide primer, 25ng genomic DNA and 1 U of Taq DNA polymerase. A high stringency touchdown and hot start thermocycling profile was used as follows: an initial denaturation step for 5 min at 94ºC followed by ten touch down cycles (94ºC/30 sec, 65-55ºC/45 sec, 72ºC/1 min). This was followed by thirty-five cycles (94ºC/30 sec, 55ºC/45 sec, 72ºC/1 min) and then a final extension cycle at 72ºC for 7 min. The PCR products were separated on 2% agarose gel in 1X TBE buffer containing ethidium bromide and photographed with a Polaroid camera.

Data analysis

The banding patterns generated by RAPD-PCR and ISSR markers analyses were compared to determine the genetic relatedness of the 14 date palm accessions. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973). Dice formula: $GSij = 2a/(2a+b+c)$, where GSij is the measure of genetic similarity between individuals *i* and j, a is the number of bands shared by *i* and j, b is the number of bands present in i and absent in j, and **c** is the number of bands present in j and absent in *i*.

The similarity matrices were used in the cluster analyses which were employed to generate dendrograms. The software used through this study were SPSS 10.0, POPGEN 3.2, XLSTAT-Pro 7.1, and Microsoft EXCEL.

RESULTS AND DISCUSSION

In the present investigation the level of polymorphism among 14 date palm accessions

was estimated using two PCR-based marker techniques, i.e. RAPDs and ISSRs. These accessions represent six Egyptian date palm cultivars (Sakkoty, Bertmoda, Malkaby, Gandila, Siwi, and Fraihy).

Randomly amplified Polymorphic DNA (RAPD) Analysis

Thirty-seven decamer RAPD primers were screened with the DNA of the 14 date palm accessions. Twenty-seven primers generated reproducible and scorable RAPD profiles. These produced multiple band profiles with a number of amplified DNA fragments ranging from 4 to 17 (Table 2 and Fig. 1). In this respect, Sedra *et al*. (1998) reported that 19 out of 123 prescreened arbitrary decamer primers revealed polymorphic and reproducible results. In the present study, the total number of fragments produced by the twenty seven primers was 282 with an average of 10.4 fragments/primer (Table 2). While, the number of polymorphic fragments ranged from 0 to 7. A maximum number of 17 amplicons were amplified with primer OPO-15, while the minimum number of fragments (4) was amplified with primer OPA-14. The highest number of polymorphic bands (7) was obtained with primers OPB-05, OPB-07, and OPZ-05. Primer OPB-07 exhibited the highest percentage (77.8%) of polymorphism. Table (2) also revealed that the total number of polymorphic amplicons obtained by the twenty-seven studied primers was 71. This corresponds to a level of polymorphism of 25.2% and an average number of polymorphic fragments/primer of 2.6. In this respect, Sedra *et al*. (1998), Motawei *et al*. (2003) and Adawy *et al*. (2004) found that in date palm the average number of polymorphic bands/primer was 1.9, 2.4, and 1.2, respectively. Moreover, the size of the amplified fragments varied with different primers, ranging from 100 to 1700 bp. Sakka *et al*. (2000) reported fragment sizes ranging from 200 to 1600 bp, while, Adawy *et al*. (2002) stated that in the RAPD analysis of five date palm cultivars using 10 primers, the fragment sizes ranged from 310 to 2800 bp. These discrepancies could be attributed to the use of different primers and different reaction conditions.

In the present study, the RAPD results revealed very low intra-varietal polymorphism. However, Hussein *et al*. (2002) pointed out the presence of intra-varietal polymorphism among five Egyptian date palm cultivars from the Delta region. These results could suggest that the date palm cultivars from Upper Egypt and Marsa-Matrouh are more genetically homogenous than those from the Delta region. Moreover, Adawy *et al*. (2004) claimed that RAPD was not able to detect intravarietal variation among five date palm cultivars (Bertmoda, Gandila, Shameia, Malkaby and Sakkoty) obtained from the Egyptian Ministry of Agriculture experiment station at Aswan governorate. The present results also revealed low level of intervarietal polymorphism among the six studied cultivars, suggesting a narrow genetic background of these cultivars.

To examine the genetic relationships among the 14 date palm accessions based on RAPD results, the scored data were analyzed using the Dice coefficient to compute the similarity matrices. These similarity matrices were used to generate a dendrogram using the UPGMA method. The estimated genetic similarities ranged from 91.4% to 99.6% revealing very high levels of genetic similarity among the studied accessions. The highest genetic similarity (99.6%) was between the accessions: Siwi/Dakhla and Siwi/Hafr-El-Baten. This was followed by 99% between the accession Siwi/Kharga and the accessions Siwi/Dakhla and Siwi/Hafr-El-Baten. In the same time, the genetic similarity between the

two accessions of cultivar Fraihy was 98.8%, while the lowest genetic similarity (91.4%) was detected between accessions Sakkoty/Aakab and Siwi/Hafr-El-Baten.

To estimate the genetic relationships among the six date palm cultivars based on RAPD results, only the common bands between the different accessions representing each cultivar were scored. This strategy produced some loci with missing values for some cultivars; such loci were disregarded in the analysis. The estimated genetic similarities ranged from 96.1% to 99.5%. The highest genetic similarity (99.5%) was between the cultivar Sakkoty and the cultivars Bertmoda and Malkaby and between cultivar Bertmoda and cultivar Malkaby. While the lowest genetic similarity (96.1%) was detected between cultivars Siwi and Gandila. In this respect, Adawy *et al*. (2004) using RAPDs estimated similarity value ranging from 97.7% (between Gandila and Sakkoty) to 93.1% (between Gandila and Bertmoda). The small difference in the percentage of similarity detected by Adawy *et al*. (2004) compared to the present results could be attributed to the fact that the present results were obtained from accessions collected from different places in Aswan, while the results of Adawy *et al*. (2004) were based on accessions from only one place. Moreover, the number of primers used to deduce the genetic similarity was different.

The UPGMA cluster analysis was carried out to represent graphically the genetic distances among the 14 date palm accessions (Fig. 3). The obtained dendrogram was divided into two main clusters; one cluster included the four accessions of cultivar Siwi. The other main cluster included two subclusters. One subcluster contained the accessions Sakkoty/Aakab and Bertmoda/Abo-El-Rish. The other subcluster contained two groups; one included the two accessions of cultivar Fraihy. The other group clustered the two accessions of cultivar Gandila together in one subgroup, while the rest of accessions clustered together in another subgroup. These results separated cultivar Siwi in one main cluster, perhaps because it is the only semi-dry cultivar included in the study. However, the RAPD technique revealed slight intravarietal polymorphism within the cultivars from Aswan and did not group the accessions belonging to each of the cultivars Sakkoty, Bertmoda, and Malkaby to different groups. This reflects the close relationships among these cultivars and suggests the occurrence of gene flow between these cultivars.

Moreover, the UPGMA cluster analysis was carried out to represent graphically the genetic distances among the six date palm cultivars (Fig.4). The obtained dendrogram separated the cultivar Siwi from all the others in one cluster. While, the second cluster included two subclusters, the first comprised Fraihy alone and the second involved two groups. One contained the cultivar Gandila, while the other included cultivars Sakkoty, Bertmoda and Malkaby. These results confirmed the distinctiveness of the cultivar Siwi. In this respect, Adawy *et al*. (2004) generated a dendrogram which clustered the five cultivars into two main clusters, where Gandila and Sakkoty constituted one cluster correlated with Malkaby. While, Shameia and Bertmoda formed the second cluster.

In the present study, the RAPD technique was successful in characterizing 5 out of the 6 tested date palm cultivars by unique positive and/or negative markers (Table 4). These markers ranged in size from 100 to 1100 bp. A total number of 17 unique markers were identified by 10 out of the 27 RAPD primers. Cultivar Fraihy was identified by the highest number of positive unique markers (5) and no negative markers. Cultivar Siwi was the only cultivar that was

characterized by three negative unique markers in addition to four positive unique markers. The cultivar Gandila was characterized by the presence of 3 positive unique markers only, while cultivar Sakkoty was identified by the presence of one unique positive marker ($OPZ\text{-}11_{440bp}$). Cultivar Bertmoda was characterized by one negative unique marker ($OPB\text{-}07_{600bp}$).

Moreover, RAPD-accession-specific markers were detected in five out of the 14 date palm accessions (Table 5). The total number of accession-specific markers was 10 comprising only 2 positive markers and 8 negative markers. The accession Sakkoty/Aakab was distinguished by the highest number of RAPD markers (3 negative unique markers and 2 positive unique markers). Two unique negative markers characterized the accession Bertmoda/Abo-El-Rish, while only one RAPD negative marker characterized each of the accessions Gandila/Aakab, Malkaby/Abo-El-Rish and Bertmoda/Aakab. Similarly, Adawy *et al*. (2002; 2004) using RAPD primers identified unique markers for the date palm cultivars they studied.

Inter Simple Sequence Repeats (ISSR) analysis

In the present study, the 10 ISSR primers produced good reproducible and scorable patterns and the amplification profiles were screened for the presence of polymorphisms among and within the 6 date palm cultivars (Fig. 2). As shown in Table (3), a total of 105 fragments were generated by the 10 primers with an average of 10.5 fragments/primer. Primer IS7 yielded the highest number of products (15 amplicons), while primer IS6 detected the lowest number (6 amplicons). The number of polymorphic markers also varied between primers, ranging from 0 to 9, with primers IS2, and IS6 yielding only monomorphic bands and IS10 generating 69.2% polymorphic bands (9 polymorphic bands out of 13). The average number of polymorphic fragments/primer among the 14 date palm accessions was 3. Moreover, the size of the amplified fragments varied with different primers, ranging from 105 to 1000 bp. These results are in agreement with those of Ben Saleh and El-Helaly (2003), using ISSR on date palm that generated 42 bands distributed between 0.2 and 2.4 kb and the average number of fragments per primer was 10.5 fragments with 100 % polymorphism. Adawy *et al*. (2002) using seven ISSR primers generated 53 fragments ranging from 298 to 1200 bp in size. The average number of fragments per primer was 7.6 fragments with 64.1% polymorphism. While, Adawy *et al*. (2004) generated 159 amplicons when using 19 ISSR primers to analyze bulked DNA samples representing five date palm cultivars and the average number of amplicons/primer were 8.4.

The scored data obtained from the ten primers were used to determine the genetic similarity among the fourteen date palm accessions using the Dice coefficient. The highest similarity percentage (100%) was observed between the accessions Siwi/Hafr-El-Baten and Siwi/Tamazough. The lowest genetic similarity (91.2%) was detected between accession Sakkoty/Abo-El-Rish and Fraihy/Hafr-El-Baten. The results also revealed that cultivars Gandila and Siwi showed very low intravarietal variation, while the other cultivars showed higher level of intravarietal variation.

To determine the genetic similarity among the six date palm cultivars only the common bands between the different accessions representing each cultivar were employed. The highest similarity percentage (100%) was observed among the cultivars Sakkoty, Bertmoda and Malkaby and the

lowest genetic similarity (96.2%) was detected between cultivar Siwi and cultivars Sakkoty, Bertmoda, Malkaby and Gandila. In addition, it was the same percentage between cultivar Fraihy and cultivars Sakkoty, Bertmoda, Malkaby and Gandila. The similarity percentage of 100% observed by the ISSRs is probably due to the low number of primers used in the analysis (10) of these closely related cultivars and consequently the low number of loci detected (105), i.e. the genomes of these date palm cultivars require screening by a larger number of primers and different techniques. In this respect, Adawy *et al.* (2004) estimated the genetic distance among four Egyptian date palm cultivars based on ISSRs; this ranged from 80.2% to 89.0%. Using ten ISSR primers, Ben Saleh and El-Helaly (2003) calculated the molecular distance among 15 Tunisian costal date palm cultivars. The Kent and Garn Gazel were the nearest varieties in the group with genetic distance of 21.13% (dissimilarity) and Ftimi and Smiti were the farest with a genetic distance of 90.45%.

Based on the 105 polymorphic ISSR fragments generated by 10 primers, a dendrogram (Fig. 5) was constructed using UPGMA cluster analysis. The obtained dendrogram was divided into two main clusters; one cluster included two subclusters, one grouped the two accessions of cultivar Fraihy, while the other included the four accessions of Siwi. The second main cluster contained two subclusters; one included the accessions Bertmoda/Abo-El-Rish and Malkaby/Aakab. The other subcluster included two groups; one of them contained accession Bertmoda/Abo-El-Rish, while the other included two subgroups. Sakkoty/Aakab was separated in one subgroup, while the other subgroup included two branches. The two accessions of Gandila were separated in one branch, and Sakkoty/Abo-El-Rish and Malkaby/Abo-El-Rish constituted the second branch. The ISSR technique revealed a high level of intravarietal differences within the cultivars Sakkoty, Bertmoda, Malkaby. While, no intravarietal differences were detected among the cultivars Fraihy, Siwi, and Gandila. Furthermore, ISSRs revealed that the cultivars from the oases (Siwi and Fraihy) were closer to each other. In addition, the cultivars from Aswan (Sakkoty, Bertmoda, Malkaby, and Gandila) shared a closely related genetic background.

Table (1): Date palm accessions, cultivar name, and the origin of each accession.

Accession No.	Cultivar	Origin	Governorate
1	Sakkoty (SAK-AK)	Al-Aakab	Aswan
2	Sakkoty (SAK-AB)	Abo-El-Rish	
3	Bertmoda (BRT-AK)	Al-Aakab	
4	Bertmoda (BRT-AB)	Abo-El-Rish	
5	Malkaby (MLK-AK)	Al-Aakab	
6	Malkaby (MLK-AB)	Abo-El-Rish	
7	Gandila (GND-AK)	Al-Aakab	
8	Gandila (GND-AB)	Abo-El-Rish	
9	Siwi (SIW-KH)	El-Kharga	New Valley
10	Siwi (SIW-DK)	El-Dakhla	
11	Siwi (SIW-HB)	Hafr El-Baten	Marsa-Matrouh (Siwa)
12	Siwi (SIW-TZ)	Tamazough	
13	Fraihy (FRA-HB)	Hafr El-Baten	Marsa-Matrouh (Siwa)
14	Fraihy (FRA-TZ)	Tamazough	

Table (2): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers among the 14 accessions.

Primer	Total # amplicons	Monomorphic amplicons	Polymorphic amplicons	% polymorphism
OPA-14	4	4	0	0.0
OPA-16	13	11	2	15.4
OPB-03	11	9	2	18.2
OPB-06	11	6	5	45.5
OPB-08	11	6	5	45.5
OPB-11	11	7	4	36.4
OPB-13	5	5	0	0.0
OPB-16	5	3	2	40.0
OPC-03	8	8	0	0.0
OPC-13	16	15	1	6.3
OPD-05	9	8	1	11.1
OPD-07	11	11	0	0.0
OPO-07	14	12	2	14.3
OPO-15	17	14	3	17.6
OPZ-11	9	8	1	11.1
OPZ-14	7	7	0	0.0
OPZ-19	14	14	0	0.0
OPZ-20	16	15	1	6.3
OPB-05	14	7	7	50.0
OPB-07	9	2	7	77.8
OPC-02	9	7	2	22.2
OPC-09	5	3	2	40.0
OPZ-04	10	6	4	40.0
OPZ-05	10	3	7	70.0
OPZ-06	15	9	6	40.0
OPZ-07	10	5	5	50.0
OPZ-08	8	6	2	25.0
Total	**282**	**211**	**71**	
Average	**10.4**	**7.8**	**2.6**	**25.2**

Table (3): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by ISSR markers among the 14 accessions.

Primer	Total # amplicons	Monomorphic amplicons	Polymorphic amplicons	% polymorphism
IS1	9	8	1	11.1
IS2	11	11	0	0.0
IS3	11	6	5	45.5
IS4	11	5	6	54.5
IS6	6	6	0	0.0
IS7	15	14	1	6.7
IS8	11	10	1	9.1
IS9	7	3	4	57.1
IS10	13	4	9	69.2
A9	11	8	3	27.3
Total	105	75	30	
Average	10.5	7.5	3.0	28.6

Table (4): The date palm cultivars characterized by unique positive and/or negative RAPD and ISSR markers, marker size and total number of markers identifying each cultivar.

	Cultivar	Unique positive markers			Unique negative markers			Grand Total
		Size of the marker band (bp)	Primer	Total # markers / cultivar	Size of the marker band (bp)	Primer	Total # markers / cultivar	
RAPD	Sakkoty	440	OPZ-11	1			0	1
	Bertmoda			0	600	OPB-07	1	1
	Gandila	100, 450 360	OPB-06 OPO-15	3			0	3
	Siwi	1100 410 250 405	OPB-07 OPB-16 OPD-05 OPO-15	4	450 480 370	OPB-16 OPO-15 OPZ-20	3	7
	Fraihy	400, 480 470 205 490	OPA-16 OPB-03 OPB-06 OPC-13	5			0	5
Total				13			4	17
ISSR	Gandila	370	IS4	1			0	1
	Siwi	140 380	IS4 IS8	2			0	2
	Fraihy	220, 420	IS4	2			0	2
Total				5			0	5

Table (5): The date palm accessions characterized by unique positive and/or negative RAPD and ISSR markers, marker size and total number of markers identifying each accession.

	Cultivar	Unique positive markers			Unique negative markers			Grand Total
		Size of the marker band (bp)	Primer	Total # markers / cultivar	Size of the marker band (bp)	Primer	Total # markers / cultivar	
RAPD	Sakkoty Aakab	500 730	OPZ-06 OPB-05	2	800 900 1200	OPZ-06 OPB-05 OPB-07	3	5
	Bertmoda Aakab			0	1600	OPZ-06	1	1
	Bertmoda Abo El-Rish			0	800 1100	OPB-07 OPZ-07	2	2
	Malkaby Abo El-Rish			0	900	OPB-07	1	1
	Gandila Aakab			0	750	OPZ-05	1	1
	Total			2			8	10
ISSR	Bertmoda Aakab	400	IS10	1			0	1
	Bertmoda Abo El-Rish			0	450, 510	IS10	2	2
	Malkaby Aakab			0	500	IS10	1	1
	Siwi Dakhla	350	IS10	1			0	1
	Fraihy Hafr-El-Baten			0	360	IS9	1	1
	Total			2			4	6

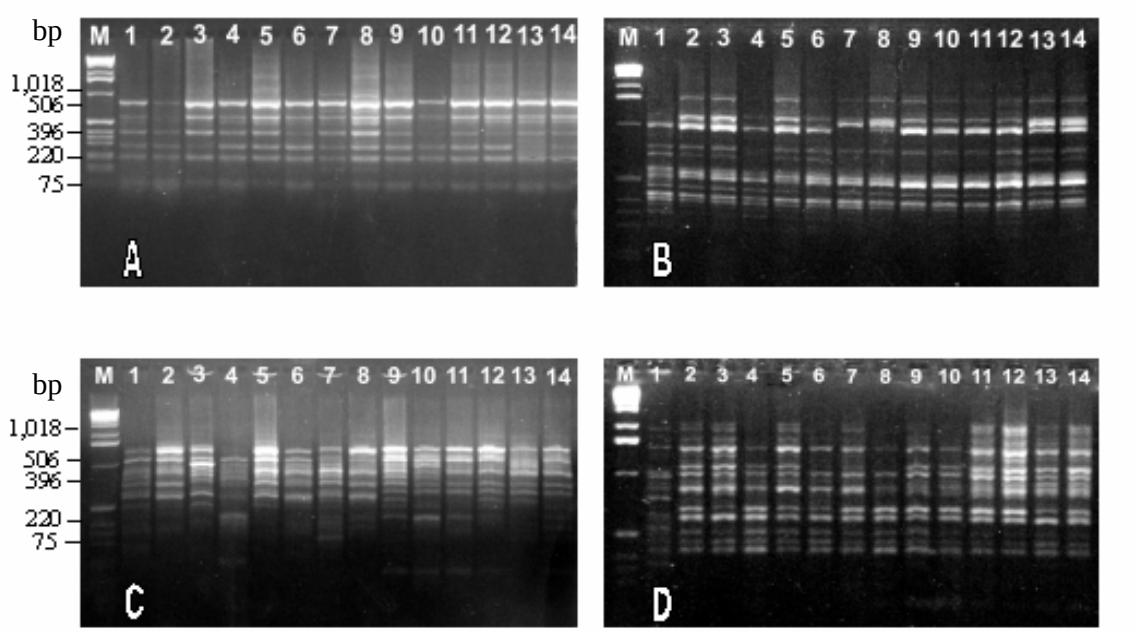

Fig. (1): RAPD profiles for the 14 date palm accessions as detected with primers OPB-06 (A), OPB-08 (B), OPB-11 (C), and OPO-07 (D). Lanes 1 to 14 represent: SAK-AK, SAK-AB, BRT-AK, BRT-AB, MLK-AK, MLK-AB, GND-AK, GND-AB, SIW-KH, SIW-DK, SIW-HB, SIW-TZ, FRA-HB and FRA-TZ. M: 1 Kb ladder DNA marker.

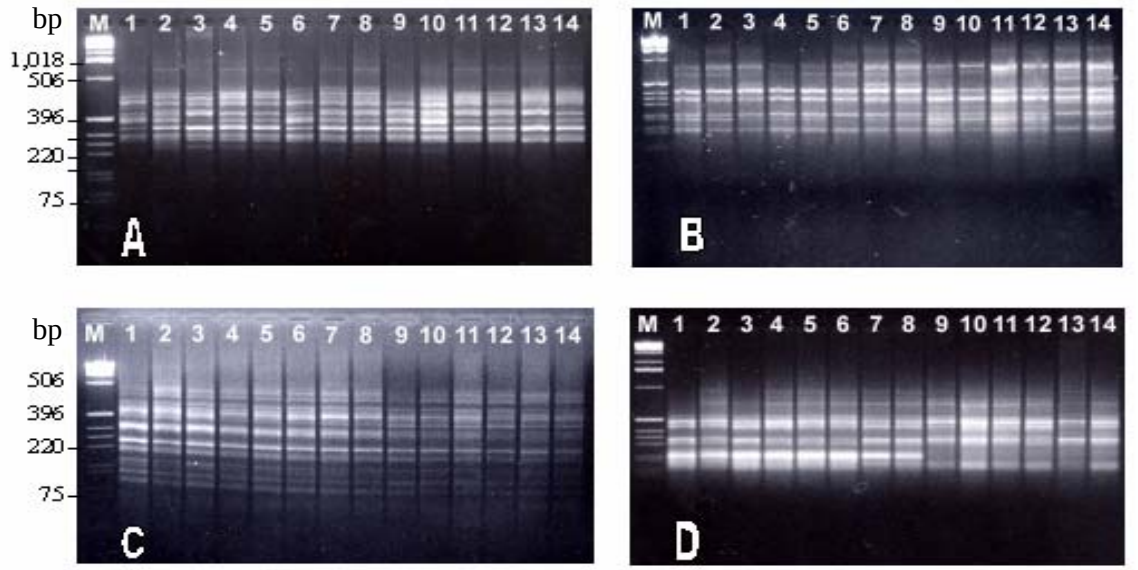

Fig. (2): ISSR profiles of the 14 date palm accessions using the primers: IS3 (A), IS4 (B), IS7 (C), IS9 (D). M: 1 Kb ladder DNA marker. Lanes 1 to 14 represent: SAK-AK, SAK-AB, BRT-AK, BRT-AB, MLK-AK, MLK-AB, GND-AK, GND-AB, SIW-KH, SIW-DK, SIW-HB, SIW-TZ, FRA-HB and FRA-TZ.

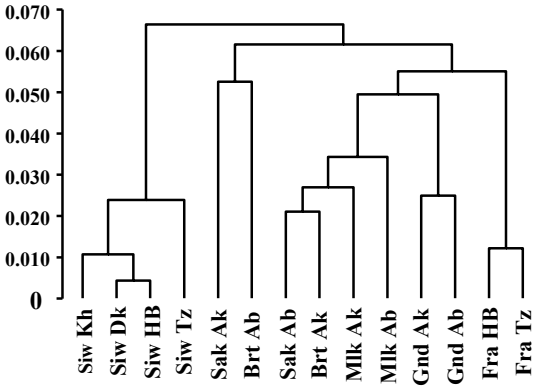

Fig.(3): Dendrogram for the 14 Date Palm accessions constructed from the RAPDs data using UPGMA and similarity matrices computed according to Dice coefficient.

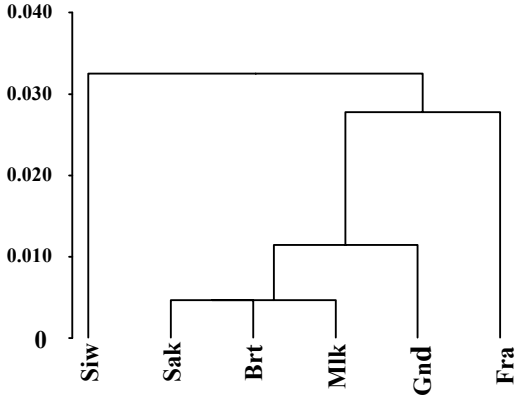

Fig.(4): Dendrogram for the 6 Date Palm cultivars constructed from the RAPDs data using UPGMA and similarity matrices computed according to Dice coefficient.

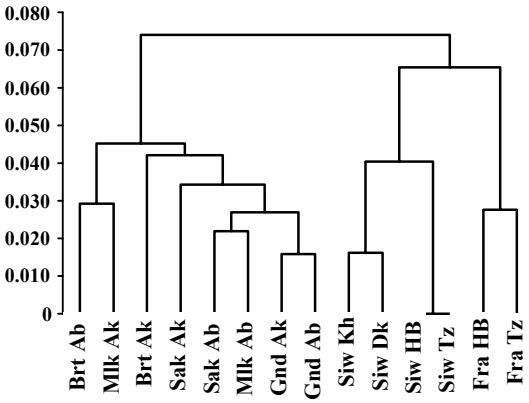

Fig.(5): Dendrogram for the 14 Date Palm accessions constructed from the ISSRs data using UPGMA and similarity matrices computed according to Dice coefficients.

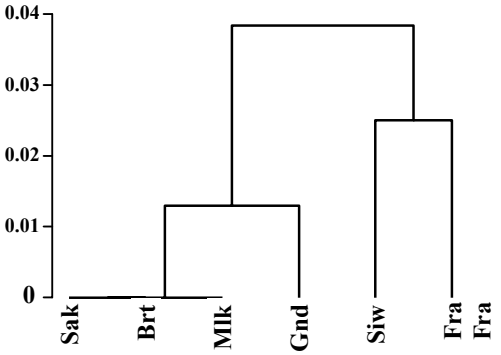

Fig.(6): Dendrogram for the 6 Date Palm cultivars constructed from the ISSRs data using UPGMA and similarity matrices computed according to Dice coefficients.

Based on the similarity matrix developed by analyzing only the common bands between the different accessions representing each cultivar a dendrogram (Fig. 6) was constructed. The obtained dendrogram was divided into two main clusters; one cluster included cultivars Siwi and Fraihy, while the other one included two subclusters, with cultivar Gandila in one subcluster, and Sakkoty, Bertmoda and Malkaby in the second

subcluster. Thus, this distribution clustered Gandila in-between two groups of cultivars, one group included the cultivars from the Oases and the other group included the cultivars from Aswan. In this respect, Adawy *et al.* (2004) developed a dendrogram constructed by cluster analysis using ISSR based genetic distance. The overall tree topology suggested a rather weak grouping association except for the cultivars Shameia and Bertmoda, which clustered together.

Among the 10 studied ISSR primers, 2 revealed unique markers characterizing 3 of the 6 date palm cultivars. The 2 primers exhibited positive markers only. The total number of unique ISSR markers was 5 (Table 4). Cultivars Siwi and Fraihy were identified by the highest number of positive unique markers (2 positive markers for each). These markers ranged in size from 140 to 420 bp. The cultivar Gandila was characterized by the presence of $IS4_{370bp}$ band, which was absent in all the other cultivars. While, Siwi could be distinguished by the presence of two bands $IS4_{140bp}$ and $IS8_{380bp}$, while the cultivar Fraihy could be distinguished by the presence of two bands $IS4_{220bp}$ and $IS4_{420bp}$. The presence of unique ISSR markers among the various date palm cultivars indicates the advantage of this approach for fingerprinting purposes.

In addition, unique markers were observed at the intravarietal level, i.e., between accessions. As shown in Table (5), the total number of unique markers on the intravarietal level was six, comprising four negative and 2 positive markers. These unique markers identified five accessions Bertmoda/Aakab ($IS10_{400bp}$, positive), Bertmoda/Abo-El-Rish ($IS10_{450bp}$, and $IS10_{510bp}$, negative), Malkaby/Aakab ($IS10_{500bp}$, negative), Siwi/Dakhla ($IS10_{350bp}$, positive), and Fraihy/Hafr-El-Baten ($IS9_{360bp}$, negative). In this context, Adawy *et al.* (2002; 2004) revealed unique markers characterizing each of four cultivars from Delta, Egypt and each of five cultivars from upper Egypt, respectively. The total number of unique ISSR markers was 24.

Therefore, the results of the present study revealed that DNA markers represent an efficient tool for estimating the genetic variability and the genetic relationships among closely related genotypes of date palm. This could represent a useful tool in date palm improving programs.

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الملخص العربي

التوصيف الجزيئي لبعض المصادر الوراثية من نخيل البلح المصري

باستخدام الواسمات الجزئية من نوع ISSR, RAPD

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تم دراسة التباين و علاقات القرابة الوراثية بين ١٤ عينة من نخيل البلح المصري (*Phoenix dactylifera* L.) تمثل ٦ أصناف مجمعة من ٦ مناطق بمصر باستخدام ٢٧ بادئ RAPD و ١٠ بادئات ISSR و قد كانت نسبة التباين الوراثي التي أظهرتها تقنيتي الـ RAPD , ISSR تقدر بـ ٢٥,٢% و ٢٨,٦% على التوالي. و هذه المستويات المنخفضة للتباين تعكس الأساس الوراثي المحدود لهذه العينات و الخلفية الوراثية المتقاربة . كما تم دراسة التشابه الوراثي بين ١٤ عينة باستخدام معامل Dice حيث كانت تتراوح ما بين ٩٦,١% إلى ٩٩,٥ % في الـ RAPD و ما بين ٩٦,٢ % إلى ١٠٠% في الـ ISSR .

أظهرت دراسة التباين ما بين الستة أصناف بناء على بيانات RAPD , ISSR أعلى نسبة تشابه وراثي بين صنف برتمودا و كل من أصناف ملكابي و سكوتي و قد تبين من الدندروجرامات المبنية على نتائج كل من الـ RAPD , ISSR أن العينات التابعة لكل من الأصناف الثلاثة فريحي و سيوي و جنديلة تقع في مجموعات مختلفة بينما حدث تغيير في مواقع بعض العينات التابعة للأصناف الأخرى في الدندروجرامات المختلفة مما يظهر تقاربها في الخلفية الوراثية و قد أمكن تحديد دلائل جزيئية مميزة لكل صنف تم استخدامها في تحديد بصمة وراثية فريدة لكل منها حيث أظهر الـ RAPD ١٧ دليلا جزيئيا مميزا لأربعة أصناف بينما اظهر الـ ISSR خمسة دلائل جزيئية مميزة لخمسة أصناف من النخيل تحت الدراسة بالإضافة إلى ذلك فقد نجحت كل من تقنيتي الـ RAPD , ISSR في تحديد دلائل جزيئية مميزة لخمسة تراكيب وراثية . مما يدل على كفاءة هذه التقنيات في التمييز بين الأصناف المتقاربة وراثيا .

الكلمات الدالة: نخيل البلح (*Phoenix dactylifera* L.) , واسمات د ن ا , RAPD , ISSR , واسمات فريدة مميزة للطراز الوراثي .