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

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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 determine morphological traits among nine date palm 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).

**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 94°C.
Each cycle consisted of denaturation at 94°C for 1 min; annealing at 36°C for 1 min; extension at 72°C for 2 min and final extension at 72°C 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 94°C; 30 s at 94°C and 90 s at 72°C. A final extension was performed at 72°C 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 (Un-weighted Pair Group Method with Arithmetical 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 Gamm, 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 Gamm 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 ZTK1 male, 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 Gamm 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 Gamm cultivars, ZPK, ZTM and ZPM males. The area covered on the Midrib was medium. 15-25% for Oshengpel, Taktakt, Gorm Agazal, Halwo Gamm 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 Gamm 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 Gamm 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 61.5 amplicons/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 phylogenetic 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 Gannm 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 agro-

nomic 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.

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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)$_3$ GC      |
|               | A-10        | (GCT)$_4$ C       |
Table (2): Vegetative characters for the Siwa Date palm cultivars.

| Characters                        | Date palm cultivars                              |
|-----------------------------------|--------------------------------------------------|
|                                  | Siwi    | Fryhee | Oshene  | Taktak  | Quaip   | Karama  | Gorm    | Gazal   | Halwo   |
| Trunk Diameter (cm)               | 60.6    | 57.3   | 40.6    | 57.9    | 67.9    | 64.5    | 54.4    | 70.3    | 53.4    |
|                                   | ±5.6    | ±4.1   | ±3.2    | ±4.9    | ±3.5    | ±2.8    | ±4.8    | ±3.1    | ±5.6    |
| Frond (Leaves)                    |         |        |         |         |         |         |         |         |         |
| Length Med35-425cm                | 332.5   | 359.2  | ---     | ---     | ---     | ---     | ---     | ---     | ---     |
|                                   | ±8.1    | ±7.9   | ±6.9    | ±7.4    | ±4.9    | ±7.1    | ±7.7    | ±9.1    | ±9.8    |
| Length Long>425cm, 5cm            | ---     | ---    | 436.5   | 475.3   | 444.8   | 477.5   | 470.4   | 480.6   | 465.7   |
| Leaf end                          | Single  | Single | Single  | Double  | Double  | Double  | Single  | Single  | Double  |
| 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      |
| Number/Front                      | 109.5   | 116.3  | 211.6   | 254.6   | 158.6   | 161.2   | 201.2   | 190.1   | 200.3   |
|                                   | ±9.1    | ±9.3   | ±15.1   | ±15.9   | ±8.9    | ±9.8    | ±12.2   | ±12     | ±21.1   |
| Area covered on the Midrib (%)    | 53      | 56     | 68      | 62      | 61      | 64      | 51      | 60      | 67      |
| Length Short<60cm                 | 38.1    | 47.8   | 58.8    | 50.49   | 46.2    | 56.7    | 53.33   | 50.7    | 52.67   |
|                                   | ±3.9    | ±6.4   | ±7.1    | ±9.1    | ±5.4    | ±3.9    | ±1.9    | ±3.7    | ±4.5    |
| Length Narrow<38mm                | 29.5    | 23.2   | 22.5    | 30.9    | 27.5    | 26.2    | 26.7    | ---     | ---     |
|                                   | ±5.1    | ±2.1   | ±3.6    | ±4.1    | ±3.5    | ±6.1    | ±9.1    | ---     | ---     |
| Length Med38-44mm                 | ---     | ---    | ---     | ---     | ---     | ---     | 39.3    | 43.52   | ---     |
|                                   |         |        |         |         |         |         | ±4.9    | ±4.4    |         |
| B/L %                            | 7.7     | 4.8    | 3.8     | 5.94    | 5.96    | 4.6     | 5.27    | 7.76    | 7.6     |
| Average Number 20-30              | ---     | ---    | 28.3    | 28.6    | 26.3    | 24.6    | ---     | ---     | ---     |
| Large>than 30                     | 35.1    | 40.1   | 41.4    | ±6.9    | ---     | 55.3    | 39.9    | 55.1    | ±4.8    |
| Area covered on the Midrib (%)    |         |        |         |         |         |         |         |         |         |
| Med 15-25%                        | ---     | ---    | 21.5    | 21.9    | ±6.1    | 18.2    | 21.1    | ---     | ---     |
|                                   |         |        | ±3.9    | ---     | ---     | ±2.9    | ---     | ---     | ---     |
| Long >25%                         | 36.3    | 33.9   | 36.1    | 26.3    | ±3.9    | 32.6    | ---     | ---     | ---     |
|                                   | ±2.9    | ±3.4   | ±3.2    | ±3.9    | ±5.1    | ---     | ---     | ---     | ---     |
| Spine Thickness                    | Thick & hard | Thick & hard | Thin & hard | Thick & hard | Thin & hard | Thick & hard | Thick & hard | Thick & hard | Thick & hard |
| Short<10cm                        | 9.5     | ±3.1   | 11.2    | ±1.5    | 11.2    | ---     | 16.7    | ---     | ---     |
|                                   | ±8.83   | ±0.9   | ---     | ---     | ---     | ---     | ---     | ---     | ---     |
| Med.10-15 cm                      | 11.5    | 12.5   | 10.4    | 12.5    | 10.4    | 16.7    | ---     | ---     | ---     |
|                                   | ±1.1    | ±2.6   | ±1.9    | ±1.5    | ±1.9    | ---     | ±3.1    | ---     | ---     |
| Long>15 cm                        | ---     | ---    | 18.4    | 18.4    | 18.4    | ---     | 19.6    | ---     | ---     |
|                                   | ±2.6    | ±2.6   | ±2.6    | ±2.6    | ±2.6    | ---     | ±4.1    | ---     | ---     |
| Arrangement and angle on the midrib | Double | Double | Double | Double | Double | Double | Double | Double | Double |
Table (3): Vegetative characters of the six males collected from different regions in Siwa oasis.

| Characters                              | ZPK   | ZTK1  | ZTK2  | ZUK   | ZTM   | ZPM   |
|-----------------------------------------|-------|-------|-------|-------|-------|-------|
| Trunk Diameter (cm)                     | 38.2  | 34.8  | 32.7  | 30.5  | 50.2  | 45.6  |
| Fron (Leaves)                           |       |       |       |       |       |       |
| Short <325cm                            | ---   | ---   | 220.6 | 200.1 | ---   | ---   |
| Length                                  |       |       |       |       |       |       |
| Medium 325-425 cm                       | ---   | 335.5±3.4 | ---   | ---   | ---   | ---   |
| Long >425cm                             | 440.2 | ---   | ---   | ---   | 480.2 | 465.4 |
| Leave end                               |       |       |       |       |       |       |
| Thickness (cm)                          | 4.5±0.9 | 3.5±0.4 | 2.1±0.1 | 2.6±0.09 | 6.6±10.9 | 5.3±8.4 |
| 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 brown | 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 |
| 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±4.5 | 65.7±2.8 | 63.4±4.5 | 72.5±1.8 | 61.6±3.5 | 69.9±4.5 |
| Leaflet (Pinna)                         |       |       |       |       |       |       |
| 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±1.9 | 7.13±2.9 | 5.62±1.5 | 8.56±1.8 | 6.76±2.6 | 8.72±2.8 |
| 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 |
| Med 15-25%                              | 20.1±1.1 | ---   | ---   | ---   | ---   | 23.2±1.1 |
| Long > 25%                              | ---   | 26.9±1.1 | 29.5±1.5 | 25.3±1.8 | 30.9±2.6 | ---   |
| Thickness                               | Thick hard | Thin hard | Thin & flexible | Thick hard | Thin & hard | Thick & hard |
| 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 | OPA-01 | OPA-05 | OPB-07 | OPC-08 | OPC-16 | OPC-18 | OPD-09 | OPH-10 | OPR-11 | OPR-18 | OPR-05 | Total |
|-------------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
| Siwi        | AF      | 2      | 6      | 3      | 6      | 2      | 3      | 5      | 3      | 5      | 1      | 7      | 6     | 49    |
|             | PF (%)  | 1      | 50     | 1      | 17     | 1      | 50     | 1      | 35     | 1      | 70     | 1     | 466   | 35(71)|
| Fryheed     | AF      | 4      | 5      | 4      | 4      | 5      | 4      | 3      | 2      | 2      | 1      | 8      | 5     | 53    |
|             | PF (%)  | 3      | 75     | 0      | 4(100) | 5      | 83     | 4      | 100    | 6      | 100    | 2      | 4(100)| 39(74)|
| Oshapel     | AF      | 3      | 6      | 3      | 6      | 3      | 7      | 5      | 3      | 5      | 4      | 6      | 6     | 57    |
|             | PF (%)  | 2      | 67     | 1      | 17     | 1      | 50     | 1      | 70     | 1      | 100    | 6      | 4(100)| 1     | 466   |
| Taktat      | AF      | 4      | 6      | 3      | 3      | 3      | 3      | 5      | 3      | 4      | 1      | 5      | 5     | 45    |
|             | PF (%)  | 3      | 75     | 1      | 17     | 1      | 50     | 1      | 70     | 1      | 100    | 6      | 5(100)| 31(69)|
| Quaip       | AF      | 3      | 6      | 2      | 6      | 2      | 7      | 6      | 4      | 6      | 1      | 4      | 5     | 52    |
|             | PF (%)  | 2      | 67     | 1      | 17     | 1      | 50     | 1      | 70     | 1      | 100    | 6      | 4(100)| 3     | 66    |
| Karanma     | AF      | 3      | 6      | 3      | 3      | 4      | 4      | 5      | 3      | 1      | 6      | 6     | 6     | 49    |
|             | PF (%)  | 2      | 67     | 1      | 17     | 1      | 50     | 1      | 70     | 1      | 100    | 6      | 6(100)| 35(71)|
| Gorm Agaza  | AF      | 4      | 6      | 2      | 3      | 3      | 5      | 3      | 4      | 1      | 6      | 6     | 6     | 46    |
|             | PF (%)  | 3      | 75     | 1      | 17     | 1      | 50     | 1      | 70     | 1      | 100    | 6      | 5(100)| 32(70)|
| Gazaly      | AF      | 2      | 5      | 3      | 6      | 3      | 5      | 3      | 5      | 1      | 5      | 5     | 5     | 46    |
|             | PF (%)  | 1      | 50     | 0      | 4(100) | 5      | 83     | 3      | 100    | 5      | 100    | 5      | 5(100)| 32(70)|
| Halw Ganm  | AF      | 3      | 5      | 2      | 5      | 3      | 4      | 7      | 3      | 2      | 10     | 5     | 5     | 52    |
|             | PF (%)  | 2      | 67     | 0      | 1      | 50     | 4      | 80     | 3      | 100    | 4      | 100    | 4     | 5(100)| 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      | 5(100)| 2      | 50     | 4      | 5(100)| 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      | 6(100)| 30(51)|
| ZTK2        | AF      | 7      | 6      | 5      | 5      | 4      | 7      | 4      | 3      | 3      | 6      | 5     | 5     | 58    |
|             | PF (%)  | 2      | 28     | 2      | 33     | 4      | 80     | 2      | 40     | 2      | 50     | 2      | 5(100)| 29(50)|
| ZUK         | AF      | 6      | 6      | 6      | 3      | 6      | 3      | 6      | 4      | 4      | 2      | 8      | 4     | 89    |
|             | PF (%)  | 1      | 17     | 2      | 33     | 5      | 83     | 3      | 100    | 1      | 33     | 2      | 5(100)| 30(51)|
| ZTM         | AF      | 6      | 7      | 6      | 3      | 4      | 4      | 5      | 4      | 3      | 5      | 5     | 5     | 58    |
|             | PF (%)  | 1      | 17     | 3      | 45     | 5      | 83     | 3      | 100    | 1      | 25     | 2      | 5(100)| 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     | 2      | 50     | 4      | 6(100)| 23(44)|
| Total       | AF      | 68     | 89     | 59     | 78     | 47     | 78     | 69     | 57     | 56     | 28     | 94     | 78    | 801   |
|             | PF (%)  | 29     | 20     | 53     | 51     | 35     | 60     | 30     | 27     | 56     | 22     | 82     | 36    | 501   |

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 | Amic-06 | Amic-07 | Amic-08 | Amic-09 | Mic-07 | Mic-08 | A-08 | A-10 | Total |
|-----------|---------|---------|---------|---------|---------|--------|--------|------|------|-------|
| Siwi      | AF      | 6       | 9       | 2       | 6       | 2      | 6      | 4    | 3    | 34.89 |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 38    |
| Fryhee    | AF      | 7       | 7       | 7       | 2       | 4      | 3      | 6    | 6    | 38    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 38    |
| Oshengpel | AF      | 4       | 7       | 3       | 1       | 2      | 6      | 3    | 6    | 32    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 32    |
| Taktakt   | AF      | 4       | 3       | 3       | 4       | 2      | 5      | 4    | 5    | 30    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 30    |
| Quaipe    | AF      | 3       | 5       | 2       | 3       | 2      | 5      | 4    | 3    | 27    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 27    |
| Karama    | AF      | 3       | 10      | 3       | 6       | 3      | 4      | 3    | 4    | 36    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 36    |
| Gorm Agazal | AF    | 5       | 3       | 4       | 4       | 4      | 4      | 5    | 6    | 35    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 35    |
| Gazaly    | AF      | 5       | 10      | 3       | 3       | 2      | 8      | 3    | 5    | 39    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 39    |
| Halwo Gamm | AF    | 4       | 10      | 2       | 4       | 2      | 7      | 4    | 5    | 38    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 38    |
| ZPK       | AF      | 2       | 4       | 3       | 4       | 4      | 4      | 6    | 4    | 31    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 31    |
| ZTK1      | AF      | 3       | 4       | 2       | 8       | 3      | 4      | 6    | 3    | 33    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 33    |
| ZTK2      | AF      | 4       | 2       | 2       | 3       | 3      | 3      | 6    | 3    | 26    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 26    |
| ZUK       | AF      | 2       | 2       | 3       | 6       | 5      | 5      | 4    | 4    | 17    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 17    |
| ZTM       | AF      | 4       | 3       | 5       | 3       | 3      | 4      | 4    | 3    | 19    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 19    |
| ZPM       | AF      | 2       | 3       | 4       | 3       | 3      | 3      | 4    | 3    | 19    |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 19    |
| Total     | AF      | 58      | 82      | 43      | 63      | 43     | 78     | 62   | 63   | 492   |
|           | PF (%)  | 100     | 100     | 100     | 100     | 83     | 83     | 50   | 66   | 385   |

**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 need to be tested 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 tested 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).