MORPHOLOGICAL AND MOLECULAR GENETIC CHARACTERIZATION OF SOFT DATE PALM (*Phoenix dactylifera* L.) CULTIVARS IN EGYPT

E. A. EISSA[1], A. B. ABD EL-RAZEK[2], S. F. EL-SHARABASY[3] AND R. M. RIZK[4]

1. *Dept. Genetics, Fac. Agric., Fayoum Univ., Fayoum, Egypt*
2. *Dept. Genetics, Fac. Agric., Ain Shams Univ., Cairo, Egypt*
3. *Central Lab. Res. and Develop. of Date Palms, Agric. Res. Center, Cairo, Egypt*
4. *National Gene Bank, Agric. Res. Center, Cairo, Egypt*

The fruits of the date palm (*Phoenix dactylifera* L.) are sweet berries with a sugar content of more than 50% (El-Sharabasy, 2009). The origin of the date palm is supposed to be in Middle East (Zohary and Speigel-Roy, 1975; Zohary and Hopf, 1988; and Amer, 2000). In Arab countries and in the Middle East the date palm is a staple food that can be produced easily under unfavorable natural and economic conditions (El-Sharabasy, 2009).

During the past decades, classical methods to evaluate genetic variation have been complemented by molecular techniques. The development of so-called "DNA markers" which are based on polymorphisms found in proteins or DNA has greatly facilitated research in a variety of many biological branches such as taxonomy, phylogenetic relationships and genetics (Carlson *et al*., 1991; Halward *et al.*, 1992 and Abdelsalam *et al.*, 1998).

There are a number of molecular techniques available for characterization of the variation at the DNA level, e.g. RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter Simple Sequence Repeats). Furthermore, these techniques are able to reveal a virtually unlimited number of markers. For genetic diversity studies, the RAPD technique shows some important advantages (Williams *et al.*, 1990). The ISSR strategy was therefore performed to access the DNA diversity among crop genotypes (Zehdi *et al*., 2004). Soliman *et al.* (2003) used RAPD markers to study three males and four females date accessions from Egypt. Soliman *et al.* (2006) identify the genetic polymorphism for semi-dry date palm in Egypt using RAPD and ISSR markers.

The genetic improvement of a crop species depends on the ability to select promising plant material. To facilitate the selection process, molecular markers that are associated with important traits can be used as selection tools. The markers can then be used to establish genetic maps,

which in turn are important tools for more refined marker-assisted selection in breeding programs as well as for in-depth genetic and systematic analyses (Soliman *et al.,* 2006). Cleary, an integrated approach is needed incorporating morphological and genetic studies to improve the knowledge of date palm taxonomy and diversity. Proteins or/and DNA attributes can be used successfully for variety identification, source of information of date palm gene bank and for studying the genetic diversity of cultivars. So, date palm can be promoted best through better characterization and evaluation (Soliman *et al*., 2006*).*

The objective of the present study was simplified over view framework on the identification, characterization, evaluation and documentation the genetic diversity of soft date palm (*Phoenix dactylifera* L.) cultivars in Egypt. Genetic diversity of nine soft date palm cultivars growing in Egypt was addressed to identify and describe DNA markers and morphological important traits as well as molecular genetic characterization relationships were examined. The achieved results have been utilized for establishing genetic markers in order to discriminate among the date palm cultivars in Egypt.

MATERIALS AND METHODS

Plant materials and samples

Young and fresh leaves of date palm were collected from the Central Laboratory Research and Development of Date Palms, Agricultural Research Center, Ministry of Agriculture and Land Reclamation during 2008 season. The date palm studied were nine females of soft date palm cultivars (Zagloul, Samani, Hayani, Bent-Eisha, Barhi, Orebi, Selmi, Amhat and Om-Elferakh). The terminology of morphological attributes basically follows Stearn (1973); El-Sharabasy and Rizk (2005); and Rizk and El-Sharabasy (2006).

Extraction of DNA

Young and fresh leaf samples were collected separately from 10 trees for each date palm cultivar. The selected leaves were normal and free from any pathogenic symptoms. All leave samples were saved in ice box and quickly transported to laboratory. Plant tissues were ground under liquid nitrogen to a fine powder, then bulked DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen).

PCR conditions and RAPD-PCR analysis

The Polymerase Chain Reaction (PCR) mixture (25 µl) consisted of 0.8 U of *Taq* DNA polymerase, 2.5 mM dNTPs, and 25 pmol of random primer (Operon Biotechnologies, Inc. Germany), Table (1), and 50 ng of genomic DNA. The reaction mixture was placed on a DNA thermal cycler (Techni 512). The PCR program included an initial denaturation step at 94°C for 2 minutes followed by 45 cycles with 94°C for 1 minute for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30

seconds and final extension at 72°C for 10 minutes were carried out. The amplified DNA fragments were separated on 1.5% agarose gel and stained with ethidium bromide and fragments sizes were estimated with the 100 bp ladder marker. The amplified pattern was visualized on an ultraviolet light transilluminator and photographed. PCR amplification was performed using eight random 10-mer arbitrary primers synthesized by Operon Biotechnologies, Inc. Germany, Table (1).

PCR conditions and ISSR-PCR analysis

ISSR-PCR reactions were conducted using four primers. The Polymerase Chain Reaction (PCR) mixture (25 µl) consisted of 0.8 U of *Taq* DNA polymerase, 2.5 mM dNTPs, and 25 pmol of random primer (Operon Biotechnologies, Inc. Germany) Table (1), and 50 ng of genomic DNA. The reaction mixture was placed on a DNA thermal cycler (Techni 512). The PCR was programmed for one cycle at 94°C for 4 minutes followed by 45 cycles of 1 minute at 94°C, 1 minute at 57°C, and 2 minutes at 72°C. The reaction was finally stored at 72°C for 10 minutes. The PCR products were separated on a 1.5 % agarose gel and fragments sizes were estimated with the 100 bp ladder marker.

Eight primers succeeded to generate reproducible polymorphic DNA products. Table (1) lists the base sequences of these DNA primers that produced informative polymorphic bands.

Data analysis

RAPD and ISSR-PCR data were processed using Quantity One Software (Bio-Rad) which identifies bands using an optimized set of parameters (as reported in Quantity One user guide for version 4.2 Windows Bio-Rad Laboratories) adjusted manually by visual inspection.

The results of band identification were then used to create a qualitative data matrix of presence (1) and absence (0) that was processed using SPSS Software, (SPSS, 1999). Pair wise similarities between accessions were calculated using Jaccard's coefficient for qualitative data (Jaccard, 1908) according to the formula: Jaccard's coefficient=a (n-d), where n: is the total number of polymorphic bands, a: the bands present in both cultivars and d: the bands absent in both accessions. The resulting similarity matrix (calculated with the Jaccard's coefficient) was used to construct a dendrogram by means of the UPGMA (Unweighted Pair-Group Method with Arithmetical average) algorithm (Sensi *et al*., 2003).

RESULTS AND DISCUSSION

Morphological attributes

A total of seventy seven morphological attributes were applied for distinguishing the soft date palm cultivars in Egypt (Table 2). Cluster analysis was conducted to generate a dendrogram (Fig. 1) illustrating possible relationships among the studied nine soft date palm cultivars based on the most useful fruit

attributes, vegetative attributes and all morphological attributes (Table 2).

According fruit attributes, cultivars are divided into two groups at a distance of 3.378. The first group comprises all cultivars characterized by elongated fruit (>5cm), high fruit volume (>15cm^3), seed base blant, cudate or truncate. Within the first group Samani and Selmi are most similar (0.863), while the Zaghloul, Hayani and Om-Elferakh are sub-grouped depend on the fruit shape (Cylindrical).

In view of vegetative attributes, Barhi was delimited in a separate group at a distance (31.306) depend on the leaf length, blade length, pinnate number, apical and middle diverge of pinnate. Regarding all morphological characters, Barhi was delimited in a separate group at a distance (22.901). In most cases, the valuable distinctive attributes are restricted to fruit shape, dimension and color as well as leave attributes which may be, for some instance, common for number of cultivars in Egypt.

To get the linkage between the studied soft date palm cultivars and the most important useful morphological attributes, data matrix were standardized and compute coordinates for plotting Biplot mapping by using perceptual mapping. Perceptual mapping using combination of taxa and attributes was shown in Fig. (2). Perceptual mapping-Biplot of fruit attributes showed the importance of fruit color at maturity, fruit apex, seed shape, and percentage of seed volume to fruit volume to grouping the soft date palm cultivars of Barhi, Orebi, Amhat and Bent-Eisha. While the other group Om-Elferakh, Zaghloul, Hayani, Samani and Selmi were grouped depend on the fruit base and pulp; seed shape, apex, length and volume. Perceptual mapping-Biplot of vegetative attributes shows the value of crown shape and spine type to determine Selmi, value of spine area in determine Orebi and Selmi, value of pinnate to determine Hayani, value of leaf length as well as blade length and pinnate length to grouping Om-Elferakh, Zaghloul, Bent-Eisha and Samani in one group.

From the point of morphological attributes and the lager number of date palm cultivars in Egypt, there are no specific vegetative morphological criteria to distinguish the close related soft date palm cultivars before fruiting stage. These results agree with El-Sharabasy and Rizk (2005).

Molecular markers

All the eight primers (Table 1) examined produced different RAPD-PCR fragment patterns (Plate 1). The number of fragments generated per primer varied between 3 to 13 bands.

Identification of RAPD-PCR markers

At the polymorphism level, a low level of polymorphism was generated utilizing the eight RAPD-PCR primers (Plate 1 and Table 3). A total number of 621 RAPD bands across all cultivars, were obtained. Of these, 41 bands were polymorphic (6.60%). The highest num-

ber of amplicons was generated from Hayani cultivar (73) amplicons and Barhi cultivar (72) amplicons while Samani cultivar generated the lowest (60) amplicons. The highest number of amplicons was generated from primer OP-F04 (108) amplicons, while the lowest was generated from primer OP-B13 (45) amplicons. A number of 29 amplicons were useful gender-specific markers in which fourteen of them were scored for the presence of a unique band for a given cultivars (positive marker), while fifteen scored for the absence of a common band (negative marker).

The highest number of cultivar-specific markers (five markers) was scored for Zaghloul cultivar followed by Orebi cultivar (four markers), while the Om-Elferakh and Barhi no generate specific markers. Primers OP-B09 generated four of cultivar-specific markers, followed by OP-A05 and OP-A09 generated three of cultivar-specific markers. Primer OP-B05, OP-C13 and OP-B17 generated two of cultivar-specific markers, while primers OP-B13 and OP-F04 generated the lowest (one marker), Table (3).

In conclusion, all RAPD-PCR primers used in the present study allowed for enough distinction among the Zaghloul, Hayani, Orebi, Amhat, Samani, Bent-Eisha and Selmi date palm cultivars. Overall comparison among cultivars across the eight primers revealed the power of RAPD in distinguishing among date palm cultivars grown in the same location and this results were in line with Zehdi *et al*. (2004) and Hemeid *et al*. (2007).

Identification of ISSR-PCR markers

ISSR is a class of molecular markers based on inter-tandem repeats of short DNA sequences. These inter repeats are highly polymorphic, even among closely related genotypes, due to the lack of functional constraints in these non-functioning regions. Similarly, a high level of polymorphism was generated utilizing the four ISSR-PCR primers (Plate 2 and Table 3). A total number of 254 ISSR bands were obtained. Of these 29 bands were polymorphic (11.42%) and 225 were monomorphic (88.58%). The highest number of amplicons was generated.

Cluster analysis was conducted to generate a dendrogram (Fig. 3) illustrating possible relationships among the studied nine soft date palm cultivars based on molecular attributes (Table 3). Orebi cultivar was delimited in separate group from the rest of studied soft date palm cultivars at the distance of 0.274. Samani was separated from the rest of cultivars at a distance 0.242. Selmi was separated in one sub-group from the rest of cultivars. Within the second sub-group, the similarity of Zaghloul and Hayani was highly at a distance of 0.102; the other cultivar of the sub-group (Barhi, Amhat, Om-Elferakh and Bent-Eisha) was similar and grouped at the distance 0.145. The results of RAPD and ISSR-PCR are in harmony with (Adawy *et al*., 2004; Soliman *et al*., 2006; Hemeid *et al.*, 2007).

To interpretation the effect of all matrix data on grouping of cultivars, morphological data (Table 2) and molecular attributes (Table 3) were compiled to generate a cluster tree. Samani was delimited from the rest of cultivars at the distance 0.472, as well as Orebi was separated from the rest of cultivars at distance 0.435. Selmi was delimited from the rest of cultivars at distance 0.414 as subgroup. Zaghloul and Hayani were more similar to each other as well as Om-Elferakh, Barhi and Amhat.

To get the linkage between the studied soft date palm cultivars in Egypt and the RAPD and ISSR polymorphism per primer, data matrix were standardized and compute coordinates for plotting Biplot mapping by using perceptual mapping (Table 4 and Fig. 4). Perceptual mapping-Biplot showed the importance of RAPD and ISSR analysis by primers OP-B09, OP-B13, HB-10, HB-12 and HB-15 to separate Samani from the rest of cultivars. The primers HB-15 and OP-B17 were valuable to distinguish Hayani among the rest of cultivars. Primer OP-B17 was the most important to distinguish Bent-Eisha cultivar. Primers OP-A09 and OP-B05 were valuable to distinguish Zaghloul cultivar.

To get the linkage between the studied cultivars of soft date palm, Spearman correlation matrix was conducted based on the molecular markers (Table 5) and morphological attributes as well as molecular marker (Table 6). On the basis of molecular marker, the lowest similarity (0.281) was recorded between Selmi and Samani. The highest similarity (0.743) was recorded between Om-Elferakh and Barhi. In corporation of molecular marker with morphological attributes, the lowest similarity (0.665) was recorded between Hayani and Orebi and the highest similarity (0.807) was recorded between Amhat and Selmi.

In conclusion the molecular marker might be the easier criteria to distinguished date palm cultivars in Egypt than morphological attributes because of there is no specific morphological criteria to distinguished the close related date palm cultivars in Egypt before fruiting stage.

SUMMARY

Some morphological important traits as well as molecular genetic characterization of nine cultivars of soft date palm (Samani, Zaghloul, Bent-Eisha, Hayani, Orebi, Om-Elferakh, Amhat, Selmi and Barhi) in Egypt were subjected to identify and described DNA markers. RAPD-PCR analysis for eight primers (OP-A05, OP-A09, OP-B05, OP-B09, OP-B13, OP-B17, OP-C13 and OP-F04) and ISSR-PCR analysis for four primers (HB08, HB10, HB12 and HB15) as well as seventy seven morphological attributes are conducted for assess morphological and genetic polymorphism of soft date palm cultivars in Egypt. Morphologically, the valuable distinctive attributes included fruit shape, dimension and color as well as leave attributes which may be, for some instance, common for number of cultivars in Egypt. All RAPD-PCR pri-

mers used in the present study allowed discriminating between Zaghloul, Hayani, Orebi, Amhat and Selmi date palm cultivars. Overall comparison among cultivars across the primers revealed the power of RAPD-PCR in distinguishing among palm cultivars grown in the same location. Similarly, a high level of polymorphism was generated utilizing the four ISSR-PCR primers. The genetic similarity matrices were estimated for the nine cultivars and used to develop dendrograms revealing the genetic relationships. Moreover, the polymorphism detected and its reproducibility suggests that RAPD and ISSR markers are reliable for identification of date palm cultivars. This data will be used to enhance sources of information of date palm gene bank of the Central Laboratory of Date Palm Research and Development, Agricultural Research Center, Egypt.

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Table (1): The nucleotide sequences of primers used for RAPD and ISSR-PCR analysis.

Marker	Primer code	Sequence 5'- 3'	Annealing Temp. °C/Sec
RAPD	OP-A05	5´ AGTCAGCCAC 3`	30/30
	OP-A09	5´ GGGTAACGCC 3`	
	OP-B05	5´ GTGACCCCTC 3`	
	OP-B09	5` AGGGAACGAG 3`	
	OP-B13	5` TGTCTCGGTG 3`	
	OP-B17	5` TGGGGGACTC 3`	
	OP-C13	5` GGACCCAACC 3`	
	OP-F04	5` GGG CGGTAC T 3`	
ISSR	HB08	5` GTGTGTGTGTGTGG 3`	57/60
	HB10	5` GAGAGAGAGAGACC 3`	
	HB12	5` CACCACCACGC 3`	
	HB15	5` GTGGTGGTGGC 3`	

Table (2): Morphological attributes used in the study of soft date palm in Egypt.

Morphological attributes	Cultivars name								
	Samani	Zaghloul	Bent-Eisha	Hayani	Orebi	Om-Elferakh.	Amhat	Selmi	Barhi
Vegetative attribute									
Trunk width (1. homogenous 2. base broad than above)	2	1	1	1	1	2	2	1	2
Crown shape (1. dense, 2. moderate dense, 3. Loose and open from the middle)	1	1	2	1	3	2	2	2	1
Leaf length (cm)	570	530	500	500	455	525	480	490	375
Leaf width (at the middle) (cm)	105	55	95	90	75	90	90	75	90
Colors of leaf (1. dark green, 2.green)	2	2	2	1	1	1	1	1	1
Midrib color (1. dark green, 2. glossy green, 3. light green)	3	2	3	2	3	1	3	2	2
Leaf curvature (1. high curved, 2. moderately curved, 3.slightly curved)	1	3	3	2	2	1	3	2	2
Leaf curvature point (1.at middle of blade, 2. at second half of blade, 2. at all blade)	2	2	1	1	3	3	3	2	1
Petiole length (cm)	50	110	50	65	60	45	35	80	45
Petiole / leaf ratio	0.09	0.21	0.1	0.13	0.13	0.09	0.08	0.16	0.12
Petiole thickness (at the middle) (cm)	5	3	3	3.5	3	4	4	4	4
Petiole width (at the middle) (cm)	10	5.5	7	8	7	8	6	6	8
Petiole shape (1. slender, 2. base stout than above)	2	1	1	1	1	2	1	2	1
Leaf base width (at the attachment point) (cm)	17	10	10	11	12	16	11	14	14
Color of leaf base abaxial surface (1.dark green , 2.light green,)	2	1	2	1	1	2	1	1	2
Blotches on leaf base abaxial surface (1.small reddish brown blotches, 2. absent)	2	1	1	1	1	1	2	1	1
Blade length (cm)	420	405	390	400	340	400	310	300	250
Blade/leaf ratio	0.74	0.76	0.78	0.8	0.75	0.75	0.64	0.61	0.67
Number of pinnate	243	213	231	151	213	201	185	175	149
Pinnate density (1.dense, 2.lax, 3.very lax)	1	1	1	3	1	2	1	1	1
Length of blade/number of pinnate ratio	1.7	1.9	1.7	2.6	1.6	2	1.7	1.7	1.7
Pinnate length (cm)	65	65	67	60	45	60	65	55	45
Pinnate width (cm)	4	2.5	3	3.5	3.5	3.25	3.5	2.5	4.5
Pinnate shape (1. lanceolate, 2.linear)	2	2	2	1	1	2	1	2	1
Pinnate apex (1. acute, 2. soft end)	1	1	2	2	1	2	2	1	1
Pinnate nature (1. semi-drooping, 2. non-drooping, 3.semi-erect)	3	2	3	2	2	1	3	3	3
Pinnate – Rachis angle (°)	57.5	40	45	60	70	50	57.5	55	60
Apical divergence (°)	67.5	50	70	90	85	70	80	75	95
Middle divergence of pinnate (°)	110	65	90	110	135	95	110	95	120
Valley angle (1.small, 2.large, 3.absent)	2	1	1	3	1	3	2	1	1
Spine area length (cm)	45	15	60	45	55	80	135	110	80
Spine area/leaf ratio	0.08	0.03	0.12	0.09	0.12	0.15	0.28	0.23	0.21
Spine shape (1. pyramids, 2. pyramids with longitudinal fold, 3.mixed)	2	1	1	1	2	2	2	3	2
Shorter spine length (cm)	9	4	5	6	5	7	3	4	6
Longer spine length (cm)	21	9	22	12	11	25	24	15	15
Spine base (1.not raised, 2.rasied, 3.raised and pulvinous)	3	1	2	1	3	3	1	2	3
Spine type (1. single, 2. di, 3. mixed)	2	1	1	1	3	3	3	3	1
Spin color (1. dark green, 2. light green, 3. yellowish green)	3	1	1	2	1	2	2	2	1
Spine nature (1. flexible, 2. rigid)	2	1	1	1	2	2	2	1	2
Spine rachis angle (°)	55	40	62.5	40	55	50	35	45	55

Table (2): Cont.

Morphological attributes	Cultivars name								
	Samani	Zaghloul	Bent-Eisha	Hayani	Orebi	Om-Elferakh	Amhat	Selmi	Barhi
Fruit attribute									
Fruit length (cm.)	5.85	6.16	3.96	5.6	3.95	6.85	3.5	5.15	3.7
Fruit width (cm.)	3.35	2.85	2.27	2.72	2.82	2.98	2.15	3.15	2.82
Fruit weight (gm)	29.0	22.4	10.1	21.8	13.2	22.6	8.9	30.0	15.56
Fruit volume (cm^3)	30	26	8.33	20	11.5	15	8.33	27.5	15
Fruit density (W/V)	0.97	0.862	1.21	1.09	1.15	1.51	1.07	1.09	1.037
Seed length (cm)	3.85	3.55	2.95	3.35	3.25	4.3	2.25	2.65	2.5
Seed width (cm)	1.18	1.12	0.8	1.02	1.05	1.1	0.9	0.88	1.1
Seed weight (gm)	2.4	2.23	1.02	1.76	1.53	2.35	0.96	1.18	1.61
Seed volume (cm^3)	2	1.7	1	1.66	0.7	1.33	1.33	1	1.33
Weight of seed/ weight of fruit	0.08	0.1	0.1	0.08	0.12	0.11	0.11	0.04	0.11
Volume of seed/ volume of fruit	0.07	0.07	0.12	0.08	0.06	0.09	0.16	0.04	0.09
Fruit shape (1. cylindrical, 2.ovate-elongate, 3. obviate-elongate, 4. Falcoid-elongate, 5.ovate, 6. obviate)	2	1	6	1	5	1	4	3	4
Fruit apex (1.obtuse, 2.blunt, 3.retuse)	2	1	3	1	1	1	1	2	1
Fruit base (1. obtuse, 2.retuse, 3. truncate,4.truncateand emarginated)	3	3	1	3	2	3	3	4	2
Fruit color (khalal) (1. pale red, 2. shiny red,3. Pale yellow , 4.orange)	4	2	2	2	1	1	3	4	2
Fruit color at maturity (1.red, 2. dark red, 3.yellow-orange mottled pale red, 4. pale brown, 5. brownish black, 6. reddish black)	3	2	5	5	2	1	6	4	1
Fruit skin nature at maturity (1.smooth and united with flesh, 2.smooth and loose from flesh)	1	1	2	2	1	1	2	1	1
Fruit skin appearances (1. shiny, 2. not shiny)	1	1	1	1	1	2	1	2	1
Fruit skin thickness (cm.)	0.16	0.12	0.05	0.04	0.06	0.09	0.02	0.05	0.05
Fruit flesh thickness (cm.)	0.95	0.85	0.65	0.8	0.75	0.94	0.65	0.85	0.8
Fruit flesh color (1. white, 2. whitish yellow, 3.cream-brown)	1	1	1	3	2	2	3	1	1
Fruit flesh texture (1. soft, 2. firm, 3. fibrous, 4.dry)	2	2	1	1	2	2	1	2	3
Fruit flavor (1. poor, 2. good, 3. excellent)	1	2	3	2	1	1	2	2	2
Fruit taste (1. delicious, 2. delicious-sweet)	2	2	1	2	1	1	1	2	1
Fruit pulp (1. stout, 2. less stout at apex)	1	2	1	2	2	2	2	2	2
fully maturation (1. early, 2. internecine, 3. late)	2	2	2	1	3	3	1	3	1
Seed shape (1. cylindrical, 2. elliptical)	1	1	1	1	2	1	2	2	2
Seed color (1.cream,2. pale brown)	1	2	2	1	2	2	2	1	2
Seed apex (1. obtuse, 2. blunt, 3.retuse)	2	3	1	2	1	3	2	1	2
Seed base (1. obtuse, 2. blunt, 3.caudate, 4.truncate)	3	4	1	4	1	4	1	2	4
Seed surface (1. smooth, 2. rough)	2	2	2	2	1	2	1	2	1
Seed transverse grooves (1.absent, 2.moderately)	2	2	2	2	1	2	1	2	1
Seed micropyle position (1. towards the apex, 2.at the middle, 3.towards the base)	2	1	3	3	2	1	1	2	3
Seed micropyle elevation (1. not sunken, 2. moderately sunken)	2	2	1	2	1	1	1	1	1
Ventral furrow shape (1. regular, 2.broadest at base, 3. broad at both ends)	1	1	3	2	3	1	1	3	1
Seed ventral furrow ends (1. Open at one end, 2. Open at both ends)	2	1	2	2	2	2	1	2	2

Table (3): Number of amplified fragments markers of nine date palm cultivars based on RAPD–PCR and ISSR–PCR analysis.

Cultivars		RAPD primers									ISSR primers				
		OP-A05	OP-A09	OP-B05	OP-C13	OP-B09	OP-B13	OP-B17	OP-F04	Total	HB08	HB10	HB15	HB12	Total
Samani	AF	11	8	8	6	6	3	8	10	60	5	6	5	3	19
	SM	0	0	0	0	1	1	0		2	0	1	2	3	6
Zaghloul	AF	11	7	7	8	6	4	10	10	63	6	5	8	9	28
	SM	0	2	1	1	1	0	0		5	0	0	0	0	0
Bent-Eisha	AF	11	7	6	6	8	7	4	13	62	6	5	8	10	29
	SM	0	0	0	0	0	0	1		1	0	0	0	0	0
Hayani	AF	12	7	7	6	13	4	12	12	73	5	7	10	10	32
	SM	1	0	0	0	0	0	1		2	0	0	2	0	2
Orebi	AF	12	7	8	10	5	6	9	11	68	3	5	5	7	20
	SM	0	0	1	0	2	0	0	1	4	0	0	0	0	0
Om-Elferakh	AF	11	7	5	6	13	5	7	13	67	3	6	10	8	27
	SM	0	0	0	0	0	0	0		0	0	0	0	0	0
Amhat	AF	11	7	6	9	11	5	7	13	69	5	5	11	9	30
	SM	1	0	0	0	0	0	0		1	2	0	0	0	2
Selmi	AF	11	8	8	10	8	5	7	12	69	4	6	11	9	30
	SM	1	1	0	1	0	0	0		3	0	0	0	0	0
Barhi	AF	11	6	6	8	13	5	10	13	72	4	4	11	9	28
	SM	0	0	0	0	0	0	0		0	1	0	0	0	1
PB		0	1	4	7	11	5	8	5	41	7	3	11	8	29
TAF		104	67	63	71	87	45	76	108	621	44	50	83	77	254
TSM		3	3	2	2	4	1	2	1		3	1	4	3	

AF=Amplified fragments, SM=Marker including either the presence or absence of a band in date palm cultivars, PB=Polymorphic bands, TAF=Total number of amplified fragments and TSM=Total No. of specific markers across date palm cultivars.

Table (4): The polymorphism percentage produced by different primers of the studied cultivars of soft date palm in Egypt.

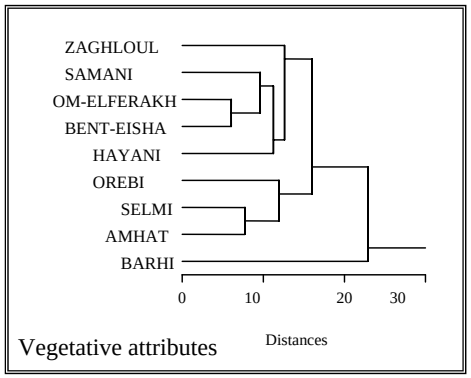
	Primer	Polymorphism per primer%								
		Samani	Zaghloul	Bent-Eisha	Hayani	Orebi	Om-Elferakh	Amhat	Selmi	Barhi
RAPD	OP-A05	0.47	0.47	0.33	0.20	0.47	0.13	0.20	0.47	0.53
	OP-A09	0.15	0.15	0.00	0.23	0.23	0.08	0.15	0.15	0.23
	OP-B05	0.27	0.27	0.45	0.45	0.18	0.00	0.36	0.36	0.45
	OP-C13	0.08	0.08	0.23	0.46	0.15	0.38	0.38	0.31	0.54
	OP-B09	0.14	0.29	0.43	0.14	0.29	0.29	0.29	0.29	0.29
	OP-B13	0.23	0.23	0.46	0.31	0.31	0.00	0.38	0.38	0.31
	OP-B17	0.00	0.10	0.00	0.20	0.20	0.10	0.00	0.00	0.10
	OP-F04	0.00	0.00	0.33	0.25	0.08	0.25	0.08	0.25	0.17
ISSR	HB-08	0.15	0.23	0.38	0.23	0.23	0.31	0.23	0.31	0.31
	HB-10	0.00	0.33	0.42	0.08	0.25	0.33	0.42	0.33	0.33
	HB-15	0.09	0.18	0.27	0.27	0.18	0.27	0.27	0.27	0.27
	HB-12	0.08	0.42	0.42	0.50	0.33	0.33	0.25	0.25	0.25

Table (5): The Spearman correlation matrix between the soft date palm cultivars, on the basis of DNA markers.

Cultivars	Samani	Zaghloul	Bent-Eisha	Hayani	Orebi	Om-Elferakh	Amhat	Selmi
Zaghloul	0.574							
Bent-Eisha	0.485	0.528						
Hayani	0.382	0.546	0.473					
Orebi	0.325	0.437	0.414	0.332				
Om-Elferakh	0.433	0.494	0.628	0.585	0.415			
Amhat	0.378	0.549	0.563	0.494	0.508	0.724		
Selmi	0.281	0.445	0.416	0.369	0.445	0.442	0.666	
Barhi	0.401	0.483	0.538	0.591	0.314	0.743	0.705	0.538

Table (6): The Spearman correlation matrix between the soft date palm cultivars, on the basis of DNA markers and morphological attributes.

Cultivars	Samani	Zaghloul	Bent-Eisha	Hayani	Orebi	Om-Elferakh	Amhat	Selmi
Zaghloul	0.790							
Bent-Eisha	0.729	0.754						
Hayani	0.712	0.794	0.752					
Orebi	0.705	0.693	0.711	0.665				
Om-Elferakh	0.771	0.729	0.730	0.753	0.741			
Amhat	0.708	0.715	0.736	0.728	0.764	0.798		
Selmi	0.737	0.728	0.749	0.710	0.783	0.752	0.807	
Barhi	0.722	0.759	0.762	0.788	0.703	0.801	0.786	0.754



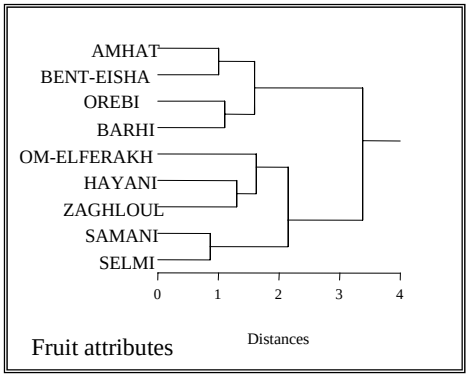
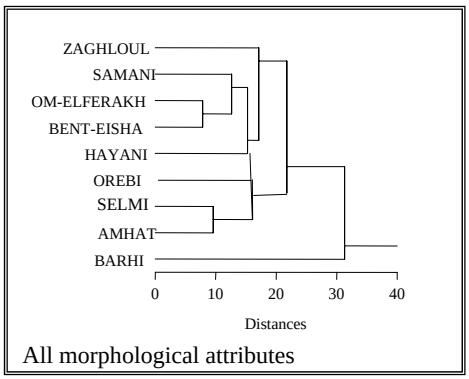
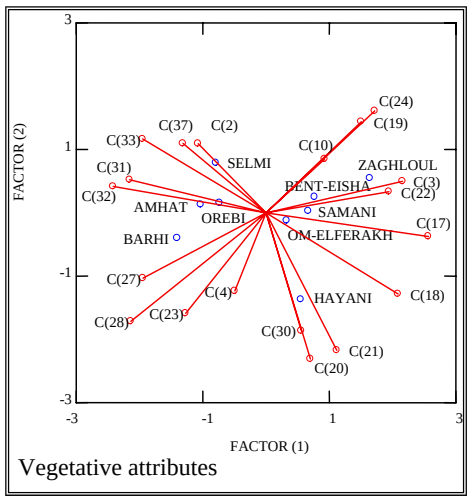


Fig. (1): Phenogram showing the relationships between nine soft date palm cultivars using distance metric is Euclidean distance average linkage.





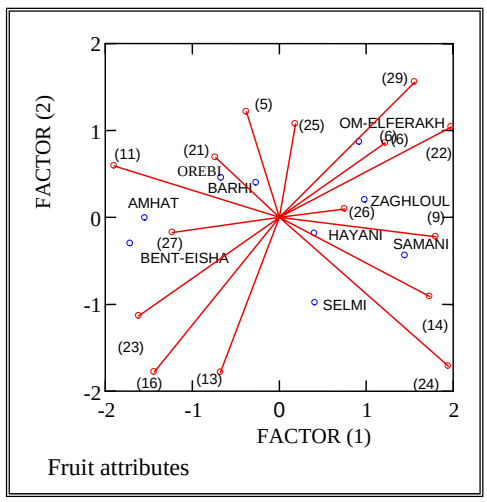


Fig. (2): Perceptual mapping (Biplot) of the studied soft date palm cultivars for combination of taxa and attributes, configuration has been standardized. For attributes see Table (2).

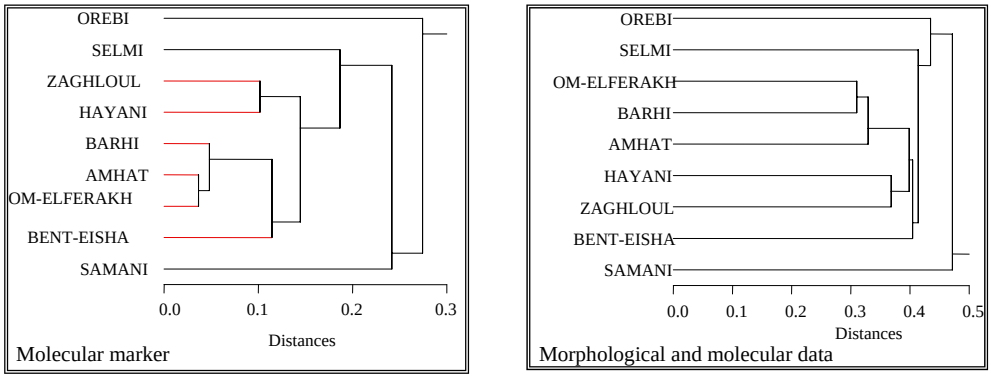


Fig. (3): Dendrogram and phenogram showing the relationships between nine soft date palm cultivars using distance metric is 1-Gamma coefficient average linkage method.

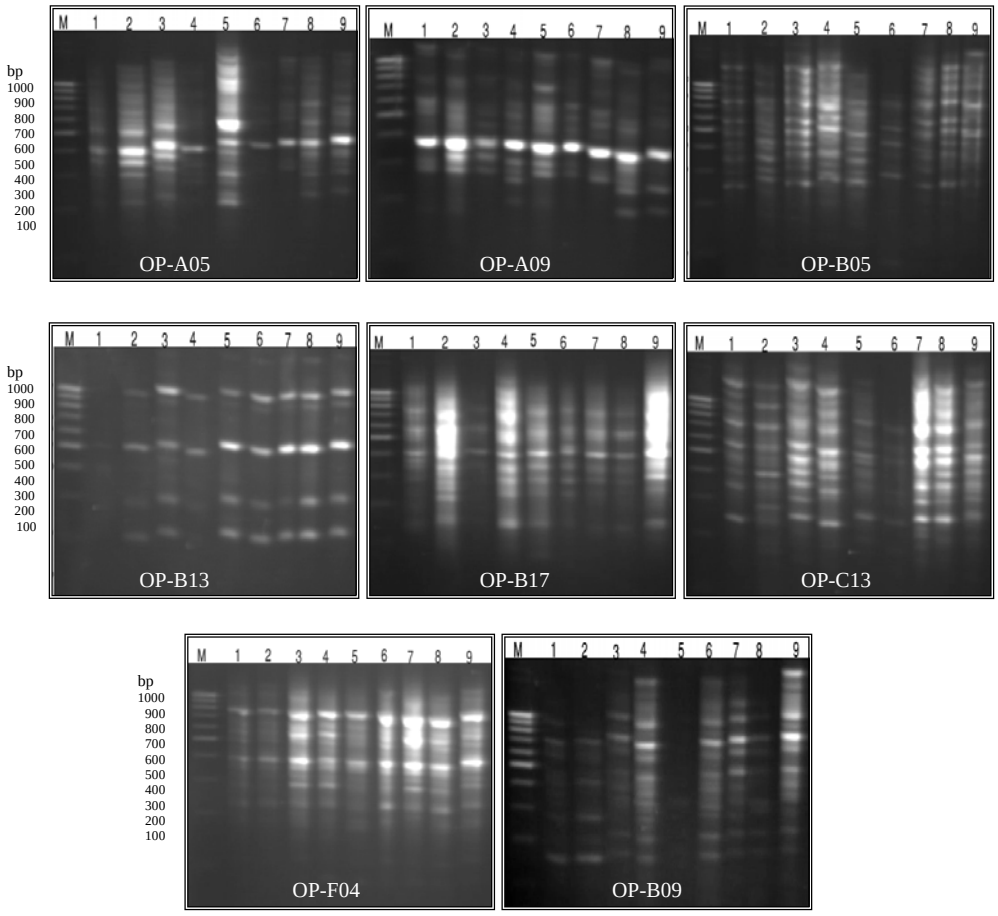


Plate (1): RAPD profile of the nine date palm cultivars amplified with eight different RAPD primers. M: 100 bp ladder marker. 1: Samani, 2: Zaghloul, 3: Bent-Eisha, 4: Hayani, 5: Orebi, 6: Om-Elferakh, 7: Amhat, 8: Selmi, 9: Barhi.

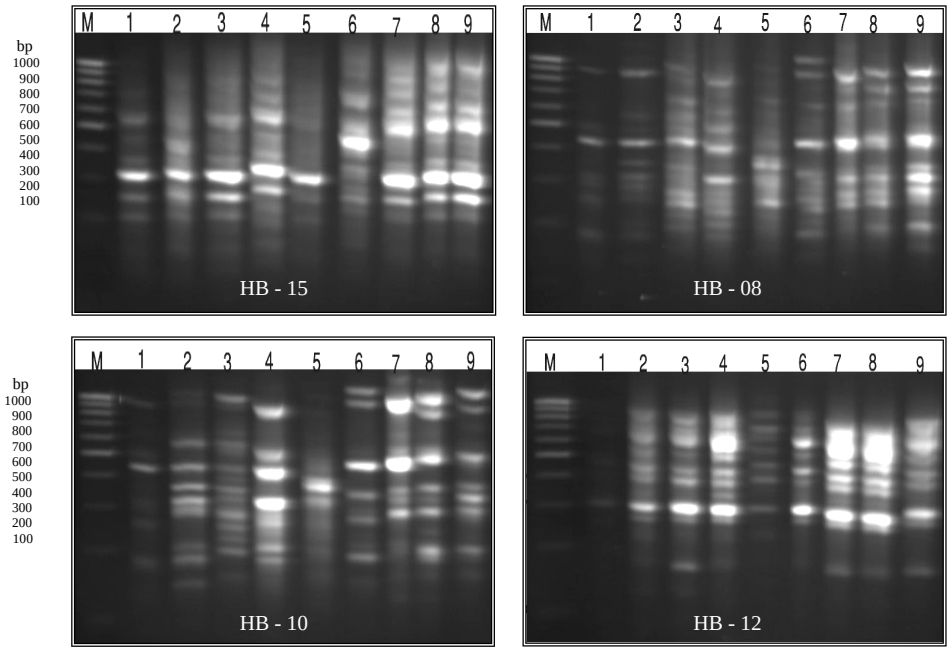


Plate (2): ISSR profile of the nine date palm cultivars amplified with four different ISSR primers. M: 100 bp ladder marker. Lanes 1 through 9 refer to date palm cultivars: 1: Samani, 2: Zaghloul, 3: Bent-Eisha, 4: Hayani, 5: Orebi, 6: Om-Elferakh, 7: Amhat, 8: Selmi, 9: Barhi.

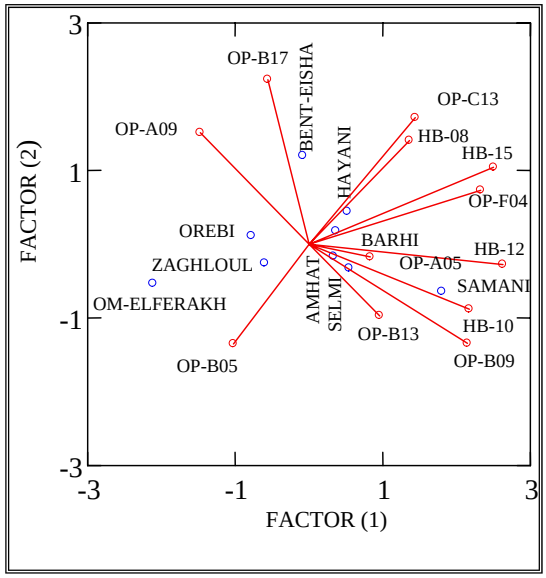


Fig. (4): Perceptual mapping (Biplot) of the studied soft date palm cultivars for combination of taxa and polymorphism per primer, configuration has been standardized.