IDENTIFICATION AND GENETIC SIMILARITY ANALYSIS OF DATE PALM (*Phoenix dactylifera* L.) COLLECTED FROM DIFFERENT REGIONS IN SIWA OASIS USING MORPHOLOGICALLY TRAITS AND MOLECULAR MARKERS.

REHAM M. ABD EL-AZEEM[1], M. H. HASHEM[2] AND A. A. HEMEIDA[3]

1. *Department of Molecular Biology, Genetic Engineering & Biotechnology Research Institute, Minufiya University, Egypt*
2. *Department of Animal Biotechnology, Genetic Engineering & Biotechnology Research Institute, Minufiya University, Egypt*
3. *Department of Bioinformatics, Genetic Engineering & Biotechnology Research Institute, Minufiya University, Egypt*

Date palm (*Phoenix dactylifera* L., 2n = 36) is a fruit tree mainly cultivated in arid regions in the Middle East, where it has been domesticated for at least 5,000 years and is believed to have originated in Mesopotamia. Date palm (*Phoenix dactylifera* L) is the major factor of oasis environmental and economic stability (Zehdi *et al.*, 2004). The major date producers in the world are located in the Middle East and North Africa (Rania *et al.*, 2008).

In Egypt, date palm is an important crop where the total number of fruitful female palm is about 10 million palm trees according to the statistics of the Central Administrations of Horticultural, Ministry of Agriculture (Rania *et al.*, 2008). This crop is of a great socioeconomic importance in oases. The oasis of Siwa located in Egypt's western desert is about 600 km away from Alexandria and 300 km South-West from Matrouh (Mediterranean coast) and about 65 km east from the Libyan borders. Siwa oasis is a natural isolated depression in the western desert of Egypt. However, little is known about the genetic characterization of Date palm cultivars. The date palm cultivation takes about 40% of all cultivated area.

The pollen of the date palm has been found to exert a direct influence on the size, shape and color of the seed and also, on the size of the fruit, on the speed of development of the fruit and on the time of ripening of the fruit. This direct influence of the male parent on the development of the date fruit is precise and definite and varies with the particular male used to fertilize the female flowers. Each male is exerting approximately the same effect on fruit of all varieties and exerting the same effect in different years. Therefore, it is important to select and identify superior male in term of fertilization (Walter, 1928).

Recently, study of genetic diversity for plant crops is the process by which variation among individuals or groups of individuals is analyzed by a specific genetically method or a combination of such methods. The most important measurements are data obtained by DNA based marker data that detect and monitor identification of different genomes. Many new markers can be identified in the same region using inter Simple Sequence Repeat (ISSR) markers linked to genes of interest. Furthermore, ISSR is informative about many loci and are suitable to discriminate closely related genotype variants and lastly, ISSR markers constitute discrete markers suitable in the DNA fingerprinting (Gupta and Varshney, 2000).

The objectives of this study were designed to the determine morphological traits among nine date palm cultivars and six male trees grow in Siwa oasis and develop molecular fingerprints based RAPD and ISSR analysis. Moreover, through the obtained data, determine genetic relationships among these cultivars by applying morphological and molecular analysis.

MATERIALS AND METHODS

Plant materials

Fifteen date palm (*Phoenix dactylifera* L.) cultivars, including 9 cultivars (Siwi, Karama, Gazaly, Halwo Ganm, Fryhee, Oshengpel, Taktakt and Quaipe) and 6 male plants were collected from Kadosa region {Male tree that produce plentiful pollen (ZPK); two Males needs to be test (ZTK1 and ZTK2) and unknown Male (ZUK)} and Mishandid region {Male tree that produce plentiful pollen (ZPM) and Male needs to be test (ZTM)}. The young leaves of different cultivars trees (one for each genotype) were randomly collected.

Measurement of the morphological traits

The morphological traits for nine cultivars and six male plants of date palm were measuring. The morphological traits were the trunk (diameter, cm), the frond (length, cm, leave end), leaf base (thickness, cm, breadth, cm), color of the dorsal surface and length (cm), leaflet, number/frond, arrangement on the midrib, area covered on the midrib (%), length (cm) and breadth (mm), spine (number), area covered on the midrib (%), thickness, length (cm), spine base, arrangement and angle on the midrib, sheath fiber (texture, pores and color).

Total genomic DNA extraction

Genomic-DNA from each cultivar leaves was isolated according to the method of Hemeida *et al.* (2007).

RAPD analysis

RAPD analysis was carried out using twelve oligonucleotide primers (Table 1) that were selected from the Operon Kit (Operon Technologies Inc., Alabameda, CA). The polymerase chain reaction mixture (25 µl) consisted of 0.8 U of *Taq* DNA polymerase; 25 pmol dNTPs; 25 pmol of primer and 50 ng of genomic DNA. PCR amplification was performed in a Biometra *T1* gradient thermalcycler for 40 cycles after initial denaturation for 3 min at 94ºC.

Each cycle consisted of denaturation at 94ºC for 1 min; annealing at 36ºC for 1 min; extension at 72ºC for 2 min and final extension at 72ºC for 10 min (Soliman, *et al.,* 2003). Amplification products were separated on 1% agarose gels at 100 volts for 1.30 hrs with 1 x TBE buffer. To detect ethidium bromide/ DNA complex, agarose gels were examined on ultraviolet transilluminator (302 nm wavelength) and photographed. Using 100 pb DNA ladder (V-gene Biotechnology Limited, shiqao, P. R. China), the lengths of the different DNA fragments were determined. For each sample, the reproducible DNA bands from two runs were scored for their presence or absence.

ISSR analysis for Date palm

Genomic-DNAs were amplified using eight primers (Table 1). ISSR reactions were carried out in 25 µl containing 30 ng of DNA; 60 pg primer; 2.5 µl 10 X *Taq* DNA polymerase reaction buffer; 1.5 unit of *Taq* DNA polymerase and 200 mM of each dNTP. For 35 cycles, amplification was performed using a program of 5 min at 94ºC; 30 s at 94ºC and 90 s at 72ºC. A final extension was performed at 72ºC for 5 min (Trifi *et al.*, 2000). Amplification products were separated on 1.4% agarose gels; stained with ethedium bromide; visualized with ultraviolet light and photographed. DNA fragment lengths were determined by comparisons with 100 pb DNA ladders run on each gel.

Analysis of RAPD's and ISSR's fragments

RAPD's and ISSR's fragments were scored as present/absent. Data matrices were entered into the NTSYS (Numerical Taxonomic and Multivariate Analysis System) program, version 2.1, Applied Biostatistics Inc. (Rohlf, 2000). Similarity coefficients were applied to construct dendrograms using the UPGMA (Unweighted Pair Group Method with Arithmetic Average) and the SAHN (Sequential Agglomerative Hierarchical Nested Clustering) routine in the NTSYS program.

RESULTS AND DISCUSSION

Morphological traits

Tables (2 and 3) represent different vegetative characters for the Siwian Date palm cultivars. Trunk diameters varied from cultivar to another. The highest value was 70.3±3.1 cm for Gazaly cultivar, while the lowest value was 30.5 cm for ZUK male. The other values were intermediate. For all cultivars, the lengths of frond (leaves) were long (> 425 cm) in Oshengpel, Taktakt, Quaipe, Karama, Gorm Agazal, Gazaly cultivars, ZTM and ZPK males while in ZTK1 male, Siwi and Fryhee cultivars were medium (325-425 cm) .In ZTK2, ZUK males the lengths of frond was Short (< 325 cm). The leaves end was double for Taktakt, Quaipe, Karama and Halwo Ganm, while single for all other cultivars and males.

The thickness, breadth and lengths of the leaf base varied from cultivars to another. The highest values were 9 cm for Quaipe, 25 cm for Halwo Ganm and 80 cm for Taktakt, respectively. In the contrary, the lowest values were 2.1±0.1 cm

for ZTK2 male, 4.9±0.9 cm for ZUK male and 15.9±0.9 for ZTK2 male, respectively. The color of dorsal surfaces was Light brown for all cultivars except Taktakt, Quaipe and Gorm Agazal ZTK1male, ZTM male (dark brown) and Oshengpel (very dark).

The numbers of leaflet (Pinna) varied from cultivar to another. The highest number was 254.6±15.9 for Taktakt cultivar and the lowest value was 100.3±2.8 for ZTK2 male. For all cultivars, leaflet lengths were short (< 60 cm). The Breadth was narrow (< 38 mm) in all cultivars except for Gazaly, Halwo Ganm cultivar, ZTM and ZPM males it was medium (38-44 mm). For the percentage of Breadth/ Length value the highest percentage was 8.72 for ZPM male and the lowest percentage was 3.8 for Oshengpel.

Apart from pinnae, the petioles usually also grow spines in the lower region. They are hard and very sharp. The date cultivator often removes the spines to prevent injury during cultural practices. The number of Spine divided to two ranges, the Average number (20-30) which was found for Oshengpel, Taktakt, Quaipe, Gorm Agazal cultivars, ZTK1, ZTK2 and ZUK males while the Large more than 30 was found for Siwi, Fryhee, Karama, Gazaly, Halwo Ganm cultivars, ZPK, ZTM and ZPM males. The area covered on the Midrib was medium. 15-25% for Oshengpel, Taktakt, Gorm Agazal, Halwo Ganm cultivars, ZPK and ZPM males, while the other cultivars had Long area covered on the midrib (> 25%). The Spine thickness was thick and hard for Siwi, Fryhee, Quaipe, Gorm Agazal, Gazaly, Halwo Ganm cultivars, ZPK, ZUK, ZTM and ZPM male, while Oshengpel, Taktakt, Karama cultivars, ZTK1 and ZTK2 males. For the spine length Taktakt, Gorm Agazal cultivars, ZPK, ZTK1 and ZTK2 males were short (< 10 cm). While, Siwi, Fryhee, Oshengpel, Quaipe, Gazaly cultivars, ZUK and ZTM males showed medium length for spine. The long length (> 15 cm) was found in Karama, Halwo Ganm cultivars and ZPM male. The arrangement and angle on the midrib was double for all cultivars except for ZPK male it was single.

Elshibli and Korpelainen (2009) indicated that hundreds of date palm cultivars and strains were recognized and selected by farmers through a long history of more than 3000 years of cultivation in Sudan. The most common characters used to identify cultivars are tree and fruit morphology as well as softness characters of fruits, which are detectable only at tree maturity. On the other hand, Hamza *et al.* (2009) showed that the morphological studies of date palm have always been considered difficult to undertake because they require a large set of phenotypic data and because they are varied due to the environment effect. On another point of view, Hamza *et al.* (2009) indicated that the majority of the phenotypic date palm studies are aimed at studying the spectrum genetic variation but they cannot allow definitive discrimination between cultivars, fruit, quality and plant behavior.

However, this study identified the relationship between some six males and nine known females in order to use them in breeding that agree with the recommendation of Hamza *et al.* (2009). Where, it was indicated that future studies should be considering the possible relations of other important phenotypic markers related to the tolerance towards oases stress. This should be backed up by others studies such as molecular ones to provide reliable tools for measuring genetic divergence.

RAPD analysis

The fifteen different genomic DNAs of Date palm (nine cultivars and six males) were assayed using the producible twelve RAPD primers. As shown in Tables (1 and 4) and Figs (1 and 2). These primers produced multiple band profiles with a number of amplified DNA fragments ranging from 1 to 8 fragments. Fingerprinting revealed a total number of 801 unambiguous DNA fragments with an average of 66.75 fragment/primer. The number of polymorphic bands ranged from 0 to 8 per primer with an average of 41.75 polymorphic bands per primer. The total number of polymorphic amplicons produced by the 12 primers was 501, thus, representing a level of polymorphism (63%, Table 4) across the 15 date palm cultivars.

Hassan *et al.* (1998) showed that RAPD assay could allow the establishment of a catalogue of cultivars grown worldwide. Other applications could include fingerprinting of date palm genotype, identification of duplicate cultivars and establishment of a core collection. Where, it was indicated that RAPD technology is an effective tool for identifying cultivars of date palm. RAPD markers should therefore be of high value for date palm germplasm characterization and genetic maintenance. In this context, the results showed that RAPD primers used in the present study allowed for enough distinction among the males and females date palm cultivars. Overall comparison among cultivars across, the twelve primers revealed the power of RAPD in distinguishing among males and females date palm grown in Siwa oasis. Rania *et al.* (2008) revealed the power of RAPD in distinguishing among palm cultivars grown in the same location. Also, indicated that the RAPD markers can be used in subsequent experiments to detect molecular markers for genes with male and female identification in palm cultivars. Moreover, Saker and Moursy (1999) stated that a low number of amplicons per a RAPD primer was sufficient to produce useful fingerprints for palm cultivar discrimination.

ISSR analysis

The ISSR analysis was performed on the bulked DNA samples representing nine cultivars and six males of Siwa oasis using eight ISSR primers composed of short tandem repeat sequences (Table 5). These eight ISSR primers were screened from ten primers for studying their ability to generate consistently amplified fragment patterns and to access polymorphism in the tested cultivars. Figures (3 and 4) illustrating the ISSR profile of the 15 cultivars of the studied date palm. A total of

492 amplicons were generated by the tested primers with an average number of 61.5amplicons/primer. Primer Amic-07 exhibited the highest number of fragments (82 amplicons), while primers Amic08 and Mic-07 revealed the least number (43 amplicons). The total number of polymorphic bands was 385 with an average of 48.1 polymorphic amplicons per primer. This represents a level of polymorphism (78%, Table 5) across the 15 date palm cultivars.

In this study, the ISSR technology was designing to enlarge the number of molecular markers that are suitable in the molecular characterization and the phylogenic relationships in order to examine males and females date palm grown in Siwa oasis. All ISSR primers used in the present study allowed for enough distinction among the studied males and females cultivars. Overall comparison among genotypes across the markers obtained by the eight primers revealed the power of ISSR primers in distinguishing among palm genotypes grown in Siwa oasis. These markers can be used in subsequent experiments to detect molecular markers for genes with male and female identification in palm cultivars. In this context, Rania *et al.*, (2008) indicated that a low number of amplicons per ISSR primer was sufficient to produce useful fingerprints for palm cultivar discrimination. Moreover, Adawy *et al.* (2002 and 2004) revealed unique markers characterizing cultivars that were studied from Delta and Upper Egypt. Also, Hussein *et al.* (2005) indicated that RAPD and ISSR-specific markers were detected in five out of 14 date palm cultivars. In another point of view, Zehdi *et al.* (2001) showed that it is clearly evident that in combination with agronomic parameters, isoenzyme and RAPD markers, ISSRs could provide the establishment of identification criteria in date palm germplasm.

Genetic similarity and genotypes relationships

Based on the matrix of genetic similarity values (Morphological traits, RAPD and ISSR compound data), UPGMA cluster analysis (Rohlf, 2000) was developed to identify genetic variation patterns among the fifteen Date palm genomes under study (Table 6). The genetic similarity estimates ranged from 0.46% to 0.81%. The highest value of genetic similarity (0.88%) was observed between Karama cultivar and ZTK1 male, while the lowest was detected between ZPM male and Siwi cultivar (similarity of 49%). This revealed moderate levels of genetic similarity among the studied Date palm genomes. Similarly, El-Khishin *et al.*, (2003) reported genetic similarity estimates ranged from 64.4% to 76.7% among five date palm cultivars.

In the present study, dendrograms based on similarity values from Morphological traits, RAPD and ISSR were constructed to reveal the genetic relationships between the nine cultivars of Date palm and six male trees. The three applied markers amplify different parts of the genomes (Amel *et al.*, 2005). This was par-

tially reflected on the topology of the phylogenetic trees drawn from the data of the three assays (Fig. 5). Therefore, the dendrogram was generated from the genetic distance matrix according to UPGMA clustering method using NTSYS-pc program. The dendrogram was divided into two main clusters. The first cluster shows two separate sub-clusters, the first one included pairs of male tree ZTK1 and Karama cultivar were grouped together, which were nearly similar (similarity 0.82), while the other sub-clusters included the Oshengpel cultivar.

In relation to the second cluster formed two sub clusters at a genetic similarity about 0.63. The first sub cluster included Siwa and Fryhee were grouped together, which were nearly similar (similarity 0.72). The present results were in good accordance with those Adawy *et al.* (2005) and Hemeida *et al.* (2007) as they showed that the overall dendrogram tree separated the Siwa Oasis cultivars together *i.e*. Siwi and Fryhee were grouped together. Abou Gabal *et al.* (2006) revealed that Siwi and Fryhee cultivars were clustered together in the same cluster. For the second sub-cluster included Gazaly and Halwo Ganm were grouped together, which were nearly similar (similarity 0.70), while the other sub-clusters included the rest of the Siwa Date palm cultivars and male trees.

In general, the results indicated that there is a conflict between diversity measurements based on morphological traits and molecular genetic analysis. Nevertheless, the lacks of similarity between agronomic and molecular diversity measurements in this study that germplasm classification and utilization should not be based on one measurements diversity alone. Another reason for significant differences between agronomic and molecular measurements that the former is invisible and, therefore, unselected by breeder, while the latter is subjected to selection. Determination of molecular diversity should not be seen as replacing traditional characterization but rather as complementing it. The results presented here agree with previous studies by El-Khishin *et al.* (2003), Adawy *et al.* (2004) and Hemeida *et al.* (2007). On this basis, it is possible to look for linkages between molecular markers and agronomically important traits, taxonomic studies and to identify genetic variation at different stages of the breeding process.

The results provide the exciting possibility of being used to address several issues, including developing DNA probes to determine sex in palm dates to increase understanding of the evolution of date palm. In combination with agronomically important morphological criteria, RAPD and ISSR assays could allow the establishment of a catalogue of cultivars grown worldwide. Other applications could include fingerprinting of date palm genotype, identification of duplicate cultivars, and establishment of a core collection.

SUMMARY

Genetic variation among 15 date palm (*Phoenix dactylifera* L.) cultivars, including nine cultivars and six male plants, collected from different regions in

Siwa oasis, was studied using morphologically traits and molecular markers (Random Amplified Polymorphic DNA and Inter Simple Sequence Repeat markers). The pre screening of 35 primers allowed selection of 20 primers which revealed polymorphism and gave reproducible results. All analyzed genotypes were distinguishable by their fingerprint patterns. A RAPD and ISSR molecular marker appears very effective for identifying male trees of date palm. Morphologically traits and molecular markers based genetic distance were used to determine the relationships between the male trees. They showed a relatively high level of polymorphism. This could be related to the mode of introduction and maintenance of the Siwa date palm germplasm involving limited foundation germplasm. Exchange of male trees between plantations and periodic development of new recombinant male trees through sexual reproduction and seedling selection may also have played a role. In addition, the selection applied by farmers concerns mainly end use quality related genes which may represent only a small fraction of the date palm genome.

REFERENCES

Abou Gabal, A. A., A. A. Abdelaziz, M. M. Harhash and H.F. El-Wakil (2006). Genetic diversity among seven Date palm landraces in Siwa oasis. Egypt. J. Genet. Cytol. 35: 117-128.

Adawy, S. S., E. H. A. Hussien, E. M. Samer and H. A. El-Itriby (2005). Genomic diversity in date palm cultivars (*Phoenix dactylifera* L.) as reveled by AFLPs in comparison to RAPDs and ISSRs. Arab J. Biotech., 8: 99-114.

Adawy, S. S., Ebtissam A. Hussein, Dina El- Khishin, H. Moharam and Hanayia A. El-Itriby (2002). Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifer*a L.) cultivars: II. RAPD and ISSR profiling. Arab J. Biotech., 5: 225-236.

Adawy, S. S., Ebtissam A. Hussein, M. M. Saker and Hanaiya A. El-Itriby (2004). 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.

Amel, HCh,. M. Khaled, M. M. Messaoud and T. Mokhtar (2005). Comparative analysis of genetic diversity in two Tunisian collections of Fig cultivars based on Random Amplified Polymorphic DNA and Inter Simple Sequence Repeats Fingerprints. Genetic Resources and Crop Evolution, 52: 563-573.

Ebtissam, A. Hussein, S. S. Adawy, S. E. M Ismail and Hanaiya A. El-Itriby (2005). Molecular characterization of some Egyptian date palm

germplasm using RAPD and ISSR markers. Arab J. Biotech., 8: 83-98.

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

Elshibli, S. and H. Korpelainen (2009). Identity of date palm ((*Phoenix dactylifera* L.) germplasm in Sudan: from morphology and chemical characters to molecular markers. Acta Hort. (ISHS), 859: 143-153.

Gupta, P. K. and R. K. Varshney. (2000). The development and use of microsatellite markers for genetic analyis and plant breeding with emphasis on bread wheat. Euphytica 113: 163-185.

Hamza, H., M. Rejeli, M. Elbekkay and A. Ferchichi (2009). New approach for the morphological identification of date palm (*Phoenix dactylfera* L.) cultivars from Tunisia. Pak. J. Bot., 41: 2671-2681.

Hassan, S., L. Philippe, T. Pierre, C. Marie-Christine and H. Serge (1998). Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica, 103: 75-82.

Hemeida, A. A., Sanaa A. R. and M. A. Taha (2007). Molecular characterization of different Date palm (*Phoenix dactylifera* L.) cultivars grown in Siwa oasis. Egypt. J. Genet. Cytol., 36: 145-162.

Younis, Rania, A., Ismail Omayma M. and S. S. Soliman (2008). Identification of sex-specific DNA markers for date palm (*Phoenix dactylifera* L.) using RAPD and ISSR techniques. Research J. Agriculture and Biological Sciences, 4: 278-284.

Rohlf, F. J. (2000). On the use of shape spaces to compare morphometric method. Hystrix, Italian J. Mammology (n.s.), 11: 8-24.

Saker, M. M. and H. A. Moursy (1999). Molecular characterization of Egyptian date palm. I: RAPD fingerprints. Arab J. Biotechnology, 2: 71-78.

Soliman S. S., B. A. Ali and M. M. M. Ahmed (2003). Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. African Journal of Biotechnology, 2: 86-86.

Trifi, M., A. Rhouma and M. Marrakchi (2000). Phylogenetic relationships in Tunisian date-palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. Agron., 20: 665-671.

Walter, T. S. (1928). Metaxenia in the datepalm Possibly a Hormone Action by the Embryo or Endosperm. The Journal of Heredity, 19: 257-268.

Zehdi, S., M. Trifi, N. Billotte, M. Marrakchi and J. C. Pintaud (2004). Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. Hereditas, 141: 278-287.

Zehdi, S., M. Trifi, Ould M. Salem, A. Rhouma and M. Marrakchi (2001). Molecular Characterization of Tunisian Date-Palm germplasm using ISSR markers. Second International Conference on Date Palms, Al-Ain, United Arab Emirates, 2001 (courtesy of UAE University).

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

Analysis type	Primer code	Sequence (5`-3`)
RAPD	OPA-01	CAG GCC CTT C
	OPA-05	AGG GGT CTT G
	OPA-19	CTG GGG ACT T
	OPB-07	GGT GAC GCA G
	OPC-05	GAT GAC CGC C
	OPC-08	TGG ACC GGT G
	OPC-16	CAC CAT CCA G
	OPD-09	CTC TGG AGA C
	OPH-18	GAA TCG GCC A
	OPR-01	GGT GCG GGA A
	OPR-04	CCC GTA GCA C
	OPR-05	GAC CTA GTG G
ISSR	Amic-06	$GGC(CA)_7$
	Amic-07	$CGA(CAG)_5$
	Amic-08	$GAA(TC)_7$
	Mic-09	$(GTG)_5$
	Mic-07	CCT ACC TAC CTA CCT
	Mic-08	$(CGA)_5$
	A-08	$(AGC)_4$ GC
	A-10	$(GCT)_4$ C

Table (2): Vegetative characters for the Siwa Date palm cultivars.

Characters			Date palm cultivars								
			Siwi	Fryhee	Oshen gpel	Taktakt	Quaip e	Karama	Gorm Agazal	Gazal y	Halwo Ganm
Trunk	Diameter (cm)		60.6 ±5.6	57.3 ±4.1	40.6 ±3.2	57.9 ±4.9	67.9 ±3.5	64.5 ±2.8	54.4 ±4.8	70.3 ±3.1	53.4 ±5.6
Frond (Leaves)	Length	Med325-425cm	332.5 ±8.1	359.2 ±7.9	---	---	---	---	---	---	---
		Long>425cm	---	---	436.5 ±6.9	475.3 ±7.4	444.8 ±4.9	477.5 ±7.1	470.4 ±7.7	480.6 ±9.1	465.7 ±9.8
	Leave end		Single	Single	Single	Dou-bled	Dou-bled	Dou-bled	Single	Single	Dou-bled
Leaf base	Thickness (cm)		6	6	7	6	9	6	6	7	8
	Breadth (cm)		15.75	18	15	17	24	12	20	21	25
	Color of the dorsal surface		Light brown	Light brown	Very dark	Dark brown	Dark brown	Light brown	Dark brown	Light brown	Light brown
	Length (cm)		45	50	55	80	70	45	70	60	55
Leaflet (Pinna)	Number/Front		109.5 ±9.1	116.3 ±9.3	211.6 ±15.1	254.6 ±15.9	158.6 ±8.9	161.2 ±9.8	201.2 ±12.2	190.1 ±12	200.3 ±21.1
	Area covered on the Midrib (%)		53	56	68	62	61	64	51	60	67
	Length	Short<60cm	38.1 ±3.9	47.8 ±6.4	58.8 ±7.1	50.49 ±9.1	46.2 ±5.4	56.7 ±3.9	53.33 ±1.9	50.7 ±3.7	52.67 ±4.5
	Breadth	Nar-row<38	29.5 ±5.1	23.2 ±2.1	22.5 ±3.6	30.9 ±4.1	27.5 ±3.5	26.2 ±6.1	26.7 ±9.1	---	---
		Med38-44mm	---	---	---	---	---	---	---	39.3 ±4.9	43.52 ±4.4
	B/L %		7.7	4.8	3.8	5.94	5.96	4.6	5.27	7.76	7.6
Spine	Number	Average 20-30	---	---	28.3 ±2.9	28.6 ±4.7	26.3 ±4.5	---	24.6 ±9.9	---	---
		Large>than 30	35.1 ±3.3	40.1 ±6.5	---	---	---	41.4 ±6.9	---	55.3 ±5.1	39.9 ±4.8
	Area cov-ered on the Midrib	Med 15-25%	---	---	21.5 ±6.1	21.9 ±3.9	---	---	18.2 ±2.9	---	21.1 ±3.6
		Long > 25%	36.3 ±2.9	33.9 ±3.4	---	---	36.1 ±3.2	26.3 ±3.9	---	32.6 ±5.1	---
	Thickness		Thick & hard	Thick & hard	Thin & hard	Thin & hard	Thick & hard	Thin & hard	Thick & hard	Thick & hard	Thick & hard
	Length	Short<10cm	---	---	---	9.5 ±3.1	---	---	8.83 ±0.9	---	---
		Med.10-15 cm	11.5 ±1.1	12.5 ±2.6	10.4 ±1.9	---	11.2 ±1.5	---	---	16.7 ±3.1	---
		Long>15cm	---	---	---	---	---	18.4 ±2.6	---	---	19.6 ±4.1
	Arrangement and angle on the midrib		Double	Double	Dou-ble	Double	Dou-ble	Double	Double	Dou-ble	Double

Table (3): Vegetative characters of the six males collected from different regions in Siwa oasis.

Characters			Male trees					
			ZPK	ZTK1	ZTK2	ZUK	ZTM	ZPM
Trunk	Diameter (cm)		38.2	34.8	32.7	30.5	50.2	45.6
Frond (Leaves)	Length	Short <325cm	---	---	220.6 ±2.5	200.1 ±5.7	---	---
		Medium 325-425 cm	---	335.5±3.4	---	---	---	---
		Long >425cm	440.2 ±5.4	---	---	---	480.2 ±2.4	465.4 ±6.8
	Leave end		Single	Single	Single	Single	Single	Single
Leaf base	Thickness (cm)		4.5 ±0.9	3.5 ±0.4	2.1 ±0.1	2.6 ±0.09	6.6 ±10.9	5.3 ±.84
	Breadth (cm)		12.2 ±2.1	9.6 ±1.1	5.6 ±1.7	4.9 ±0.9	21.6 ±2.2	25.9 ±1.9
	Color of dorsal surface		Light brown	Dark brown	Light	Light brown	Dark brown	Light brown
	Length (cm)		47.4 ±1.9	25.3 ±4.1	15.9 ±0.9	20.1 ±2.1	56.7 ±3.5	50.3 ±2.1
Leaflet (Pinna)	Number/Front		186.1 ±2.9	137.4 ±4.5	100.3 ±2.8	100.9 ±1.8	190.4 ±4.1	200.6 ±3.4
	Area covered on the Midrib (%)		76.6	65.7	63.4	72.5	61.6	69.9
	Length	Short < 60 cm	44.6 ±3.1	43.3 ±2.1	35.8 ±4.2	35.6 ±3.6	50.7 ±6.1	52.6 ±4.1
	Breadth	Narrow < 38mm	30.2 ±2.1	30.9 ±1.9	20.2 ±1.5	30.5 ±1.2	---	---
		Med 38-44mm	---	---	---	---	34.3 ±2.6	45.9 ±4.4
	B/L %		6.77	7.13	5.62	8.56	6.76	8.72
Spine	Number	Average 20-30	---	20.3 ±1.1	22.6 ±2.3	28.7 ±1.4	---	---
		Large more than 30	42.4 ±5.1	---	---	---	55.2 ±2.8	39.5 ±3.7
	Area covered on the Midrib	Med 15-25%	20.1	---	---	---	---	23.2
		Long > 25%	---	26.9	29.5	25.3	30.9	---
	Thickness		Thick & hard	Thin & hard	Thin & flexible	Thick & hard	Thick & hard	Thick & hard
	Length	Short < 10 cm	9.8 ±2.7	9.5 ±1.1	8.7 ±0.9	---	---	---
		Med 10-15 cm	---	---	---	14.2 ±1.8	13.8 ±2.9	---
		Long > 15 cm	---	---	---	---	---	18.9 ±2.3
	Arrangement and angle on the midrib		Single	Double	Double	Double	Double	Double

Table (4): Number of amplified fragments (AF), Polymorphic fragments (PF) and Percentages of polymorphism across the nine Date palm cultivars and six male plants based on RAPD analysis.

Cultivars		Primers												Total
		OPA-01	OPA-05	OPA-19	OPB-07	OPC-05	OPC-08	OPC-16	OPD-09	OPH-18	OPR-01	OPR-04	OPR-05	
Siwi	AF	2	6	3	6	2	3	5	3	5	1	7	6	49
	PF (%)	1 (50)	1(17)	3(100)	5(83)	2(100)	3(100)	2(40)	1(33)	5(100)	1(100)	7(100)	4(66)	35 (71)
Fryhee	AF	4	5	4	6	4	6	5	3	2	1	8	5	53
	PF (%)	3 (75)	Zero	4(100)	5(83)	4(100)	6(100)	2 (40)	1(33)	2(100)	1(100)	8(100)	3(60)	39 (74)
Oshengpel	AF	3	6	3	6	3	7	5	3	5	4	6	6	57
	PF (%)	2 (67)	1(17)	3(100)	5(83)	3(100)	7(100)	2 (40)	1(33)	5(100)	4(100)	6(100)	4(66)	43 (75)
Taktakt	AF	4	6	3	3	3	3	5	3	4	1	5	5	45
	PF (%)	3 (75)	1(17)	3(100)	2(66)	3(100)	3(100)	2 (40)	1(33)	4(100)	1(100)	5(100)	3(60)	31 (69)
Quaipe	AF	3	6	2	6	2	7	6	4	6	1	4	5	52
	PF (%)	2 (67)	1(17)	2(100)	5(83)	2(100)	7(100)	3 (50)	2(50)	6(100)	1(100)	4(100)	3(60)	38 (73)
Kara-ma	AF	3	6	3	3	4	4	5	5	3	1	6	6	49
	PF (%)	2 (67)	1(17)	3(100)	2(66)	4(100)	4(100)	2 (40)	3(60)	3(100)	1(100)	6(100)	4(66)	35 (71)
Gorm Agazal	AF	4	6	2	3	3	3	5	3	4	1	6	6	46
	PF (%)	3 (75)	1(17)	2(100)	2(66)	3(100)	3(100)	2 (40)	1(33)	4(100)	1(100)	6(100)	4(66)	32 (70)
Gazaly	AF	2	5	3	6	3	5	3	3	5	1	5	5	46
	PF (%)	1 (50)	Zero	3(100)	5(83)	3(100)	5(100)	Zero	1(33)	5(100)	1(100)	5(100)	3(60)	32 (70)
Halwo Ganm	AF	3	5	2	5	3	4	7	3	3	2	10	5	52
	PF (%)	2 (67)	Zero	2(100)	4(80)	3(100)	4(100)	4 (57)	1(33)	3(100)	2(100)	10(100)	3(60)	38 (73)
ZPK	AF	7	6	7	7	4	7	5	5	3	2	8	5	66
	PF (%)	2 (28)	2(33)	6(86)	4(57)	2(50)	4(57)	3 (60)	3(60)	3(100)	1(50)	6(75)	1(20)	37 (56)
ZTK1	AF	7	7	5	5	4	8	4	5	2	3	4	5	59
	PF (%)	2 (28)	3(43)	4(80)	2(40)	2(50)	5(63)	2 (50)	3(60)	2(100)	2(66)	2(50)	1(20)	30 (51)
ZTK2	AF	7	6	5	5	4	7	4	3	3	3	6	5	58
	PF (%)	2 (28)	2(33)	4(80)	2(40)	2(50)	4(57)	2 (50)	1(33)	3(100)	2(66)	4(67)	1(20)	29 (50)
ZUK	AF	6	6	6	6	3	6	4	4	4	2	8	4	89
	PF (%)	1(17)	2(33)	5(83)	3(50)	1(33)	3(60)	2 (50)	2(50)	4(100)	1(50)	6(75)	Zero	30 (51)
ZTM	AF	6	7	6	6	3	4	4	5	4	3	5	5	58
	PF (%)	1 (17)	3(43)	5(83)	3(50)	1(33)	1(25)	2 (50)	3(60)	4(100)	2(66)	3(60)	1(20)	29 (50)
ZPM	AF	7	6	5	5	2	4	2	5	3	2	6	5	52
	PF (%)	2 (28)	2(33)	4(80)	2(40)	Zero	1(25)	Zero	3(60)	3(100)	1(50)	4(67)	1(20)	23 (44)
Total	AF	68	89	59	78	47	78	69	57	56	28	94	78	801
	PF (%)	29 (43)	20 (22)	53 (90)	51 (65)	35 (74)	60 (77)	30 (43)	27 (47)	56 (100)	22 (79)	82 (87)	36 (46)	501 (63)

ZPK: Male tree that produce plentiful pollen from Kadosa region,

ZTK1 and ZTK2: two Males needs to be test from Kadosa region,

ZUK: unknown Male from Kadosa region

ZPM: Male tree that produce plentiful pollen from Mishandid region and

ZTM: Male needs to be test from Mishandid region.

Table (5): Number of amplified fragments (AF), Polymorphic fragments (PF) and Percentages of polymorphism across the nine Date palm cultivars and six male plants based on ISSR analysis.

Cultivars		Primers								Total
		Amic-06	Amic-07	Amic-08	Amic-09	Mic-07	Mic-08	A-08	A-10	
Siwi	AF	6	9	2	6	2	6	4	3	38
	PF (%)	6 (100)	9 (100)	2 (100)	6 (100)	2 (100)	5 (83)	2 (50)	2 (66)	34 (89)
Fryhee	AF	7	7	2	4	3	6	3	6	38
	PF (%)	7 (100)	7 (100)	2 (100)	4 (100)	3 (100)	5 (83)	1 (33)	5 (83)	34 (89)
Oshengpel	AF	4	7	3	1	2	6	3	6	32
	PF (%)	4 (100)	7 (100)	3 (100)	1 (100)	2 (100)	5 (83)	1 (33)	5 (83)	28 (88)
Taktakt	AF	4	3	3	4	2	5	4	5	30
	PF (%)	4 (100)	3 (100)	3 (100)	4 (100)	2 (100)	4 (80)	2 (50)	4 (80)	26 (87)
Quaipe	AF	3	5	2	3	2	5	4	3	27
	PF (%)	3 (100)	5 (100)	2 (100)	3 (100)	2 (100)	4 (40)	2 (50)	2 (66)	23 (85)
Karama	AF	3	10	3	6	3	4	3	4	36
	PF (%)	3 (100)	10(100)	3 (100)	6 (100)	3 (100)	3 (75)	1 (33)	3 (75)	32 (89)
Gorm Agazal	AF	5	3	4	4	4	6	4	5	35
	PF (%)	5 (100)	3 (100)	4 (100)	4 (100)	4 (100)	5 (83)	2 (50)	4 (80)	31 (89)
Gazaly	AF	5	10	3	3	2	8	3	5	39
	PF (%)	5 (100)	10(100)	3 (100)	3 (100)	2 (100)	7 (88)	1 (33)	4 (80)	35 (90)
Halwo Ganm	AF	4	10	2	4	2	7	4	5	38
	PF (%)	4 (100)	10(100)	2 (100)	3 (100)	2 (100)	6 (86)	2 (50)	4 (80)	34 (89)
ZPK	AF	2	4	3	4	4	4	6	4	31
	PF (%)	2 (100)	3 (75)	2 (66)	1 (50)	3 (75)	4 (100)	2 (33)	2 (50)	19 (61)
ZTK1	AF	3	4	2	8	3	4	6	3	33
	PF (%)	3 (100)	3 (75)	1 (50)	2 (66)	2 (66)	4 (100)	2 (33)	1 (33)	18 (55)
ZTK2	AF	4	2	2	3	3	3	6	3	26
	PF (%)	4 (100)	1 (50)	1 (50)	2 (66)	2 (66)	3 (100)	2 (33)	1 (33)	16 (62)
ZUK	AF	2	2	3	6	5	5	4	4	31
	PF (%)	2 (100)	1 (50)	2 (66)	1 (50)	4 (80)	5 (100)	Zero	2 (50)	17 (55)
ZTM	AF	4	3	5	3	3	4	4	3	29
	PF (%)	4 (100)	2 (66)	4 (80)	2 (66)	2 (66)	4 (100)	Zero	1 (33)	19 (66)
ZPM	AF	2	3	4	4	3	5	4	4	29
	PF (%)	2 (100)	2 (66)	3 (75)	3 (75)	2 (66)	5 (100)	Zero	2 (50)	19 (66)
Total	AF	58	82	43	63	43	78	62	63	492
	PF (%)	58 (100)	76 (93)	37 (86)	46 (73)	37 (86)	69 (88)	20 (32)	42 (67)	385 (78)

ZPK: Male tree that produce plentiful pollen from Kadosa region,
ZTK1 and ZTK2: two Males needs to be test from Kadosa region,
ZUK: unknown Male from Kadosa region,
ZPM: Male tree that produce plentiful pollen from Mishandid region and
ZTM: Male needs to be test from Mishandid region.

Table (6): Similarity indices (%) calculated by NTSYS program among the nine Date palm cultivars and six males based on morphological characters and Molecular analysis.

	Date palm cultivars													
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
ZTK1 (1)	0.81													
ZTK2 (2)	0.52	0.50												
ZUK (3)	0.48	0.51	0.65											
ZTM (4)	0.72	0.73	0.53	0.50										
ZPM (5)	0.70	0.67	0.55	0.48	0.72									
Siwi (6)	0.49	0.53	0.68	0.64	0.52	0.46								
Fryhee (7)	0.52	0.53	0.67	0.62	0.53	0.47	0.72							
Oshengpel (8)	0.71	0.75	0.54	0.53	0.71	0.72	0.49	0.53						
Taktakt (9)	0.51	0.53	0.70	0.68	0.52	0.51	0.66	0.66	0.50					
Quaipe (10)	0.57	0.57	0.70	0.64	0.59	0.53	0.64	0.59	0.57	0.73				
Karama (11)	0.78	0.82	0.52	0.48	0.70	0.70	0.52	0.52	0.75	0.50	0.59			
Gorm Agazal (12)	0.52	0.53	0.66	0.70	0.55	0.50	0.62	0.62	0.51	0.70	0.64	0.52		
Gazaly (13)	0.55	0.52	0.66	0.61	0.58	0.52	0.67	0.66	0.50	0.62	0.65	0.50	0.69	
Halwo Ganm (14)	0.52	0.49	0.64	0.60	0.50	0.54	0.57	0.59	0.48	0.65	0.69	0.49	0.64	0.70

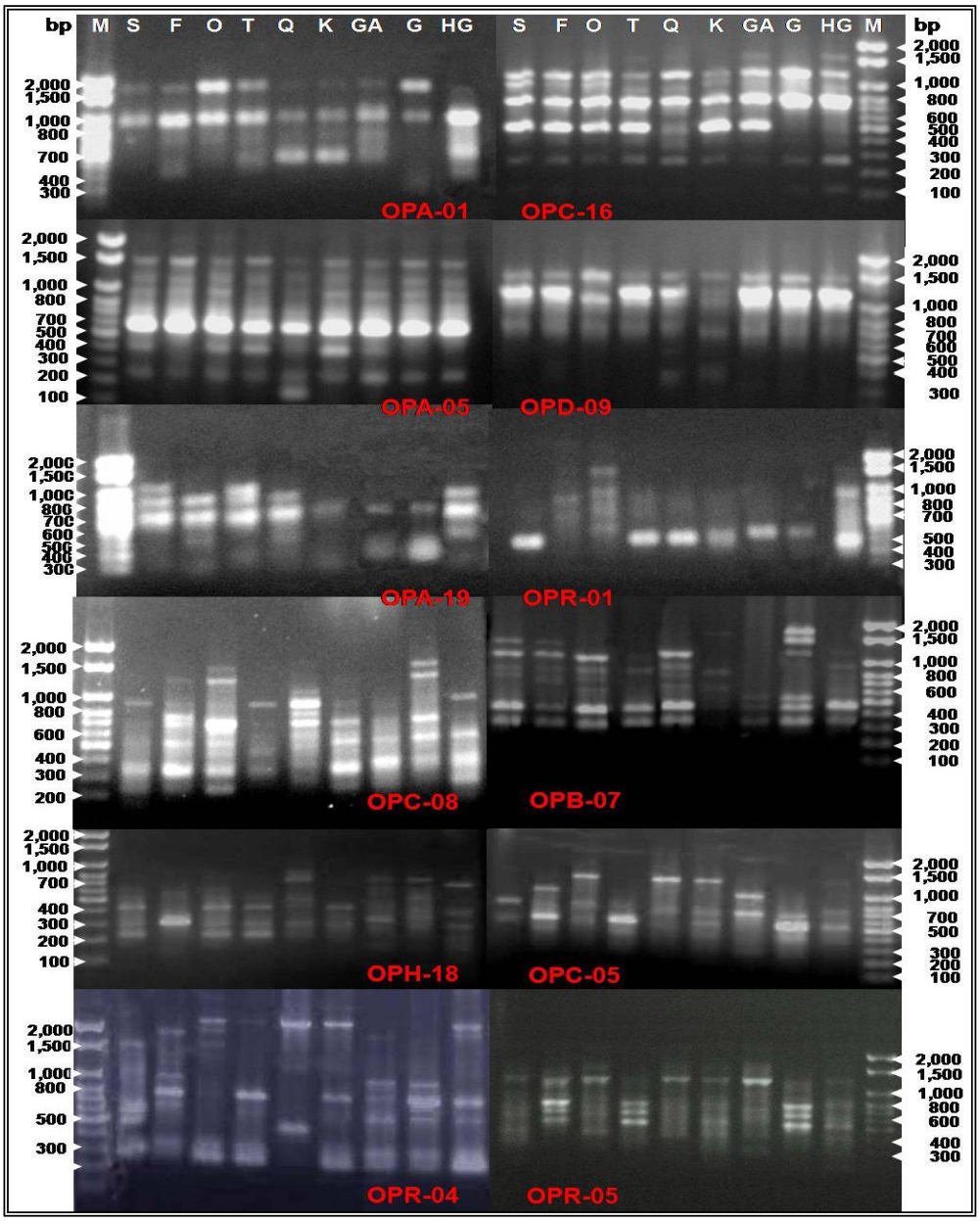

Fig. (1): Photographs showing RAPD products of the nine different cultivars of Date palm using twelve random primers. Siwi (S), Fryhee (F), Oshengpel (O), Taktakt (T), Quaipe (Q), Karama (K), Gorm Agazal (GA), Gazaly (G), Halwo Ganm (HG) and M: DNA marker.

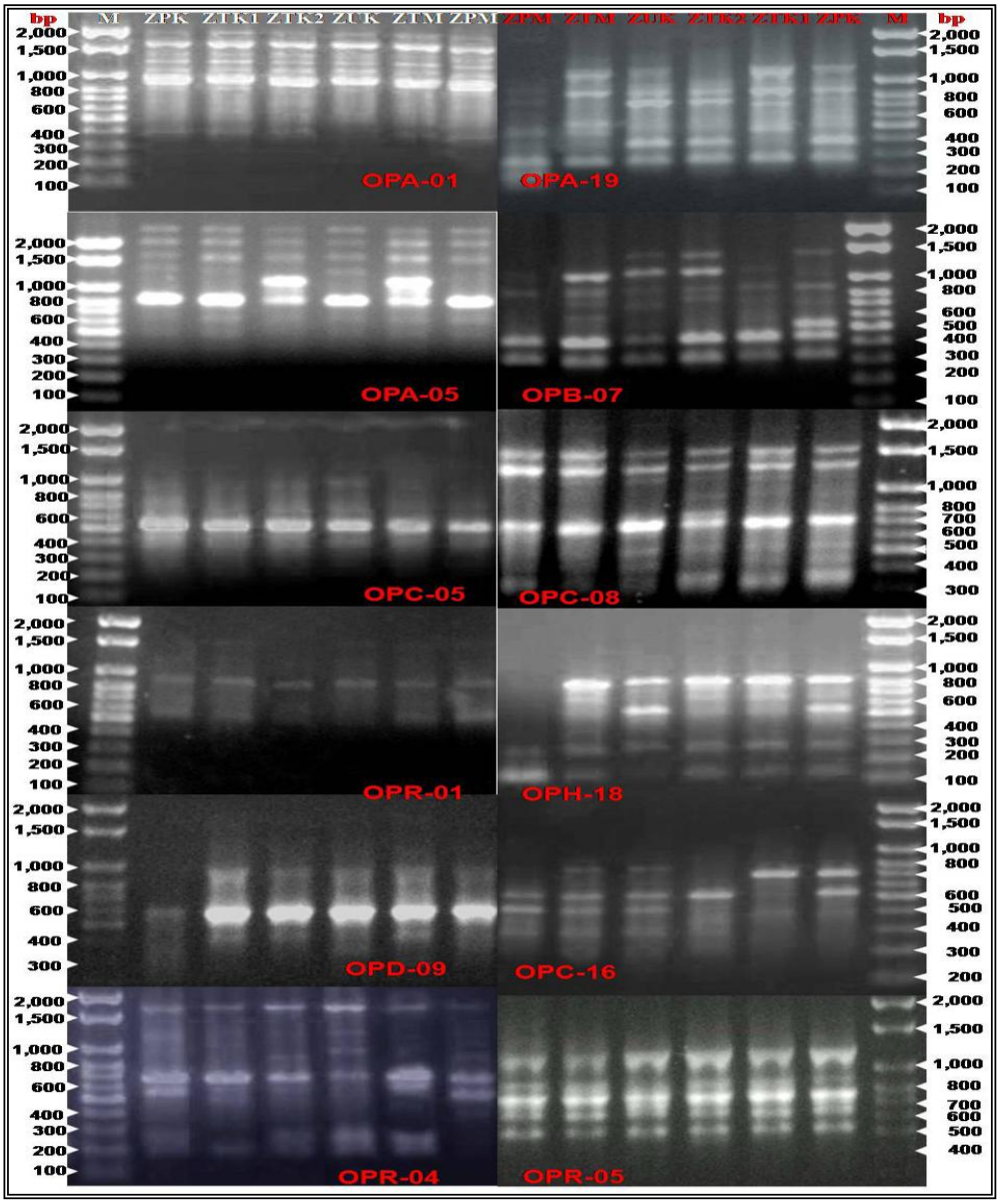

Fig. (2): Photographs illustrating DNA fingerprinting of different male plants using twelve random primers. ZPK: Male tree that produce plentiful pollen from Kadosa region, ZTK1 and ZTK2: two Males needs to be test from Kadosa region, ZUK: unknown Male from Kadosa region. ZPM: Male tree that produce plentiful pollen from Mishandid region and ZTM: Male needs to be test from Mishandid region.

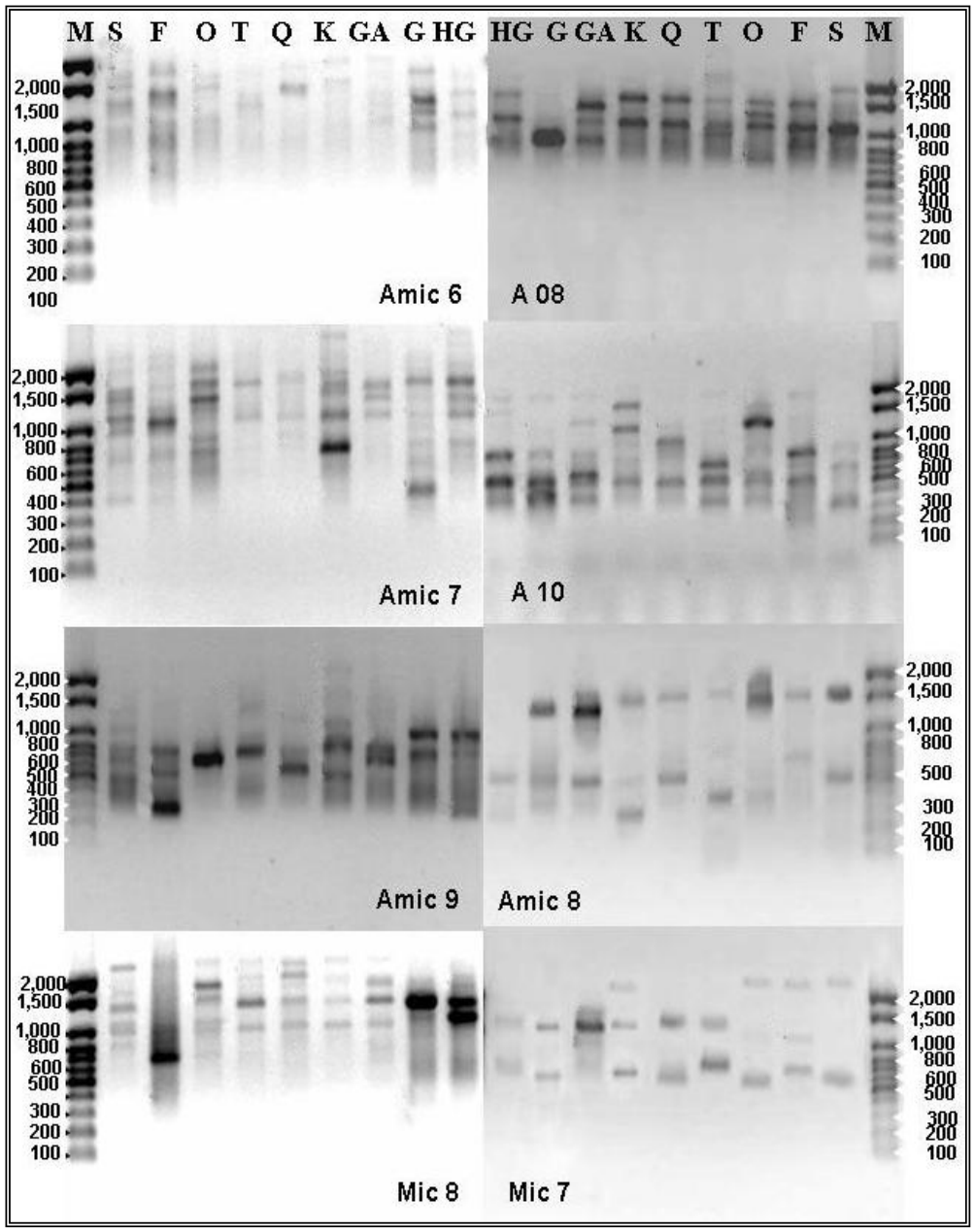

Fig. (3): Photographs showing ISSR products of the nine different cultivars of Date palm using eight ISSR primers. Siwi (S), Fryhee (F), Oshengpel (O), Taktakt (T), Quaipe (Q), Karama (K), Gorm Agazal (GA), Gazaly (G), Halwo Ganm (HG) and M: DNA marker.

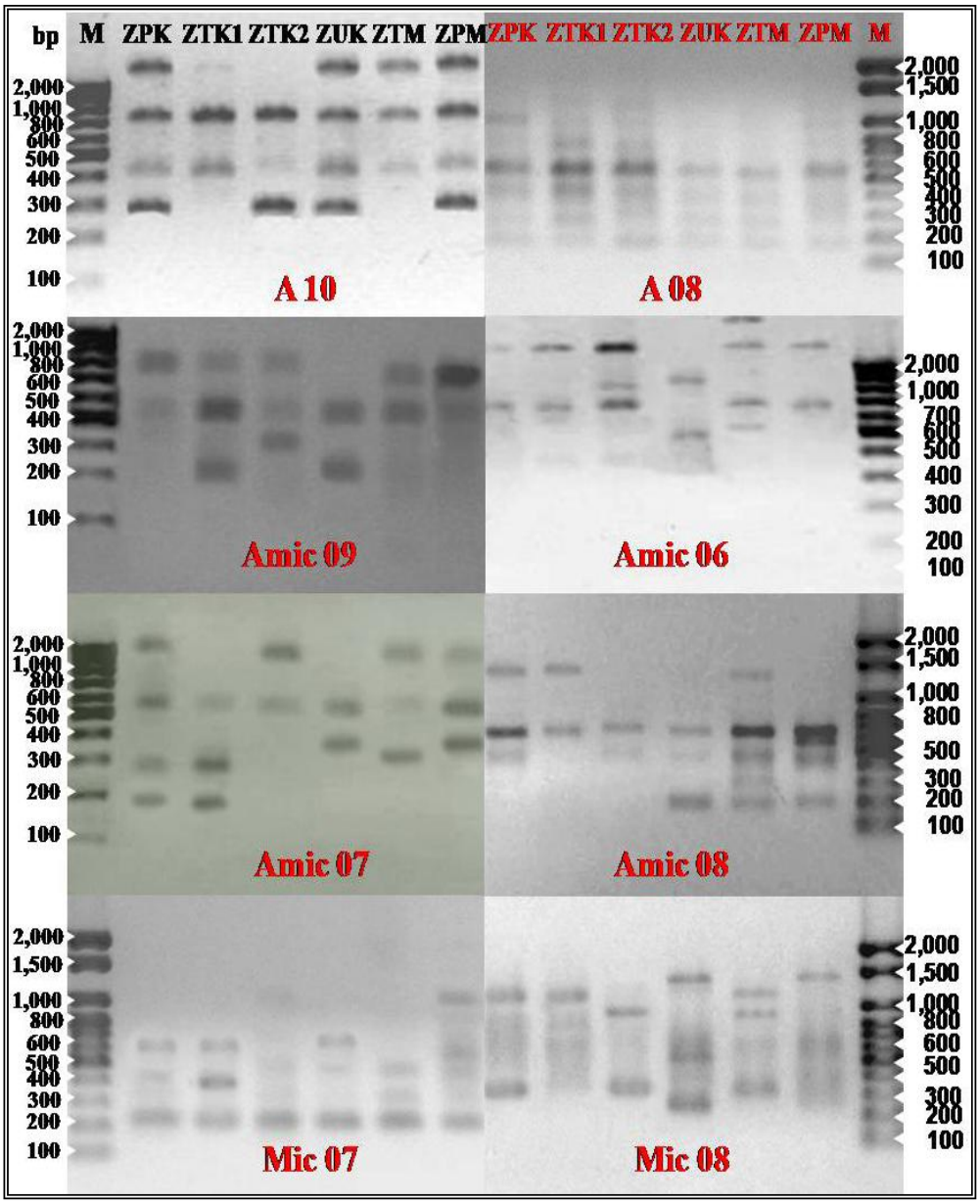

Fig. (4): Photographs illustrating DNA fingerprinting of different male plants using eight ISSR primers. ZPK: Male tree that produce plentiful pollen from Kadosa region, ZTK1 and ZTK2: two Males needs to be test from Kadosa region, ZUK: unknown Male from Kadosa region. ZPM: Male tree that produce plentiful pollen from Mishandid region and ZTM: Male needs to be test from Mishandid region.

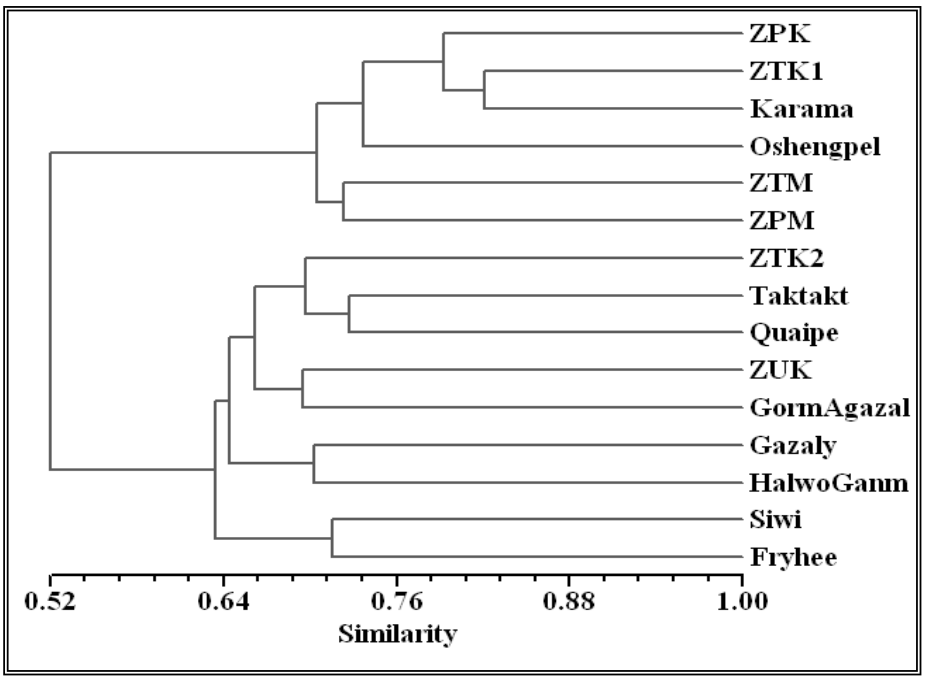

Fig. (5): Dendrogram obtained from UPGMA cluster based on morphological and combined RAPD-ISSR data from the Date palm germplasm (nine cultivars and six males).