Genomic diversity in date palm *(Phoenix dactylifera L.)* as revealed by AFLPs in comparison to RAPDs and ISSRs

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ABSTRACT

Fourteen date palm (Phoenix dactylifera L.) accessions collected from different locations in Egypt representing six Egyptian cultivars: Sakkoty, Bertmoda, Malkaby, Gandila, Fraihy and Siwi were assayed using 16 AFLP primer combinations. AFLP analysis generated a total of 657 amplicons representing a level of polymorphism of 45.8%. The genetic similarity and relationships were estimated among the 14 accessions and among the six cultivars according to Dice coefficient.The AFLP-based dendrograms clustered the genotypes of some cultivars together, i.e. Fraihy and Gandila. The genotypes of Siwi cultivar were clustered together also, but they exhibited some degree of intravarietal variation. The other three cultivars (Sakkoty, Bertmoda, and Malkaby) showed higher degree of intravarietal variation. Moreover, at the intervarietal level, the AFLP assay separated the oases cultivars i.e., Siwi and Fraihy, from the cultivars from Aswan, i.e. Sakkoty, Bertmoda, Malkaby and Gandila. AFLP analysis permitted the characterization of each cultivar by specific unique markers.Data from RAPD's and ISSR's, previously obtained on the same 14 accessions were combined with AFLP's to generate more accurate relationships based on large and versatile genome coverage. The dendrogram based on the combined data from the different types of markers (RAPD, ISSR and AFLP) was closest to the AFLP-based dendrogram. To evaluate the efficiency of the different marker systems, the sum effective number of alleles (SENA), the average expected heterozygosity for polymorphic markers (Hav(p)), the effective multiplex ratio (E) and marker index (MI) were calculated. The AFLP exhibited considerably high sum effective number of alleles (205.7) compared to RAPD and ISSR (45.1 and 17.8, respectively). The average heterozygosity was also higher in AFLP (0.39) than in RAPD and ISSR (0.36 and 0.35, respectively). The MI was 117.3 in AFLP while it was 95.9 and 10.4 in RAPD and ISSR, respectively. Thus, the results indicated that AFLP is more effective in detecting high level of polymorphism. The correlation coefficient was considerably high between RAPD and ISSR (0.68), and it was lower between RAPD and AFLP (0.23) than that between AFLP and ISSR (0.34). The results confirmed that different marker systems differ in the mechanism of detecting polymorphism, genome coverage and the ease of application. Therefore, they could complement each other to draw more accurate conclusions.

Key words: *Date palm, AFLP, Genotype unique markers, SENA, Expected heterozygosity for polymorphic loci ($H_{av\,(P)}$), Marker index (MI).*

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an arborescent, dioecious, highly heterozygous, monocotyledonous plant, with a very slow growth rate and a late reproductive phase. Palm tree is an excellent candidate for cultivation in arid and semi-arid regions of the world due to its high tolerance to environmental stresses. It is of great economic importance to oasis agriculture and creates favorable conditions for improving secondary crops. It is one of the most important fruit crops in Egypt, North Africa and in the Arab World. In spite of its economic importance, improvement programs in this crop are very limited. Therefore, the development of accurate fingerprint characterizing the different genotypes would be of great value in improvement of this important crop. In this concern, morphological characters have traditionally provided signatures of varietal genotype and purity. Recently, molecular markers have provided complementary and accurate tools for genetic analysis and germplasm characterization. These markers have been applied by different authors to tropical or subtropical species for which very limited research resources are available (Henry, 1998).

The AFLP technique (Vos *et al.,* 1995) is based on the PCR amplification of selected restriction fragments of a total genomic DNA digest. The AFLP markers are usually dominant markers. These markers are usually revealed in denaturing sequencing polyacrylamide gels, using radioactive or fluorescence labeling, allowing simultaneous identification of a large number of products in the same reaction, covering many loci in a single assay. This technique combines the reliability of RFLPs with the advantages of PCR methods. Therefore, the AFLP technique permits the development of more accurate comprehensive fingerprints (Vos *et al.*, 1995). AFLPs have been successfully used to study the genomes of different plant species (Aranzana *et al*., 2001; Baldoni *et al*., 2000; Bellin *et al*., 2001; Carr *et al*., 2003; Dehmer, 2003; Hagen *et al*., 2001; Shenghua *et al*., 2002 and Tavaud *et al*., 2001). AFLPs have been also successfully employed to identify date palm cultivars (Adawy *et al.,* 2004b; Cao and Chao, 2002; Chao and Devanand, 2003; Diaz *et al*., 2003 and El-Khishin *et al*., 2003). Different authors discussed the importance of integration between AFLP and other techniques to avoid erroneous identification and to evaluate genetic relatedness and variability within populations more accurately (Goulão *et al*., 2001; Martins *et al*., 2001 and Vignani *et al*., 2002). Several researchers compared the efficiency of the AFLP technique in the genome analysis of different plant species with different molecular markers such as RFLPs, RAPDs, ISSRs and SSRs and concluded that AFLP is superior over the other techniques (Berio *et al*., 2001; Hurtado *et al*., 2001; Lanham and Brennan, 1999; McGregor *et al*., 2000 and Virk *et al*., 2000).

Little is currently known about the molecular characterization of date palm cultivars, though a great effort is now on its way in this particular discipline. Thus, in the present investigation AFLP analysis has been carried out to estimate the level of variability and assess the genetic relationships among accessions collected from different locations and representing six date palm cultivars. Moreover, an attempt has been made to identify unique DNA markers characterizing each cultivar. In addition, the efficiency of AFLP markers in the date palm genome analysis has been compared with RAPD and ISSR markers used in a previous study (Hussein *et al.,* 2005).

MATERIALS AND METHODS

Plant material

Fourteen date palm accessions were collected from different locations in Egypt (Fig 1). These accessions represent six date palm cultivars: Sakkoty, Bertmoda, Malkaby, Gandila, Fraihy (dry cultivars), and Siwi (semi-dry cultivar).

Extraction and Purification of Genomic DNA

Genomic DNA extraction, purification an bulking were performed as described by Hussein *et al.* (2005).

AFLP analysis

This was performed as described by Vos et al (1995) by using the large genome system AFLP I (Gibco BRL, USA) (Cat.No. 10544). About 300 nanograms of DNA was digested with EcoR1 and Mse1 enzymes and digested fragments were ligated with adaptors. The ligated DNA fragments were amplified in 25 µl volume. Pre-amplification using primers with-one 3' selective nucleotide was carried out. Each reaction (25 µl final volume) contained the following: 75 ng each of primers EcoRI-core and MseI-core; 0.2 mM of each dATP, dCTP, dGTP, dTTP (Boehringer Manheim); 1.0 u Taq (HT Biotechnologies);1x reaction buffer (HT Biotechnologies); and 300 ng of digested ligated DNA. Samples were subjected to 30 cycles of the following PCR programme: 94ºC for 30 s, 60ºC for 30 s, 72ºC for 60 s. PCR products were diluted by the addition of 50 µl of buffer (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA).

Diluted pre-amplification products were used as templates for selective amplifications. According to the manufacturer's instructions, selective amplification reactions (final volume 20 µl) contained the following: 16 ng *Eco*RI primer, 15 ng *Mse*I primer, 0.2 mM of each dATP, dCTP, dGTP, dTTP, 0.5 u Taq and 1x reaction buffer. The following amplification profile was used: 1 cycle of 94ºC for 30 s, 65ºC for 30 s, 72ºC for 60 s; 11 cycles of 94ºC for 30 s, 60ºC for 30 s, 72ºC for 60 s and 23 cycles of 94ºC for 30 s, 56ºC for 30 s, 72ºC for 60 s. Half volume of formamide dye (98% formamide, 10 mM EDTA pH 8.0, 0.5 mg ml^{-1} Xylene Cyanol FF) was added. Samples were denatured for 5 min in a boiling water bath and thereafter electrophoresed through preheated (80 W for 30 min) denaturing polyacrylamide gels at a constant power of 80 W for 1 hr 45 min.

Data analysis

The genetic similarity coefficient, similarity matrices and cluster analysis were estamited as mentioned in Hussein *et al.* (2005). The sum of effective number of alleles (SENA) was calculated from the formula: $SENA = \sum [(1 / \sum {P_i}^2) - 1]$ Where the p_i is the frequency of the *i*-th allele (Powell *et al.,* 1996). The observed heterozygosity was calculated as the ratio between heterozygous genotypes and the total analyzed genotypes for each locus. However, the arithmetic mean of the expected heterozygosity for the polymorphic loci ($H_{av(p)}$) was calculated for each marker class as follows: $H_{av(p)} = \sum H_p/n_p$ and the fraction of polymorphic loci (β) is calculated as: $\beta = n_p / (n_p + n_{np})$, where n_p is the number of polymorphic loci and n_{np} is the number of non-polymorphic loci. Then, the average heterozygosity is calculated as follows: $H_{av} = \beta (\sum H_p/n_p)$ (Powell *et al.,* 1996). The number of polymorphic markers per gel lane, called marker index (*MI*), is simply the product of effective multiplex ratio and the average expected heterozygosity for the polymorphic markers and calculated from the formula: $MI = EH_{av(p)}$, where the effective multiplex ratio (E) is the number of loci polymorphic in the germplasm set of interest

analyzed per experiment and calculated from the formula: $E=n\beta$ (Powell *et al.,* 1996).

The software used through this study were SPSS 10.0, POPGEN 3.2, XLSTAT-Pro 7.1, and Microsoft EXCEL.

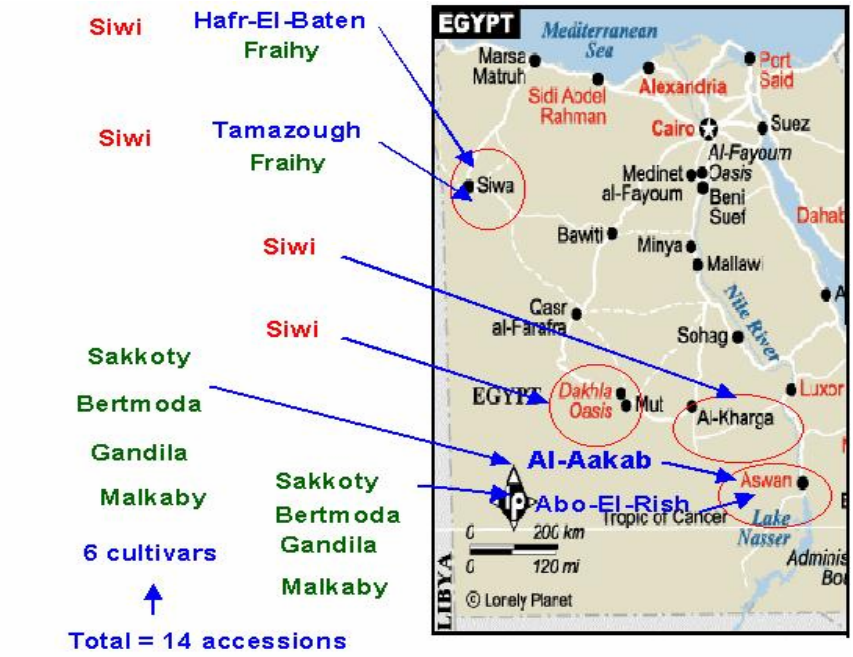

Fig. (1): Names of the 14 date palm accessions collected from six locations in Egypt.

RESULTS AND DISCUSSION

Molecular markers are efficient tools for cultivar identification and estimation of relatedness through DNA fingerprinting. In the present investigation, AFLP was employed to assess the genetic polymorphism *within* and *among* six Egyptian date palm cultivars (Sakkoty, Bertmoda, Malkaby, Gandila, Siwi, and Fraihy).

Polymorphism and genetic relationships as detected by AFLPs

Among 20 AFLP primer combinations prescreened, sixteen were selected for their scorable results. AFLP analysis using the 16 selective primer combinations generated a total of 657 amplicons. The mean number of amplicons per assay was 41.1. Meanwhile, the number of polymorphic amplicons was 301 representing a level of polymorphism of 45.8% and an average number of polymorphic bands of 18.8 per AFLP primer combination (Table 1). The size of the AFLP amplified fragments ranged from 50 bp to 800 bp. The number of distinguishable bands detected after selective amplification varied among the different primer combinations (Figs. 2 A and B). The highest number of amplicons (74) was exhibited by the primer combination (EaacXMcat), whereas the lowest number was 15 as revealed by primer combination EaggXMctg (Table 1). The level of polymorphism ranged from 17.6% to 80.8%. In this context, Cao and Chao (2002), initially, screened 32 primer combinations using 2 date palm cultivars and found that the different primer pairs produced 50-70 bands ranging in

size from 50-700 bp. Moreover, these authors used four primer sets to detect the polymorphism among 21 date palm cultivars and detected 328 polymorphic bands. Diaz et al. (2003) employed five AFLP primer combinations to analyze 3 date palm cultivars yielding 310 AFLP fragments with 220 polymorphic fragments revealing an average polymorphism of 72%. El-Khishin et al. (2003) profiled five Egyptian date palm cultivars using six AFLP primer combinations that generated a total of 433 amplicons with a mean of 72.1 amplicons per assay. The level of polymorphism ranged from 42.42% to 59.02%. On the other hand, Adawy et al. (2004b) employed 28 AFLP primer combinations to examine the phylogenetic relationships among five Upper Egypt date palm cultivars. The 28 primer combinations produced 1135 bands with 41.59% polymorphism across cultivars. The average number of polymorphic bands was 16.86 per AFLP primer combination. These discrepancies in the number of AFLP amplicons and the percentage of polymorphisms could be attributed to the use of different date palm cultivars and/or different primer combinations.

To determine the genetic relationships among the 14 accessions, the scored data (1 for presence and 0 for absence) resulting from the 16 primer combinations were used to compute the similarity matrices through Dice coefficient. The genetic similarity estimates ranged from 80.4% to 99%. This confirmed the high level of genetic similarity among the studied cultivars. The highest genetic similarity (99%) was between Siwi/El-Kharga and Siwi/El-Dakhla. While the lowest genetic similarity (80.4%) was detected between Malkaby/Aakab and Siwi/Tamazough. The genetic relationships among the six cultivars, were estimated using the common bands between the different accessions representing each cultivar, the genetic similarity estimates ranged from 96.1% to 99.9%. The highest genetic similarity (99.9%) was between Sakkoty and Bertmoda, while the lowest genetic similarity (96.1%) was detected between Fraihy and Gandila. Devanand and Chao (2003) using four AFLP primer combinations screened the accessions of two date palm cultivars Medjool (23 accessions) and Deglet Noor (33 accessions), they found a genetic similarity of ≥ 99% among the accessions of Deglet Noor. They suggested that this high genetic similarity could be due to the founder effect from initial introduction. They added that the small differences in bands among some accessions could be explained by natural mutations. El-Khishin *et al.* (2003) reported genetic similarity estimates ranging from 64.4% to 76.7% among five date palm cultivars. This revealed moderate levels of genetic similarity among the studied cultivars. Adway *et al.* (2004b) studied the genetic relationships among five date palm cultivars based on the AFLP data polymorphism and estimated the genetic similarity as ranging from 83.2% to 90.9%. The highest genetic similarity (90.9%) was between Shameia and Sakkoty; this was followed by 90.2% between Sakkoty and Malkaby, while the lowest genetic similarity (83.2%) was detected between Gandila and Bertmoda.

The AFLP-based dendrograms obtained from UPGMA cluster analysis of genetic distances (GDs) is presented in Fig (3 A and B). The results showed that the AFLP assay clustered the genotypes of some cultivars together, i.e. Fraihy and Gandila, while the genotypes of Siwi cultivar were clustered together also, but they showed some degree of intravarietal variation. The other three cultivars (Sakkoty, Bertmoda, and Malkaby) showed higher degree of intravarietal variation. The cultivar Malkaby showed the highest degree of intravarietal variation (Fig. 3

A). These intravarietal variations were expected due to the dioecious nature of date palm trees. Furthermore, the dendrogram revealing the relationships at the cultivars level, comprised two main clusters, one containing Siwi and Fraihy while the other cluster revealed two subclusters; Malkaby was separated in one subcluster while all the other cultivars formed the second subcluster.

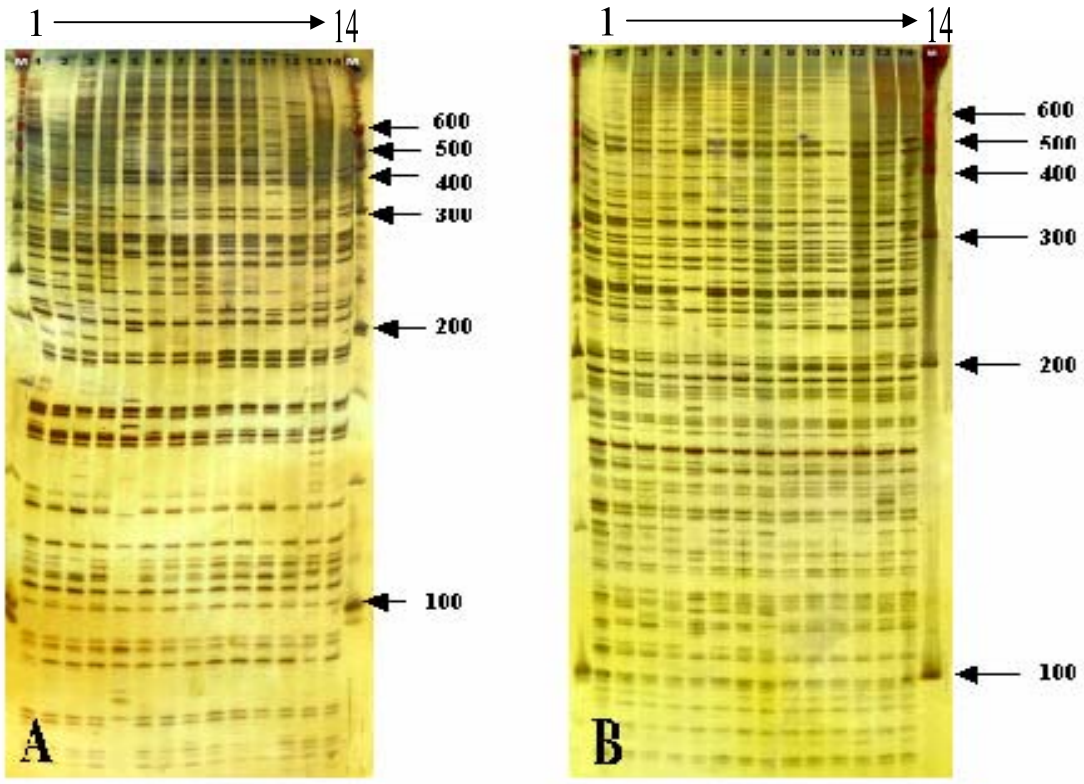

Fig. (2): AFLP profiles of the 14 date palm accessions as revealed by the primer combination E_{act} X M_{cta}. (A) and the primer combination E_{agc} X M_{caa}. (B).Lanes 1 to 14 represent: SAK-AK, SAK-AB, BRT-AK, BRT-AB, MLK-AK, MLK-AB, GND-AK, GND-AB, SIW-KH, SIW-DK, SIW-HB, SIW-TZ, FRA-HB and FRA-TZ. M: DNA molecular weight marker (100 bp Ladder).

Therefore, the overall tree separated the oases cultivars together, i.e., Siwi and Fraihy, while the cultivars Sakkoty, Bertmoda, Malkaby, and Gandila, from Aswan, were grouped together. The present results are not in good accordance with those of Adawy *et al.* (2004b) as they showed that the dendrogram based on the AFLP analysis separated the cultivar Bertmoda from all the other tested cultivars, the cultivars Gandila and Malkaby were the most genetically similar and Shameia and Sakkoty come next. While, El-Seme'a (2003) revealed that Sakkoty and Malkaby are very close, Shamia and Bartamuda are very close too, while Gondeila is in-between the two clusters of cultivars.

As shown in Table (2), the AFLP analysis permitted the distinction among the

six studied date palm cultivars and the characterization of each cultivar by specific unique markers. A total of 25 unique markers (15 positive and 10 negative markers) were identified by the 16 AFLP primer combinations. The total number of unique markers per cultivar ranged from 1 to 10. The cultivar Fraihy was characterized by the highest number of unique positive markers (7) in addition to 3 unique negative markers. Cultivar Siwi was identified by 4 positive and 1 negative unique markers. Gandila exhibited 4 unique positive markers and one unique negative marker, while cultivar Sakkoty exhibited one unique negative marker. Cultivar Malkaby showed 4 unique negative markers only, while cultivar Bertmoda did not show any unique marker neither positive nor negative. Table (2) also illustrates that the different primer combinations identified different numbers of unique markers. Moreover, AFLP-accession-specific markers were detected in eight out of the 14 date palm accessions (Table 3). The total number of accession-specific markers was 95 comprising only 8 positive markers and 87 negative markers. The accession Malkaby/Aakab was distinguished by the highest number of AFLP markers (62 negative unique markers). Ten unique negative markers plus one unique positive marker characterized the accession Siwi/Tamazough. Six AFLP negative markers identified the accession Sakkoty/Aakab, while four unique negative markers and one unique positive marker identified Bertmoda/Aakab. Only one AFLP negative marker characterized each of the accessions Gandila/Aakab, Gandila/Abo-El-Rish and Siwi/Hafr-El-Baten. While, the accession Fraihy/Hafr-El-Baten was distinguished by 6 positive and 1 negative unique AFLP markers. The present results clearly demonstrate the efficiency of the AFLP marker system in date palm cultivar fingerprinting using a few number of primer combinations. This could be attributed to the high multiplex ratio of the AFLP technique. These results are in consistence with the findings of different authors on different plant species i.e. Berio *et al*. (2001), Cao and Chao (2002), Hussein *et al.* (2003) and Adawy *et al.* (2004a, b and c). Adawy *et al.* (2004b) obtained 58 positive (UPM) and 133 negative markers (UNM) through assaying five Egyptian date palm cultivars using AFLP technique. The number of UPM ranged from 6 to 22 and the number of UNM ranged from 15 to 57 in the different cultivars.

In a current work (Hussein *et al*. 2005) the authors of the present investigation used different markers, i.e., RAPDs and ISSRs on the same 14 accessions representing the six date palm cultivars. This work has been extended in the present study to fingerprint these accessions with AFLP. The different types of markers, i.e., RAPD, ISSR, and AFLP, revealed different levels of genetic similarity among the six date palm cultivars. This could be due to the difference in the polymorphism detection mechanisms by the different types of markers. DNA sequence variation at primer binding sites and DNA length differences between primer binding sites produce the RAPD polymorphisms. ISSR polymorphism is the result of differences in the number of repetitive di-tri- or tetra-nucleotide units and the number and the site of anchors, if present. Whereas, AFLP technique reflects restriction size variation. Therefore, combining the data obtained from the different types of markers may reveal moreinformative genetic relationships.

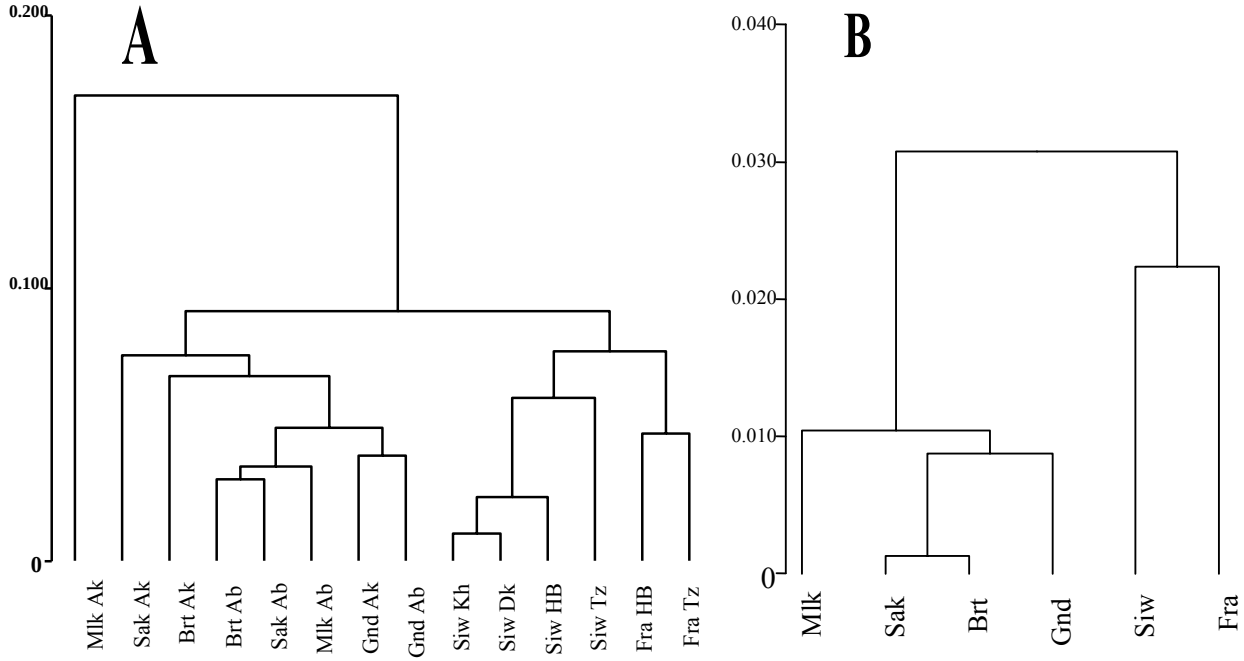

Fig. (3): Dendrograms for the 14 date palm accessions (A) and the 6 cultivars (B) constructed from the AFLPs data using Unweighed Pair-group Arithmetic (UPGMA) and similarity matrices computed according to Dice coefficients.

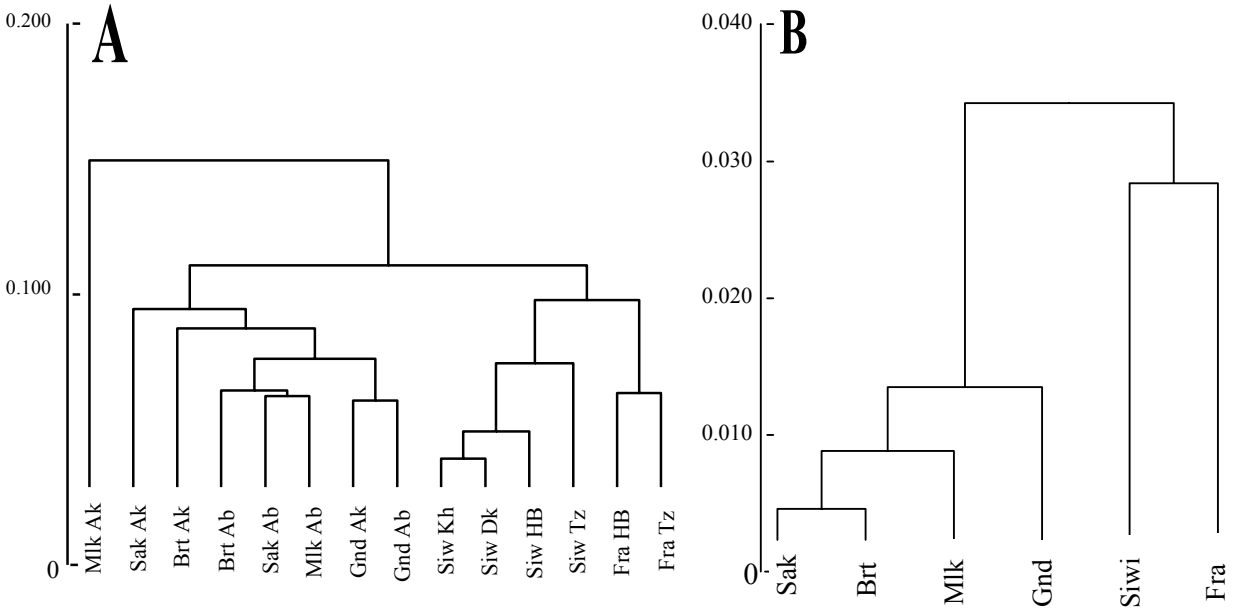

Fig. (4): Dendrograms for the 14 date palm accessions (A) and the 6 cultivars (B) constructed from the combined data of RAPDs, ISSRs, and AFLPs using Unweighed Pair-group Arithmetic (UPGMA) and similarity matrices computed according to Dice coefficients.

Combined RAPDs, ISSRs, and AFLPs data

The data scored from RAPDs, ISSRs, and AFLPs were combined and computed to generate more accurate relationships based on large and versatile genome coverage. The highest genetic similarity (98.9%) was between Siwi/El-Kharga and Siwi/El-Dakhla. While, the lowest genetic similarity (86.1%) was detected between Malkaby/Aakab and Siwi/Tamazough. Moreover, the estimated genetic similarity among the six date palm cultivars, revealed that the highest genetic similarity (99.8%) was between Sakkoty and Bertmoda and the lowest genetic similarity (96.5%) was detected between Fraihy and Gandila. The general high genetic similarity and the low polymorphism observed in the present study *among* and *within* the six studied cultivars reflect a narrow genetic pool in date palm cultivars. Similar conclusion was drawn by Goulão *et al.* (2001) and Hussein *et al.* (2002) in apple and Cotton, respectively. On the other hand, Sedra *et al.* (1998) hypothesized that the relatively low polymorphism and the lack of evident organization observed among the date palm varieties grown in Morocco could be related to the mode of introduction and maintenance of germplasm.

The cluster analysis of the 14 studied accessions using the different marker systems revealed three dendrograms exhibiting unique topology with some similarities; for example the grouping of the accessions of each of the cultivars Fraihy, Siwi and Gandila in separate groups. However, the reshuffling in the position of some of the accessions within the different dendrograms could be attributed to the narrow genetic background of these date palm cultivars, the dioecious nature of date palm or the occurrence of gene flow among these accessions. Moreover, incomplete similarity of the dendrograms could be also attributed to the nature of evolutionary mechanisms underlying the variation measured by the different types of markers (Hussein *et al.,* 2002). However, the dendrogram based on the combined data from RAPD, ISSR, and AFLP analyses (Fig. 4 A&B) revealed relationships closest to those illustrated by the AFLP-based dendrogram. The dendrogram separated the Malkaby/Aakab accession from all the other date palm genotypes included in the study. The other date palm accessions clustered into two subclusters. The dendrogram confirmed that the genotypes Siwi/El-Kharga and Siwi/El-Dakhla are the most genetically similar among the studied genotypes, while Malkaby/Aakab was the most distinct accession. Cultivars Fraihy and Gandila did not show any intravarietal variation.

Moreover, at the intervarietal level the dendrogram was divided into two main clusters; one cluster included cultivars Siwi and Fraihy, while the other one included two subclusters. One of these two subclusters contained cultivar Gandila while the other included cultivars Sakkoty, Bertmoda and Malkaby. This distribution clustered Gandila in-between two cultivar groups, one group represented the cultivars from the Oases and the other group included the remaining cultivars from Aswan. In this respect, Adawy *et al.* (2004b) developed a dendrogram for five Egyptian date palm cultivars based on the combined data from RAPD, ISSR, and AFLP analyses that showed the same grouping pattern as generated by AFLP and thus suggesting that AFLP is the most effective marker system.

Table (1): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by AFLP markers among the 14 accessions.

Primer Combination	Total # amplicons	Monomorphic amplicons	Polymorphic amplicons	% polymorphism
E_{aac} X M_{cat}	74	23	51	68.9
E_{aca} X M_{caa}	67	42	25	37.3
E_{aca} X M_{cat}	49	30	19	38.8
E_{acg} X M_{ctt}	26	15	11	42.3
E_{act} X M_{cag}	17	14	3	17.6
E_{act} X M_{cta}	45	26	19	42.2
E_{agc} X M_{caa}	62	35	27	43.5
E_{agc} X M_{cag}	35	25	10	28.6
E_{agc} X M_{cta}	21	13	8	38.1
E_{agc} X M_{ctc}	26	5	21	80.8
E_{aca}X M_{ctc}	39	16	23	59.0
E_{acg}X M_{cat}	30	12	18	60.0
E_{act}X M_{caa}	62	47	15	24.2
E_{agg}X M_{caa}	56	33	23	41.1
E_{agg}X M_{ctc}	33	13	20	60.6
E_{agg}X M_{ctg}	15	7	8	53.3
Total	657	356	301	45.8
Average	41.1	22.3	18.8	

Table (2): The date palm cultivars characterized by unique positive and/or negative AFLP 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 combination	Total # markers / cultivar	Size of the marker band (bp)	Primer combination	Total # markers / cultivar	
Sakkoty			0	93	E_{acg} X M_{ctt}	1	1
Malkaby			0	88, 89	E_{agg} X M_{ctc}	4	4
				180	E_{aca} X M_{ctc}		
				94	E_{aca} X M_{cat}		
Gandila	490, 495	E_{agg} X M_{ctc}	4	161	E_{agg} X M_{caa}	1	5
	202	E_{act} X M_{caa}					
	130	E_{aca} X M_{caa}					
Siwi	125	E_{agg} X M_{caa}	4	147	E_{aac} X M_{cat}	1	5
	122	E_{act} X M_{caa}					
	179	E_{aac} X M_{cat}					
	164	E_{agc} X M_{caa}					
Fraihy	140	E_{agg} X M_{caa}	7	133	E_{agg} X M_{caa}	3	10
	201	E_{act} X M_{caa}		700, 800	E_{aca} X M_{ctc}		
	96, 157	E_{aca} X M_{ctc}					
	275, 280	E_{aca} X M_{caa}					
	350	E_{acg} X M_{ctt}					
Total			15			10	25

Table (3): The date palm accessions characterized by unique positive and/or negative AFLP markers, marker size and total number of markers identifying each accession.

Accession	Unique positive markers			Unique negative markers			Grand Total
	Size of the marker band (bp)	Primer combination	Total # markers / accession	Size of the marker band (bp)	Primer combination	Total # markers / accession	
Sakkoty (Aakab)			0	290 200 180 70 310 283	E_{aac} X M_{cat} E_{aca} X M_{ctc} E_{acg} X M_{cat} E_{act} X M_{caa} E_{aca} X M_{cat} E_{act} X M_{cta}	6	6
Bertmoda (Aakab)	230	E_{aca} X M_{ctc}	1	320, 325 148 350	E_{aac} X M_{cat} E_{agg} X M_{caa} E_{agc} X M_{caa}	4	5
Malkaby (Aakab)			0	81, 82, 95, 96, 122, 123, 135, 154, 155, 164, 260, 265, 287, 340, 550 100, 127, 128, 143, 165, 220 133, 260, 263 201 105 80, 84, 100, 112 159, 160, 194, 195 72, 87, 105, 183, 240, 295, 300 75, 82, 375 600 75, 82, 121, 270 125, 126, 235, 260 75, 78, 79, 80, 129, 130 150, 160, 220	E_{aac} X M_{cat} E_{agg} X M_{ctc} E_{agg} X M_{ctg} E_{agg} X M_{caa} E_{act} X M_{caa} E_{acg} X M_{cat} E_{aca} X M_{ctc} E_{aca} X M_{caa} E_{aca} X M_{cat} E_{acg} X M_{ctt} E_{act} X M_{cta} E_{agc} X M_{caa} E_{agc} X M_{cag} E_{agc} X M_{cta}	62	62
Gandila (Aakab)			0	225	E_{act} X M_{cta}	1	1
Gandila (Abo-El-Rish)			0	85, 88	E_{agg} X M_{caa}	2	2
Siwi (Hafr-El-Baten)			0	300	E_{act} X M_{cag}	1	1
Siwi (Tamazough)	186	E_{aca} X M_{ctc}	1	255, 256 191, 250 155 283, 285 96, 98, 120	E_{aac} X M_{cat} E_{act} X M_{caa} E_{agg} X M_{ctg} E_{agc} X M_{caa} E_{agc} X M_{ctc}	10	11
Fraihy (Hafr-El-Baten)	158 184, 225, 243, 290 240	E_{agc} X M_{cag} E_{aca} X M_{ctc} E_{agc} X M_{ctc}	6	194	E_{agc} X M_{ctc}	1	7
Total			8			87	95

Comparison among the efficiency of RAPD, ISSR, and AFLP markers in the date palm genome analysis

As shown in Table (4), the AFLP and ISSR assays revealed the highest percentage of polymorphism per experiment (2.9%), while the RAPD assay revealed the lowest percentage of polymorphism (0.9%). Moreover, the RAPD assay exhibited the lowest multiplex ratio (10.4), while the AFLP showed the highest multiplex ratio (41.1). The ISSR marker revealed a multiplex ratio of 10.5. The variation obtained by these types of markers might be due to the mode of action of each marker with the genomic DNA. In this respect, Powell *et al.* (1996) stated that, although AFLPs, ISSRs, and RAPDs are considered to be random markers and are capable of detecting single nucleotide mutations as well as insertion/deletions, however, their relative sensitivity to these types of mutations is expected to vary, because assay for polymorphism with differing length of the genomic sequences and each exhibits its own sensitivity and level of resolution of differences in band size. Moreover, in accordance with the present results, Paran *et al.* (1998) demonstrated that AFLP primers were four times as efficient as RAPD primers in detecting polymorphism in pepper. Adawy *et al.* (2004b) used different molecular markers, i.e., RAPDs, ISSRs, and AFLPs on the same date palm cultivars from Upper Egypt and pointed out that the AFLPs were the most effective in that all primer pairs tested detected polymorphism.

To evaluate the efficiency of the different marker systems, the sum effective number of alleles (SENA), the average expected heterozygosity for polymorphic markers ($H_{av(p)}$), the effective multiplex ratio (E), and marker index (MI) were calculated. As shown in Table (5), the AFLP technique exhibited considerably high sum effective number of alleles (205.7) compared to RAPD and ISSR (45.1, 17.8, respectively). The average heterozygosity was higher in AFLP (0.39) than in RAPD and ISSR (0.36 and 0.35, respectively). Thus, the results indicated that AFLP is more effective in detecting high level of polymorphism. Moreover, AFLP scored the highest marker index value (117.3). This could be due to the very high effective multiplex ratio (301) detected in the present study by this type of markers. The RAPD markers scored a marker index of 25.9 followed by the ISSR assay (10.4). In this respect, Powell *et al.* (1996) found that SENA values in soybean (*Glycine* spp.) were lowest for RAPDs and AFLPs (in *G. max,* 70.9 and 40.9, respectively, and in *G. soja,* 55.8 and 36.1, respectively) compared to RFLPs and SSRs. H_{av} values calculated for AFLPs and RAPDs were not significantly different.

Table (4): A summary of the results obtained by the different types of markers.

Assay type	Total No. of products	No. polymorphic products	Multiplex ratio	% polymorphism per assay	% polymorphism per experiment
RAPD(27)	282	71	10.4	25.2	0.9
ISSR(10)	105	30	10.5	28.6	2.9
AFLP(16)	657	301	41.1	45.8	2.9

Table (5): Sum effective number of alleles (SENA), expected heterozygosity for polymorphic products (Hav(p)), effective multiplex ratio (E), and marker index (MI) of the different marker systems.

Marker type	SENA	($H_{av(P)}$)	E	MI
RAPD	45.1	0.36	71	25.9
ISSR	17.8	0.35	30	10.4
AFLP	205.7	0.39	301	117.3

Table (6): Correlation coefficients among the RAPD, ISSR, and AFLP similarity matrices.

Marker type	RAPD	ISSR	AFLP
RAPD	1		
ISSR	0.68	1	
AFLP	0.23	0.34	1

To estimate the compatibility and the degree of correlation among the similarity matrices revealed by RAPDs, ISSRs and AFLPs, the correlation coefficients among them were calculated (Table 6). The estimated correlation coefficients were considerably high between RAPDs and ISSR (0.68). This may be due to the similarity between the nature of both markers as the mechanism of amplification is similar with little difference. Moreover, the correlation coefficient between RAPDs and AFLPs was lower (0.23) than that obtained between AFLP and ISSR (0.34). The correlation coefficients obtained in the present study were in disagreement with those obtained by Powell *et al.* (1996) for soybean. In addition, Goulão *et al.* (2001) showed that in apple the genetic similarity matrices obtained with RAPD and AFLP data were highly correlated (r = 0.737; *p* = 1.00) indicating that both analyses mostly provide similar results, despite some slight differences in the dendrograms. Berio *et al.* (2001) found in *Osteopermum* germplasm, that the correlation between the two data sets (AFLP and RAPD) was significant, with r = 0.74138. However, Degani *et al.* (2001) stated that, the correlation coefficient between the similarity values calculated from the AFLP data and RAPD data is extremely low (r = 0.0037), indicating no correlation between the two matrices. Moreover, Awad (2003) revealed that in Citrus the correlation coefficient between RAPDs and AFLPs was lower than that obtained between AFLPs and microsatellites. This may be due to poor associations in marker loci between AFLPs and RAPDs, which lead to a low correlation between distances. However, different marker systems differ in the mechanism of detecting polymorphism, genome coverage and the ease of application. Therefore, they could complement each other to draw more accurate conclusions.

REFERENCES

Adawy, S.S., Hussein, Ebtissam H.A., Saker, M.M. and El-Itriby, Hanaiya A. (2004a). Intra- and Inter-varietal variation of Upper Egypt date palm cultivars (*Phoenix dactylifera* L.): I. As revealed by RAPD and ISSR markers. Proceed. Int. Conf. Genet. Eng. & Appl., Sharm El-Sheikh, South Sinai, Egypt (April, 8-11, 2004): 165-179.

Adawy, S.S., Saker, M.M., Mohamed, Amina A. and El-Itriby, Hanaiya A. (2004b). Genetic relationships of Upper Egypt date palm cultivars (*Phoenix dactylifera* L.): II. As revealed by AFLP markers. Proceed. Int. Conf. Genet. Eng. &

Appl., Sharm El-Sheikh, South Sinai, Egypt. (April, 8-11, 2004): 181-195.

Adawy, S.S., Assem, Shireen K., Hussein, Ebtissam H.A. and El-Itriby, Hanaiya A. (2004c). Development of AFLP markers and genotyping of elite maize inbred lines. Arab J. Biotech., 7(1): 53-64.

Aranzana, M.J., Arús, P., Carbó, J. and King, G.J. (2001). AFLP and SSR markers for genetic diversity analysis and cultivar identification in peach [*Prunus persica* (L.) Batsch]. Acta Hort. (ISHS), 546: 367-370.

Awad, Nahla A. (2003). Genetic Analysis in Some Citrus Accessions using PCR-based Molecular Markers. Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Cairo University.

Baldoni, L., Pellegrini, M., Mencuccini, M., Angiolillo, A. and Mulas, M. (2000). Genetic relationships among cultivated and wild olives revealed by AFLP markers. Acta Hort. (ISHS), 521: 275-284.

Bellin, D., Velasco, R. and Grando, M.S. (2001). Intravarietal DNA polymorphisms in grapevine (*Vitis vinifera* L.). Acta Hort. (ISHS), 546: 343-349.

Berio, T., Morreaale, G., Giovannini, A., Allavena, L., Arru, L., Faccioli, P., Terzi, V. and Pecchioni, N. (2001). RAPD and AFLP genetic markers for the characterization of *Osteospermum* germplasm. Acta Hort. (ISHS), 546: 171-176.

Cao, B.R. and Chao, C.T. (2002). Identification of date palm cultivars in California using AFLP markers. HortScience, 37: 966-968.

Carr, J., Xu, M. and Korban, S.S. (2003). Estimating genetic diversity in New Guinea Impatiens using AFLP markers. Acta Hort. (ISHS), 623: 161-168.

Chao, C.T. and Devanand, P.S. (2003). Genetic variation within 'Medjool' and 'Deglet Noor' date (*Phoenix dactylifera* L.) cultivars in California detected by fluorescent-AFLP markers. J Hort Sci Biotech, 78(3): 405-409.

Degani, C., Rowland, L.J., Saunders, J.A., Hokanson, S.C., Ogden, E.L., Golden Goldhirsh, A. and Galletta, G.J. (2001). A comparison of genetic relationship measures in strawberry (*Fragaria* X *ananassa* Duch.) based on AFLPs, RAPDs, and pedigree data. Theor. Appl. Genet., 117: 1-12.

Dehmer, K.J. (2003). Molecular genome analyses as tools for efficient *ex situ*-conservation and utilization of plant genetic resources. Acta Hort. (ISHS), 623: 151-160.

Devanand, P.S. and Chao, C.T. (2003). Identification of genetic strains of Medjool and Deglet Noor date palm cultivars in California using amplified fragment length polymorphism (AFLP) markers. Acta Hort. (ISHS), 623: 333-340.

Diaz, S., Pire, C., Ferrer, J. and Bonete, M.J. (2003). Identification of *Phoenix dactylifera* L. varieties based on amplified fragment length polymorphism (AFLP) markers. Cell. Mol. Biol. Lett., 8(4): 891-899.

El-Khisin, Dina A., Adawy S.S., Hussein, Ebtissam H.A. and El-Itriby, Hanaiya A. (2003). AFLP fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars. Arab J. Biotech., 6(2): 223-234.

El-Seme'a, Ezz El-Din G.H.G. (2003). Propagation of dry varieties of date palm. Ph.D. Thesis, Department of Pomology, Faculty of Agriculture, Cairo University.

Goulão, L., Cabrita, L., Oliveira, C.M. and Leitão, J.M. (2001). Comparing RAPD and AFLP analysis in discrimination and estimation of genetic similarities among Apple (*Malus domestica* Borkh.) cultivars. Euphytica, 119: 259-270.

Hagen, L.S., Lambert, P., Audergon, J.M. and Khadari, B. (2001). Genetic relationships between Apricot (*Prunus*

armeniaca L.) and related species using AFLP markers. Acta Hort. (ISHS), 546: 205-208.

Henry, R.J. (1998). Practical applications of molecular markers to tropical and subtropical species. Acta Hort. (ISHS), 461: 107-112.

Hurtado, M.A., Badenes, M.L., Llácer, G., Westman, A., Beck, E. and Abbott, G.A. (2001). Contribution to apricot genetic analysis with RFLP, RAPD and AFLP markers. Acta Hort. (ISHS), 546: 417-420.

Hussein, Ebtissam H.A., Al-Said. M.Sh., El-Itriby, Hanaiya A. and Madkour, M.A. (2002). Genotyping Egyptian cotton varieties (*G. barbadense*) using molecular markers. Poster, Biotechnology and Sustainable Development: Voices of the South and North Conf., Bibliotheca Alexandria Conference Center, March 16-20 (2002), Alexandria, Egypt.

Hussein, Ebtissam H.A., Abd-alla, S.M., Awad, Nahla A., and Hussein, M.S. (2003). Genetic analysis in some Citrus accessions using microsatellites and AFLP-based markers. Arab J. Biotech., 6(2): 180-201.

Hussein, Ebtissam H.A., Adawy, S.S., Ismail, S.E.M and El-Itriby, Hanaiya A (2005). Molecular characterization of some Egyption date palm germplasm using RAPD and ISSR markers. Arab J. Biotech., 8(1):83-98.

Lanham, P.G. and Brennan, R.M. (1999). Genetic characterization of gooseberry (*Ribes grossularia* subgenus *Grossularia*) germplasm using RAPD, ISSR and AFLP markers. J. Hort. Sci. Biotech., 74: 361-366.

Martins, M. Farinha, A., Ferreira, E., Cordeiro, V., Monteiro, A., Tenreiro, R. and Oliveira, M. (2001). Molecular analysis of the genetic variability of Portuguese Almond collections. Acta Hort. (ISHS), 546: 449-452.

McGregor, C.E., Lambert, C.A., Greyling, M.M., Louw, J.H. and Warnich, L. (2000). A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica, 113: 135-144.

Paran, I., Aftergroot, E., and Shifriss, C. (1998). Variation in *Capsicum annuum* revealed by RAPD and AFLP markers. Euphytica, 99: 167-173.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J. , Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Sedra, M.H., Lashermes, P., Trouslot, P., Combes, M.C., Hamon, S. (1998). Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica, 103: 75-82.

Shenghua, L., Chengquan, F., Wenqin, S. and Feng, Z. (2002). AFLP molecular markers of 10 species of *Pyrus* in China. Acta Hort. (ISHS), 587: 233-236.

Tavaud, M., Zanetto, A., Santi, F. and Dirlewanger, E. (2001). Structuration of genetic diversity in cultivated and wild cherry trees using AFLP markers. Acta Hort. (ISHS), 546: 263-269.

Vignani, R., Monica, S., Elisa, M. and Mauro, C. (2002). Genomic variability in *Vitis vinifera* L. "Sangiovese" assessed by microsatellite and non-radioactive AFLP test. Electronic Journal of Biotechnology [online].15 April 2002, vol. 5, no.1 [cited 24 August2003]. ISSN0717-3458 Available from:http://www.ejb.org/content/vol5/issue1/full/2.html.

Virk, P.S., Zhu, J., Newbury, H.J., Bryan, G.J., Jackson, M.T. and Ford-Lloyd, B.V. (2000). Effectiveness of different classes of molecular marker for classifying and

revealing variation in rice (*Oryza sativa*) germplasm. Euphytica, 112: 275-284.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23(21): 4407-4414.

الملخص العربي

دراسة التباين الجينومي في نخيل البلح *(Phoenix dactylifera L.)* باستخدام تقنية AFLP's و مقارنتها بتقنيتي RAPD و ISSR

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تم دراسة التباين و علاقات القرابة الوراثية بين ١٤ عينة من نخيل البلح المصري (*Phoenix dactylifera* L.) مجمعة من ٦ مناطق في مصر و تمثل ٦ أصناف هم : سكوتي و برتمودا و ملكابي و جندبلة و فريحي و سيوي و ذلك باستخدام ١٦ توليفة من بادئات الـ AFLP .

أظهر الـ AFLP ٦٥٧ شظية و بنسبة تباين ٤٥,٨ % و تم تقدير العلاقات الوراثية بين الـ ١٤ عينة و الستة أصناف بناء على التشابه الوراثي باستخدام معامل Dice . اظهر الدندروجرام الناتج من تقنية الـ AFLP أن التراكيب الوراثية التابعة لبعض الأصناف المدروسة كانت مجمعة في مجموعات منفصلة مثل صنفي فريحي و جندبلة . كما ظهرت التراكيب الوراثية التابعة لصنف سيوي أيضا في مجموعة واحدة إلا أنها أوضحت بعض التباين داخل الصنف , بينما أظهرت أصناف سكوتي و برتمودا و ملكابي مستوى أعلى من التباين داخل الصنف , أما على مستوى التباين بين الأصناف فقد فصل الـ AFLP أصناف الواحات (سيوي و فريحي) عن أصناف أسوان (سكوتي و برتمودا وملكابي وجندبلة) . وقد تم تجميع نتائج التحليلات السابقة من الرابد و ISSR للـ ١٤ عينة مع نتائج AFLP's كبيانات تجميعية لإظهار علاقات قرابة اكثر دقة تعتمد على تغطية اكبر للجينوم حيث كان الدندروجرام الناتج من الأنواع المختلفة من الواسمات الجزيئية (الرابد و ISSR وAFLP) أكثر تطابقا مع دندروجرام الـ AFLP . كذلك فانه تم مقارنة كفاءة هذه التقنيات الثلاث في تحليل جينوم النخيل و ذلك عن طريق تقدير مجموع العدد الفعال من الأليلات (SENA) و حساب متوسط الـ Heterozygosity و الـ Multiplex ratio و معامل الواسم (MI) لكل واسم حيث أظهرت واسمات ال AFLP مجموع عالي للعدد الفعال من الأليلات ٢٠٥,٧ مقارنة ب RAPD , ISSR (٤٥,١ , ١٧,٨ على التوالي) . كذلك أظهرت واسمات الـ RAPD , ISSR و الـ AFLP نسبة heterozygosity ٠,٣٦ , ٠,٣٥ , ٠,٣٩ على التوالي . و قد كانت قيمة الـ MI ١١٧,٣ في الـ AFLP بينما كانت ٩٥,٩ , ١٠,٤ في الرابد و الـ ISSR على التوالي .و من كل هذه النتائج اتضح أن AFLP اكثر كفاءة في تحديد مستوى التباين و كانت قيمة معامل الارتباط بين الـ RAPD و ISSR هي ٠,٦٨ و بين الـ RAPD و AFLP هي ٠,٢٣ بينما كانت هذه القيمة للـ AFLP وISSR ٠,٣٤ . و قد أكدت النتائج أن الأنظمة المختلفة للواسمات الجزيئية تختلف في ميكانيكية اكتشاف التباين و تغطية الجينوم و سهولة تطبيقها لذلك فهي تعتبر مكملة لبعضها و يمكن أن تكون ذات فائدة كبيرة في تحديد الطرز الوراثية و حفظ الأصول الوراثية و في برامج التربية .