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Genetic Relatedness among Six Common *Phoenix dactylifera* (Palmae) Cultivars in Ismailia Region, Egypt

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ABSTRACT

SDS-PAGE electrophoretic analysis of young leaves protein was used to asses the genetic relatedness among 54 specimens belonging to 6 common cultivars of *Phoenix dactylifera* L. (Aglany, Amry, Haiany, Bent Aisha, Samany, and Zaghloul), that were collected from 7 different locations in Ismailia region. A total of 10 different protein bands were collectively detected in the gels of the 54 studied specimens. The resulted protein profile comprised one monomorphic band. The maximum number of bands observed was 10 bands found in one specimen of Zaghloul cultivar. While the minimum number of bands observed was three bands found in one specimen of Aglany cultivar and two specimens of Bent Aisha cultivar. The data of the allele frequencies of the six studied date palm cultivars revealed that all the alleles in Haiany cultivar were monomorphic, except in three loci with the lowest percentage of polymorphic loci (30%). While Bent Aisha and Aglany cultivars have the highest polymorphism (70% and 80%, respectively). In addition, it was found that the ratio of gene diversity/locus varied greatly within the specimens of the six studied date palm cultivars. Agglomerative cluster analysis, based on the genetic distances of the studied 54 specimens, revealed the variations and relatedness among the six collected cultivars.

Key words: Allele frequency, Genetic distances, Ismailia region**,** Palmae, *Phoenix dactylifera* cultivars**,** SDS-PAGE electrophoresis.

INTRODUCTION

Phoenix dactylifera is amongst few plant species that have been developed into agricultural crops and became so closely connected with human life. The exact origin or gene center of the date palm has been lost in history, but evidence of date palm cultivation goes as far back as 4000 B.C. in what is now known as southern Iraq. References to date palms have also been found in Ancient Egypt, and there seems to be a consensus that the earliest form of date palm cultivation coincided with the oldest civilizations and originated in North-East Africa, stretching northeast into the delta of the Euphrates and Tigris.

From there, date palms have spread either purposely or accidentally to the Middle East, North Africa and south Sahel, areas of east and south Africa, southwestern USA, Central and south America and even in southern Europe (Spain and Italy; Barreveld, 1993). As a result, two thousand or more different cultivars of date palm are known to exist all over the world, but only few important ones have been evaluated for their agronomic performance and fruit quality (Al-Hooti *et al.*, 1997).

Egypt is in the first among the Arab countries on the base number of trees. Date trees that are cultivated in upper and Lower Egypt reach about 9.3 million trees. They are distributed along 1500 km from the Mediterranean coastal area in the north, to the high Dam in the south. It is widely spreading in the western Egyptian Oasis including Sewa, El-Baharyia, El-Farafra, El-Dakhla, El-Kharga, El-Faiyum and also in southern and northern Sinai, Suez Canal region and the Red Sea coastal region (Brown and Bahgat, 1938). Ismailia governorate is known for its soft and semi-dry date palm cultivars. It is reported that it has approximately 436,894 female palms with a total production of about 43379 tons yearly (Ministry of Agriculture, 2001).

Studies of genetic polymorphism have been carried out at the level of protein coded by structural genes. Protein polymorphism arises from changes in one or more bases in the structural genes. These changes may result in amino acid substitution in the polypeptide produced at translation stage, thus affecting the physical properties of the translated protein especially its net charge. In order to detect the change in net charge, protein can be subjected to gel electrophoresis (Caprette, 2005). The electrophoretic banding patterns of protein have provided a valid source of taxonomic evidences and were used to address the relatedness among taxa at the generic, species or even cultivar levels. This was investigated with several authors in different taxa, e.g. genus *Lathyrus* (Badr *et al*., 2000), genus *Lens esculentum* (Hassan, 2001) and *Psidium guajava* (Hassan *et al*., 2002).

Besides, to explore the genetic relatedness among studied taxa, numerical analysis was performed using computer programs, providing a logical means for expressing relationships among closely related specimens, on one hand, and specimens of uncertain positions, on the other hand (Sneath and Sokal, 1973).

Davis and Heywood (1973) stated that all kinds of characters may be employed in numerical analyses as long as they are inherent and are not greatly affected by experimental or observational uncertainties.

The present study was carried out to investigate the genetic relatedness among the six common *Phoenix dactylifera* cultivars of Ismailia, Egypt, using

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SDS-PAGE protein markers and numerical analysis methods. This will ensure true-to-type offshoots and will help in identifying new cultivars with different traits. Consequently, enhancing and promoting date palm culture in the newly reclaimed soils and may lead the palm breeder to produce high quality date palm fruits.

MATERIALS AND METHODS

Sampling of plant materials

Fifty four Palm trees of the six *P. dactylifera* cultivars (Aglany, Amry, Bent Aisha, Haiany, Samany, and Zaghloul) were sampled from 7 locations representing different habitats in Ismailia region (from West Kantarah to Ismailia-Cairo Desert Highway). Young leaf samples from each cultivar of *P. dactylifera* were collected from 3 different locations (Table 1). The collected young leaves were ground in liquid nitrogen and the macerates were washed several times with acetone to get rid of pigments by centrifugation at 10000 rpm for 20 min. The supernatants were discarded and the pellets were air dried and kept frozen at -20^{o}C until used (Laemmli, 1970).

Extraction and SDS-PAGE Electrophoresis

Samples of 500 µg leaf macerates were mixed with 500 µl extraction buffer (0.2 M Tris HCl, 10% glycerol, 2% SDS, 0.2% mercaptoethanol). The mixture was vortexed several times then centrifuged for 20 min. at 12000 rpm. Total leaf proteins in the supernatant were qualitatively analysed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli (1970). All gels were run in Bio-Rad system following the manufacturer's instructions, using resolving and stacking gel with 12.5% and 2.5% acrylamide concentration, respectively. The samples were heated at 100°C for 5 minutes and centrifuged at 14000 rpm for 10 minutes prior to loading. Samples were loaded into the wells (10 µl per well). Control wells were loaded with standard protein marker with the following molecular weights; 96.00 KDa, 66.00 KDa, 45.00 KDa, 30.00 KDa, 20.10 KDa, and 14.40 KDa.

Electrophoresis was carried out at a constant current of 60 mA. Then the gels were stained for proteins by immersing in 1% Comassie blue R250 stain. The stain solution was discarded and the gels were rinsed in water before adding destain solution (10% Trichloroacetic acid; TCA), shaken for several hours until the background staining is cleared.

Documentation of results

The gels were photographed and then protein bands were scored manually. Bands occurrence recorded as 1, while band absence recorded as 0.

Data analysis

(1) Allele frequencies

The frequency of a prescribed allele is simply the proportion of all alleles of the gene that are of the prescribed type. The frequency of any prescribed allele in a sample is therefore equal to twice the number of homozygotes for the allele plus the number of heterozygotes for that allele divided by two times the number of individuals in the sample (Hartl and Clark, 1989).

(2) Proportion of polymorphic loci

The proportion of the polymorphic loci, at all loci assayed, has commonly been used as a measure of genetic diversity. However, the magnitude of this proportion depends on the sampling of loci. In this study, a locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. One individual carrying a rare variant allele will result in the locus being classified as variable. In many instances, a locus is classified as polymorphic.

$$\text{The proportion of polymorphic loci} = \frac{\text{Number of polymorphic loci} \times 100}{\text{Total number of loci}}$$

(3) Ratio of gene diversity per locus

The ratio of gene diversity per locus is a measure of diversity similar to the proportion of polymorphic loci, with additional advantage that it takes account of the magnitude of square allele frequencies at each locus.

$$\text{Ratio of gene diversity per locus} = \Sigma \left\{ 1 - \left(p_i^2 + p_j^2 \right) \right\} / N$$

Where $\boldsymbol{p_i}$ is the frequency of dominant allele (1), $\boldsymbol{p_j}$ is the frequency of recessive allele (0) and **N** is the total number of loci. POPGENE version 1.31 computer program (Yeh *et al.*, 1999) was used to calculate all genetic parameters.

(4) Numerical Analysis

The fifty four fresh specimens were used as Operational Taxonomic Units (OTU's). A total of 24 leaf protein characters were employed in the analysis. Agglomerative cluster analysis of the OTU's could be illustrated in a tree-like structure similar to the one for phylogenetic trees. The standardized data were scaled by the mean (the mean of each variable was subtracted) then the dissimilarity distance between the OTU's was calculated using both UPGMA (Unweight Pair Group Method with Arithmetic Averages) and average Euclidean distance coefficient methods (Sneath and Sokal, 1973). This was conducted by using Systat-pc version 7.0.1 computer program (Wilkinson *et al.*, 1992).

RESULTS

SDS-PAGE

A total of 10 different bands were collectively detected in the gels of the 54 studied specimens (Table 2) and presented diagrammatically in Figure (1). The minimum number of bands observed among the studied cultivars was 3 bands. They were recorded in one individual of Aglany cultivar (lane Ag. 2.5), and 2 individuals of Bent Aisha cultivar (lanes BA.

Table (1): Details of the collected *P. dactylifera* specimens; abbreviations, replica numbers, location numbers and names and the specimen cultivars codes.

Cultivars	Abbreviations	Replica numbers	Location		Specimen code
			Numbers	Names	
		1	1	East Kantara, Erook valley	Ag. 1.1
		2			Ag. 2.1
		3			Ag. 3.1
		1	4	Abu Sweir, El-Kateeba village	Ag. 1.4
Aglany	Ag.	2			Ag. 2.4
		3			Ag. 3.4
		1	5	Abu Sweir, El Omda village	Ag. 1.5
		2			Ag. 2.5
		3			Ag. 3.5
		1	3	West Kantara, Ismailia – Port Said Highway (17 Km)	Am. 1.3
		2			Am. 2.3
		3			Am. 3.3
		1	4	Abu Sweir, El-Kateeba village	Am. 1.4
Amry	Am.	2			Am. 2.4
		3			Am. 3.4
		1	5	Abu Sweir, El Omda village	Am. 1.5
		2			Am. 2.5
		3			Am. 3.5
		1	1	East Kantara, Erook valley	BA. 1.1
		2			BA. 2.1
		3			BA. 3.1
		1	2	West Kantara, Ismailia – Port Said Highway (11 Km)	BA. 1.2
Bent Aisha	BA.	2			BA. 2.2
		3			BA. 3.2
		1	5	Abu Sweir, El Omda village	BA. 1.5
		2			BA. 2.5
		3			BA. 3.5
		1	1	East Kantara, Erook valley	Ha. 1.1
		2			Ha. 2.1
		3			Ha. 3.1
		1	2	West Kantara, Ismailia – Port Said Highway (11 Km)	Ha. 1.2
Haiany	Ha.	2			Ha. 2.2
		3			Ha. 3.2
		1	5	Abu Sweir, El Omda village	Ha. 1.5
		2			Ha. 2.5
		3			Ha. 3.5
		1	3	West Kantara, Ismailia – Port Said Highway (17 Km)	Sa.1.3
		2			Sa. 2.3
		3			Sa. 3.3
		1	6	Ismailia – Cairo Desert Highway	Sa. 1.6
Samany	Sa.	2			Sa. 2.6
		3			Sa. 3.6
		1	7	Ismailia, Suez Canal University,	Sa. 1.7
		2		farm of the Faculty of Agriculture	Sa. 2.7
		3			Sa. 3.7
		1	3	West Kantara, Ismailia – Port Said Highway (17 Km)	Za. 1.3
		2			Za. 2.3
		3			Za. 3.3
		1	6	Ismailia – Cairo Desert Highway	Za. 1.6
Zaghloul	Za.	2			Za. 2.6
		3			Za. 3.6
		1	7	Ismailia, Suez Canal University,	Za. 1.7
		2		farm of the Faculty of Agriculture	Za. 2.7
		3			Za. 3.7

2.2 and BA. 3.2). While the maximum number of bands observed was 10. They were recorded in one individual of Zaghloul cultivar (lane Za. 1.7).

The resulted protein profile comprised band number 1 which was a monomorphic band that was found in all the studied specimens. Band number 2 was recorded in all the studied cultivars except two individuals of Aglany cultivar (lanes Ag. 2.5 and Ag. 3.5) and one individual of Amry cultivar (lane Am. 1.3). Band number 3 was recorded in almost all the studied specimens of the six cultivars except 5 individuals of Aglany cultivar (lanes Ag. 3.1, Ag. 1.4, Ag. 2.4, Ag. 3.4, and Ag. 1.5) and one individual of Bent Aisha (lane BA. 1.5). Band number 4 was detected in all specimens of the six cultivars except 2 individuals of Aglany cultivars (lanes Ag. 1.5 and Ag. 2.5), 4 individuals of Bent Aisha cultivar (lanes BA. 1.1, BA. 2.2, BA. 3.2, and BA. 1.5), and 3 individuals of Haiany cultivar (lanes Ha. 1.5, Ha. 2.5, and Ha. 3.5). Band number 5 was recorded in all specimens of Haiany cultivar, 4

individuals of Aglany cultivar (lanes Ag. 1.1, Ag. 3.1, Ag. 1.4, and Ag. 2.4), 6 individuals of Bent Aisha cultivar (lanes BA. 1.1, BA. 2.1, BA. 3.1, BA. 1.2, BA. 2.5, and BA. 3.5), 2 individuals of Samany cultivar (lane Sa. 1.7 and Sa. 3.7), and 2 individuals of Zaghloul cultivar (Za. 1.7 and Za. 2.7). This band, however, was absent in the rest of the specimens.

Band number 6 was present in approximately half of the studied specimens, while it was absent in the other.

Band number 7 was recorded in all Haiany cultivar, 3 individuals of Aglany cultivar (lanes Ag. 1.1, Ag. 2.1, and Ag. 1.4), 6 individuals of Amry cultivar (lanes Am. 3.3, Am. 1.4, Am. 2.4, Am. 3.4, Am. 1.5, and Am. 2.5) 5 individuals of Bent Aisha cultivar (lanes BA. 1.1, BA. 2.1, BA. 1.5, BA. 2.5, and BA. 3.5), 4 individuals of Samany cultivar (lanes Sa. 3.3, Sa. 1.6, Sa. 1.7, and Sa. 2.7), 6 individuals of Zaghloul cultivar (lanes Za. 3.3, Za. 1.6, Za. 2.6, Za. 1.7, Za. 2.7, and Za. 3.7). This band, however, was absent in the rest of the specimens.

Band number 8 was detected in all Amry, Haiany and Samany cultivars, 3 individuals of Aglany cultivar (lanes Ag. 1.1, Ag. 1.4, and Ag. 1.5), 7 individuals of Bent Aisha cultivar (lanes BA. 1.1, BA. 2.1, BA. 3.1, BA. 1.2, BA. 1.5, BA. 2.5, and BA. 3.5), and 7 individuals of Zaghloul cultivar (Za. 1.3, Za. 2.3, Za. 3.3, Za. 1.6, Za. 2.6, Za. 1.7, and Za. 3.7). It was absent, however, in the rest of the specimens. Band number 9 was present in most of the studied specimens except in 8 individuals of Aglany cultivar (lanes Ag. 2.1, Ag. 3.1, Ag. 1.4, Ag. 2.4, Ag. 3.4, Ag. 1.5, Ag. 2.5, and Ag. 3.5), 3 individuals of Amry cultivar (lanes Am. 1.3, Am. 2.3, and Am. 3.3), 3 individuals of Bent Aisha cultivar (lanes BA. 2.2, BA. 3.2, and BA. 1.5), one individual of Samany cultivar (Sa. 1.7), and one individual of Zaghloul cultivar (Za. 3.6). Band number 10 was absent in almost all the studied specimens except that of Haiany cultivar (lane Ha. 2.3), Samany cultivar (lane Sa. 1.7), and Zaghloul cultivar (lanes Za. 2.6, Za. 1.7, and Za. 2.7).

The data of the six different *P. dactylifera* cultivars (Table 3) revealed that all the loci in Haiany cultivar are monomorphic except in 3 loci (locus 4, 6, and 10), where it recorded the lowest percentage of polymorphic loci 30%. Bent Aisha and Aglany cultivars have the highest percentage of polymorphism (70% and 80%, respectively). Meanwhile, Amry, Samany, and Zaghloul cultivars showed a moderate percentage of polymorphism (40%, 50%, and 60%, respectively). It was clearly observed that the sharing loci between two or more cultivars, with high allele frequency (dominant allele), were almost the same at all the polymorphic loci. This appeared clearly from the average of allele frequency data. For example, the average frequency of recessive allele (0) showed a highest value in each of Aglany and Bent Aisha cultivars. They were 0.67 and 0.56, respectively. A slight difference, nonetheless, existed between the averages of allele frequency in both of Haiany and Zaghloul cultivars. They were 0.20 and 0.37, respectively (Table 3).

In addition, it was found that the ratio of gene diversity/locus within the studied specimens of the six studied Date palm trees cultivars varied greatly. It ranged from a minimum of 0.08 in Haiany cultivar to a maximum of 0.31 in Bent Aisha cultivar (Table 3).

Table (2): SDS-PAGE protein band patterns of the six studied *P. dactylifera* cultivars.

Cultivars	Specimen code numbers	Protein band numbers									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Aglany	**1.1**	1	1	1	1	1	1	1	1	1	0
	2.1	1	1	1	1	0	0	1	0	0	0
	3.1	1	1	0	1	1	0	0	0	0	0
	1.4	1	1	0	1	1	0	1	1	0	0
	2.4	1	1	0	1	1	0	0	0	0	0
	3.4	1	1	0	1	0	1	0	0	0	0
	1.5	1	1	0	0	0	1	0	1	0	0
	2.5	1	0	1	0	0	1	0	0	0	0
	3.5	1	0	1	1	0	1	0	0	0	0
Amry	**1.3**	1	0	1	1	0	1	0	1	0	0
	2.3	1	1	1	1	0	1	0	1	0	0
	3.3	1	1	1	1	0	1	1	1	0	0
	1.4	1	1	1	1	0	1	1	1	1	0
	2.4	1	1	1	1	0	1	1	1	1	0
	3.4	1	1	1	1	0	1	1	1	1	0
	1.5	1	1	1	1	0	1	1	1	1	0
	2.5	1	1	1	1	0	1	1	1	1	0
	3.5	1	1	1	1	0	0	0	1	1	0
Bent Aisha	**1.1**	1	1	1	0	1	0	1	1	1	0
	2.1	1	1	1	1	1	0	1	1	1	0
	3.1	1	1	1	1	1	0	0	1	1	0
	1.2	1	1	1	1	1	0	0	1	1	0
	2.2	1	1	1	0	0	0	0	0	0	0
	3.2	1	1	1	0	0	0	0	0	0	0
	1.5	1	1	0	0	0	1	1	1	0	0
	2.5	1	1	1	1	1	1	1	1	1	0
	3.5	1	1	1	1	1	1	1	1	1	0
Haiany	**1.1**	1	1	1	1	1	0	1	1	1	0
	2.1	1	1	1	1	1	1	1	1	1	0
	3.1	1	1	1	1	1	1	1	1	1	0
	1.2	1	1	1	1	1	0	1	1	1	0
	2.2	1	1	1	1	1	0	1	1	1	0
	3.2	1	1	1	1	1	0	1	1	1	1
	1.5	1	1	1	0	1	0	1	1	1	0
	2.5	1	1	1	0	1	0	1	1	1	0
	3.5	1	1	1	0	1	0	1	1	1	0
Samany	**1.3**	1	1	1	1	0	0	0	1	1	0
	2.3	1	1	1	1	0	0	0	1	1	0
	3.3	1	1	1	1	0	1	1	1	1	0
	1.6	1	1	1	1	0	1	1	1	1	0
	2.6	1	1	1	1	0	0	0	1	1	0
	3.6	1	1	1	1	0	0	0	1	1	0
	1.7	1	1	1	1	1	1	1	1	0	1
	2.7	1	1	1	1	0	0	1	1	1	0
	3.7	1	1	1	1	1	0	0	1	1	0
Zaghloul	**1.3**	1	1	1	1	0	0	0	1	1	0
	2.3	1	1	1	1	0	1	0	1	1	0
	3.3	1	1	1	1	0	1	1	1	1	0
	1.6	1	1	1	1	0	1	1	1	1	0
	2.6	1	1	1	1	0	1	1	1	1	1
	3.6	1	1	1	1	0	0	0	0	0	0
	1.7	1	1	1	1	1	1	1	1	1	1
	2.7	1	1	1	1	1	1	1	0	1	1
	3.7	1	1	1	1	0	1	1	1	1	0

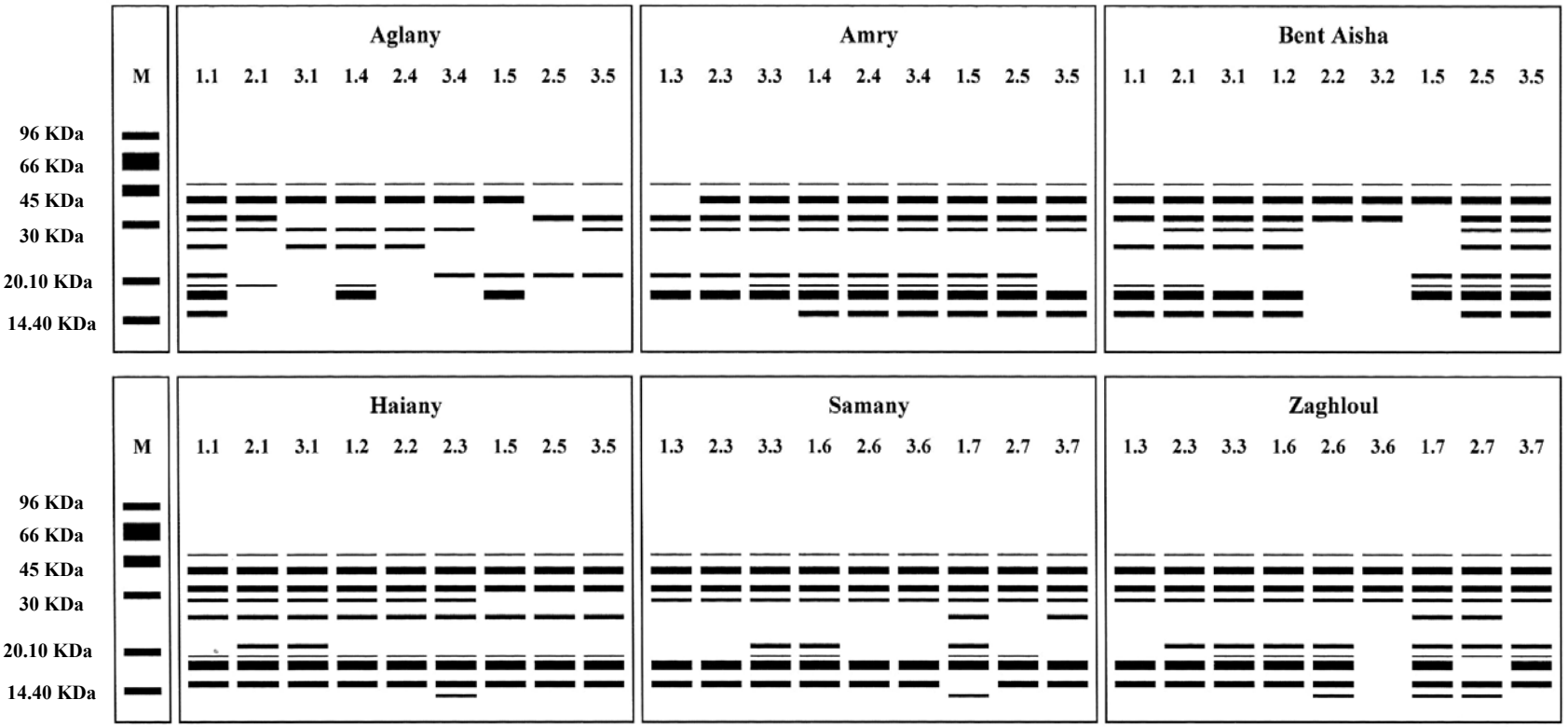

Figure (1): Diagrammatic presentation for the electrophoretic patterns of leaf protein of six common *P. dactylifera* cultivars. (Lane M; Marker protein, Lane numbers; cultivars collected from different locations; see Table 1)

Table (3): Allele frequencies belonging to the six studied *P. dactylifera* cultivars.

Cultivars	Allele frequencies																						Average of allele frequency		Ratio of gene diversity/ locus	Percentage of polymorphic loci
	Locus 1		Locus 2		Locus 3		Locus 4		Locus 5		Locus 6		Locus 7		Locus 8		Locus 9		Locus 10							
	0.00	1.00	0.0	1.00	0.8	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00				
Aglany	0.00	1.00	0.4	0.60	0.8	0.20	0.40	0.60	0.80	0.22	0.72	0.28	0.83	0.17	0.83	0.17	0.94	0.06	1.00	0.00	0.67	0.33	0.30	80.00		
Amry	0.00	1.00	0.5	0.65	0.0	1.00	0.00	1.00	1.00	0.00	0.55	0.65	0.67	0.33	0.00	1.00	0.67	0.33	1.00	0.00	0.44	0.60	0.19	40.00		
Bent Aisha	0.00	1.00	0.0	1.00	0.5	0.65	0.72	0.28	0.67	0.33	0.83	0.17	0.72	0.28	0.39	0.61	0.67	0.33	1.00	0.00	0.56	0.47	0.31	70.00		
Haiany	0.00	1.00	0.0	1.00	0.0	1.00	0.67	0.33	0.00	1.00	0.39	0.61	0.00	1.00	0.00	1.00	0.00	1.00	0.94	0.06	0.20	0.80	0.08	30.00		
Samany	0.00	1.00	0.0	1.00	0.0	1.00	0.00	1.00	0.89	0.11	0.83	0.17	0.78	0.22	0.00	1.00	0.55	0.65	0.94	0.06	0.40	0.62	0.14	50.00		
Zaghloul	0.00	1.00	0.0	1.00	0.0	1.00	0.00	1.00	0.89	0.11	0.39	0.61	0.67	0.33	0.39	0.61	0.55	0.65	0.83	0.17	0.37	0.65	0.23	60.00		

Numerical analysis

The cluster analysis of the 54 OTU's for the 24 studied leaf protein characters yielded the dendrogram shown in Figure (2). The resulted dendrogram separated the six studied *P. dactylifera* cultivars into two main groups (I and II) at a dissimilarity level of 6.12. Group I included Aglany and Amry cultivars at a relatively low dissimilarity level of 2.06. However, group II was further sub-divided into two sub-groups (1 and 2) at a dissimilarity level of 4.10. Sub-group "1" included both Haiany and Bent Aisha in one cluster at 2.05 dissimilarity level, while sub-group "2" included both Samany and Zaghloul cultivars.

Discussion

The characterization of the genetic diversity usually involves the description of variation among morphological traits and partitioning of such variation into genetic and environmental components. Continuous variation in most morphological traits is assumed to be under polygenic control (Bawa *et al*., 1991). Thus, morphological data are utilized to analyze variation at many unspecified loci. However, the genetic information provided by morphological characters is often limited. These limitations have resulted in deployment of biochemical techniques (Gottlieb, 1977; Crawford, 1989; Olfelt *et al*., 2001).

Genetic variations and relatedness among certain group of plants can be detected at the molecular level by studying the changes in the structure of their DNA or the proteins coded by specific genes. Genetic markers includes total storage seed proteins, isozymes, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), and simple sequence repeats (SSRs) (e.g. Doll and Brown, 1979; Saghai-Maroof *et al.*, 1984; Nevo *et al*., 1986; Chalmers *et al*., 1991; Peterson *et al*., 1994; Sanchez de la Hoz *et al*., 1996; Chtourou-Ghorbel *et al*., 2001; Sakka *et al*., 2004).

Characterization of genetic diversity has long been a major goal in species, subspecies; and cultivar discrimination as well as plant breeding. SDS-protein marker considered as one of the powerful techniques for determining the variations among cultivars (Yupsanis *et al*., 1992; Abdel-Salam *et al*. 1998; Badr *et al*., 1998; Badr *et al*., 2000; Hassan, 2001; Hassan *et al*., 2002; Perin *et al*., 2006). Besides, the electrophoretic surveys of proteins play an important role in the quantitative evaluation and management of genetic resources. This is because distribution of genetic variation provides guidelines for sampling strategies and germplasm preservation (Black *et al*., 1995; Karcicio and Izbirak, 2003).

Moreover, genetic variations can be estimated by detecting changes in amino acid sequence or electrophoretic mobility of proteins (Bawa *et al*., 1991). In addition, changes in amino acid sequence or electrophoretic mobility of proteins are presumed to result from alterations in DNA coding of these proteins. Sequencing of amino acids is cumbersome and expensive. Therefore, most population genetic surveys are based on the polymorphism detected by mobility

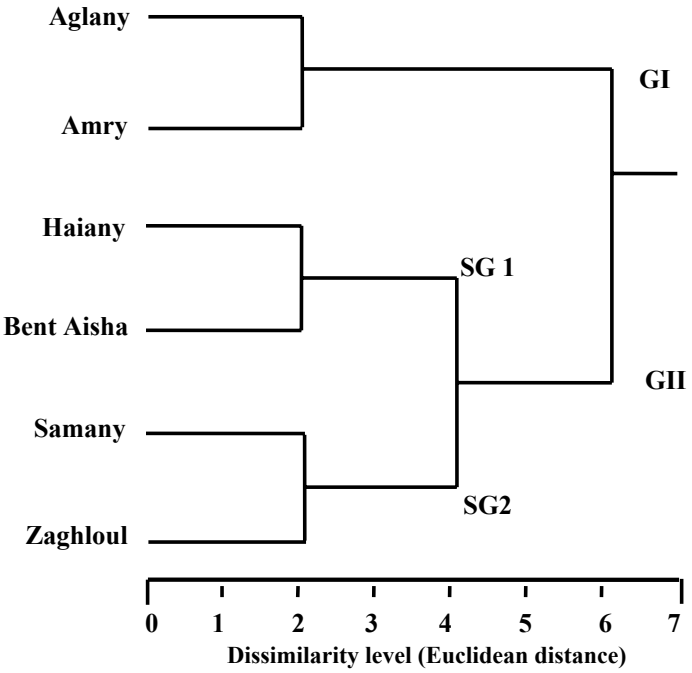

Figure (2): A dendrogram resulted from UPGMA clustering analysis based on the leaf protein characters for the 54 specimens of the six studied *P. dactylifera* cultivars (**GI**, Group I; **GII**, Group II; **SG1**, Subgroup 1; **SG2**, Subgroup 2).

differences of proteins as revealed by electrophoresis (Hamrick and Rickwood, 1990; Bawa *et al*., 1991; Olfelt *et al*., 2001).

In the present study, SDS-PAGE analysis was used to distinguish between fifty four genotypes belonging to six *P. dactylifera* cultivars. At the SDS protein pattern level, the resulted electrophoretic profiles detected variations among the studied cultivars. Based on the genetic diversity measures, Aglany and Bent Aisha cultivars showed the highest genetic diversity, this is also observed from the high percentage of their polymorphic loci. On the other hand, the lowest genetic diversity was obviously detected in Haiany cultivar. Moreover, it is well noted that Haiany cultivar could be considered as one of the most popular cultivar in Ismailia region due to its high productivity (Mousa, 1981). This high productivity could be attributed to the introduced pollen rather than diversity in the mother (female palm).

The agglomerative clustering based on genetic distance among the studied cultivars clearly discriminate the six studied *P. dactylifera* cultivars into two main groups (I and II) at a dissimilarity level of 6.12. Group I included Aglany and Amry cultivars at a relatively low dissimilarity level of 2.06 indicating that they were closely related to each other. This finding was in concomitant with the morphological results of the same cultivars found by Abdalla (1986) indicating that both cultivars sharing several genetic codes related to phenetic ones. Moreover, it supported the idea of Max *et al*. (1999) and Fjellheim *et al*. (2001), who stated that both morphological and molecular data are valuable for detecting the intra-specific variations. However, group II was further sub-divided into two sub-groups (1 and 2) at a dissimilarity level of 4.10. Sub-group "1" included both Haiany and Bent Aisha in one cluster at 2.05 dissimilarity level, while sub-group "2" included both Samany and Zaghloul cultivars. These results were consistence with the morphological findings of Ibrahim and Khulif, (1997) and indicating that cultivars of each sub-group 1 and 2 separately sharing a common gene pool.

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العلاقات الوراثية لستة سلالات شائعة من نخيل البلح في منطقة الإسماعيلية، مصر

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الملخص العربـــى

أظهرت نتائج التفريد الكهربي للبروتينات في عينات الأوراق الحديثة لسلالات نخيل البلح قيد الدراسة والتي تضمنت 54 عينة نباتية تمثل السلالات الستة المنزرعة (عجلاني وعمري وحياني وبنت عيشة وسماني وزغلول) في السبعة مناطق التي تم اختيارها في منطقة الإسماعيلية.

تم تعريف عشرة أشرطة بروتين تواجدت جميعها في الهُلام (الجل) الخاص بالعينات محل الدراسة. وقد ظهر شرط البروتين رقم (1) وحيد الشكل (التركيب الكيماوي) في جميع أنماط البروتينات. وتواجد الحد الأقصى لأشرطة البروتين (10 أشرطة) في أحد أفراد سلالة زغلول فقط. بينما تم تسجيل الحد الأدنى لأشرطة البروتين (3 أشرطة) في أحد أفراد سلالة عجلاني وفردين من سلالة بنت عيشة.

وقد أوضحت نتائج تكرار الأليلات، الخاصة بالسلالات الستة، أن جميع أليلات سلالة حياني وحيدة الشكل في ماعدا ثلاث مواضع جينية حيث سجلت أقل نسبة مئوية (30%) للمواقع الجينية عديدة الشكل. ومقارنة بذلك، سجلت سلالتي عجلاني وبنت عيشة أعلى نسبة مئوية (70%، 80%) للمواقع الجينية عديدة الشكل، على التوالي. إضافة إلى ذلك، فلقد وجد أن نسبة التنوع الجيني إلى الموقع الجيني اختلفت اختلافات كبيرة بين السلالات الستة. وفي الوقت ذاته، فإن استخدام التحليل التجميعي المبني على أساس المسافات الوراثية بين العينات موضوع الدراسة (54 عينة نباتية) والوثيقة الصلة بشكل واضح إلى مجموعتين رئيسيتين (المجموعة الأولى والمجموعة الثانية) فى مستوى اختلاف يقارب من 06.12 ولقد تضمنت المجموعة الأولى السلالتين عجلانى وعمري بينما تضمنت المجموعة الثانية الأربعة سلالات الأخرى فى مجموعتين فرعيتين (المجموعة الفرعية "1" والمجموعة الفرعية "2") فى مستوى إختلاف يقارب من 04.10 ولقد إشتملت المجموعة الفرعية "1" على كلا من السلالتين حياني وبنت عيشة، بينما تضمنت المجموعة الفرعية "2" على السلالتين الأخريين سماني وزغلول.