

The phylogenetic map between three pollinizers and their impact on fruit set, yield and fruit quality of Zaghloul and Samani date palms

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ABSTRACT

Fingerprinting analysis using RAPD has been performed to investigate the phylogenetic relationships among three males and two females of the Egyptian date-palms (*Phoenix dactylifera* L.) during the seasons of 2012 and 2013. The RAPD-PCR with nine primers detected a total number of fifty six fragments, thirty nine bands of them were polymorphic with a ratio of 69.64% (out of them seven unique bands with a ratio of 12.5%), and seventeen bands were monomorphic with a ratio of 30.36%. The genetic variability and relationships among the five accessions of the Egyptian date palms were estimated in the term of similarity using Dice coefficient. The phylogenetic tree revealed high similarity value between New Valley and both of Samani and Zaghloul (1.000 and 0.731, respectively), whereas it revealed a middle value between Rashied pollinizer and both of Samani and Zaghloul (0.667 and 0.402, respectively). The similarity value was middle between the pollinizer from El Nobararia and Zaghloul (0.447) from one site and low similarity value between El Nobararia and Samani (0.282). Depending on these results, it could be concluded that Rashied pollinizer seems to be the most suitable for Zaghloul female date palm, while El Nobararia pollinizer is the best for Samani female date palm. From another side, the both pollinizers recorded the best results concerning fruit set, bunch weight, palm yield and fruit physical properties (weight, length, diameter, and size). In addition, both pollinizers enhanced the chemical properties (TSS, and both of total reducing and non-reducing sugars). The above results were observed in both studied seasons.

Key words: Bunch weight, Egyptian Date palms, Fruit quality, Fruit set, Phylogenetic tree and RAPD – PCR markers, Pollinizers, Yield, Fruit quality.



INTRODUCTION

Date palm *Phoenix dactylifera* L. is a dioecious species belonging to Palmaceae family. It is largely grown in the oasis areas in West Asia and North Africa where it constitutes the basis for the livelihood of the local communities (Al Ugaydy, 2000). In Egypt, date palm ranked the third crop after orange and grape. Artificial and hand pollination is considered the only way for successful fruit set and commercial date production. There is common agreement among date growers that the success of fruit production depends mainly on the success of pollination. The pollen grain source is considering a critical and limited factor in palm production and fruit quality characteristics. It is generally known that pollen grains from different male palms affect the yield and fruit quality, it is known as phenomenon “Metaxenia” (Swingle, 1928). In some date palm cultivars, better fruit set resulted from pollen of some males than others due to compatibility of male and female varieties. Many investigators proved that pollen grains from different male date palms that not only influence the size and shape of seed “xenia” but also has a direct effect on fruit set, yield and fruit physical and chemical characteristics of “metaxenia”. Mothew *et al.* (1975), El-Hammady *et al.* (1977), El-Salhy *et al.* (1997) and Mustafa *et al.* (2014) reported that pollen sources affected fruit and seed characteristics and exhibited metaxenic effect depending on female cultivar used.

The use of DNA markers provides a powerful tool to certify the identity of the cultivars at the seedling stage through the variety of fingerprinting. Random

Amplified Polymorphic DNA (RAPD) markers can be used for fast screening of nuclear genome variations (Williams *et al.*, 1990). RAPD markers have been used for germplasm characterization in date palm (Aitchitt *et al.*, 1995; Askari *et al.*, 2003; Trifi *et al.*, 2000; Soliman *et al.*, 2003).

The aim of this investigation was to maximize the fruit retained and yield as well as to improve yield and fruit quality of both Zaghloul and Samani date palms by selecting the most suitable pollinizer source (New Valley, Rashied and El Nobararia) for each cultivar under Giza governorate conditions using the molecular studies as indication to detect phylogenetic relationships between different rarities of date palms.

MATERIALS AND METHODS

Plant Material

This study was carried out during 2012 and 2013 seasons on 20 years old Zaghloul and Samani female date palms grown on sandy soil with 8x8 meters apart under drip irrigation system at a private orchard located at Abou Rawash region, Giza Governorate, Egypt. The selected palms were healthy, nearly uniform in growth vigor and fruiting and received regular horticultural practices. Moreover, date palm thinned at one bunch per eight leaves rate (8-1 leaf/bunch ratio). In addition, male palms as a pollen source were selected from three locations namely New Valley, Rashied and El Nobararia. So, the accessions represent two female date-palm cultivars (Zaghloul, Samani) and three males as pollen grains source selected from Rashied, El Nobararia and

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New Valley districts. Samples were collected from leaves surrounding the palm meristem of 3 to 5 palms from each location represented in Table (1).

Table (1): The investigated date palms used under the experiment conditions.

| Investigated palms | Gender |
|--------------------|--------|
| Zaghloul | Female |
| Samani | Female |
| Rashied | Male |
| El Nobarria | Male |
| New Valley | Male |

Pollination was achieved by inserting male strands into each of the female bunch. Five bunches on each female palm were received pollens from the three different males (one bunch for each male on the same female palm). To prevent contamination of pollens, every bunch was bagged after inserting the male strands with newspaper bags which were removed after 3 weeks. The experiment was arranged in a complete randomized design including three replications for each treatment. The investigation included two main studies.

Molecular Studies

Molecular studies aimed to determine the phylogenetic tree and relationships between different varieties of date palm in steps:

a) DNA extraction:

DNA was extracted from five Date palm varieties by Bio basic kits protocol.

b) PCR- Amplification of RAPD:

Amplification reaction was carried out in 25µl reaction mixture contained 2µl of genomic DNA, 3µl of the primer, 2.5µl of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase and 200 mm of each dNTPs. The following PCR program was used in a DNA Thermocycler (PTC-100 PCR version 9.0-USA). Initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 42°C for 90 sec. for annealing temperature, 72°C for 90 Sec. and final extension at 72°C for 2 min. Products by RAPD- PCR were separated on 1.5% agarose gels in 1X TAE buffer and detected by staining with ethidium bromide according to Sambrook *et al.*, (1989). DNA ladder 100bp was used and PCR products were visualized by UV-transilluminator and photographed by gel documentation system, Biometra - Bio Documentations, the amplified bands were scored as (1) for presence and (0) for the absence of all studied date palm varieties according to gel analyzer protocol.

c) RAPD analysis

A set of nine random 10-mer primers (Table 2) was used in the detection of polymorphism among the five date palm accessions. These primers were synthesized at RAPD-PCR was carried out according to the procedure given by Williams *et al.*, (1990) with minor modifications. The amplification reaction was carried

out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgCl₂, 2 mM dNTPs, 1 µM primer, 1 µl Taq DNA polymerase and 25ng templates DNA. PCR amplification was performed in a Perkin-Elmer/Gene Amp® PCR System 9700 (PE Applied Bio systems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 40°C for 1 min, and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 5 min at 72°C in the final cycle.

Table (2): Code and sequences of nine RAPD primers.

| Sequence (5'→3') | Primer code |
|------------------|-------------|
| CAGGCCCTTC | OPA-02 |
| AATCGGGCTG | OPA-04 |
| GAAACGGGTG | OPA-07 |
| GGTGACGCAG | OPB-07 |
| CTGCTGGGAC | OPB-10 |
| TCAGAGCGCC | OPO-10 |
| GTCAGAGTCC | OPO-13 |
| AGCATGGCTC | OPO-14 |
| CAATCGCCGT | OPO-19 |

Horticultural study

Initial fruit set and fruit retention percentages were evaluated one month after pollination and at harvest time. Five female strands per each bunch were randomly selected from each replicate. Final fruit set and fruit retention percentages were calculated using the following equations:

$$\text{Fruit set \%} = \frac{Ns}{Nt} \times 100$$

$$\text{Fruit retention \%} = \frac{Nr}{Nr - Nf} \times 100$$

Where; Ns=Number of setting fruits/strand, Nt=Total number of flowers/strand, Nr=Number of retained fruits/strand, Nf=Number of flower scars/strand.

Yield was estimated as average bunch weight (kg) for each treatment crossing the number of bunches/palm. All bunches were harvested at the first week of September at the peak of color development. Bunch weight was recorded and 30 fruits were picked at random from each bunch for determination of physical properties such as fruit weight (g), fruit volume (cm³), fruit dimensions (cm), fruit pulp and seed (%). Also chemical properties such as total soluble solids %, acidity %, reducing sugars %, non-reducing sugars % and total sugars were measured as outlined in A.O.A.C. methods (A.O.A.C., 1995).

Statistical analysis

The experiment was designed as Completely Randomized Design (CRD) and the results were analyzed as one way analysis of variance (ANOVA). In case where the differences were significant, the means were further assessed for differences through Least Significant Difference (LSD) test according to Snedecor

and Cochran (1980). The value $P < 0.05$ is considered as significant level. Statistical computer software,

MSTATC (Michigan State University, USA), was applied for computing both the ANOVA and LSD.

RESULTS

Molecular Studies

Using RAPD-PCR technique of nine primers revealed 56 fragments where 39 of them were polymorphic bands 69.64% polymorphism and 17 were monomorphic bands 31.36% as show in Fig. 1 and Table (3). The relationships revealed high value between Zaghoul and Samani with Rashied, the medium value was between El Nobarria pollinizer with two females. On the other hand, the phylogenetic tree revealed the lowest values between New Valley with Zaghoul and Samani Cv. these results showed in Figure (2) and showed that, in Proximity Matrix Table (4).

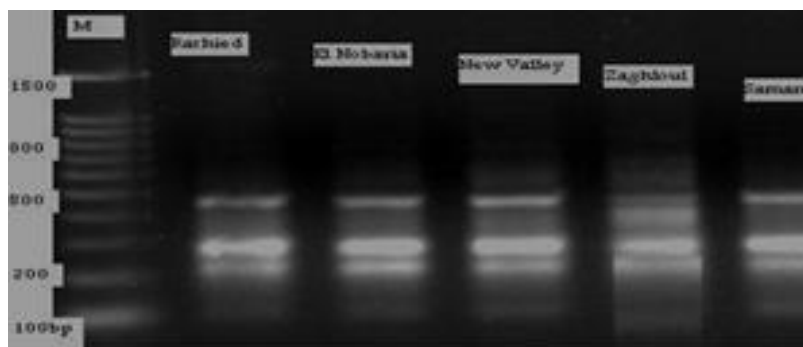


Figure (1): Example of RAPD- PCR banding patterns of the five date palm cultivars amplified with the nine 10-mer random primers; OPO-19; MS = 100-bp ladder of nine primers.

Table (3): Total number, monomorphic, polymorphic of bands and percentage of polymorphism as revealed by nine RAPD primers on five date palm genotypes.

| Primer | Total bands | Monomorphic bands | Polymorphic bands | Unique bands | % polymorphism |
|-------------|-------------|-------------------|-------------------|--------------|----------------|
| OPA-02 | 6 | 2 | 4 | 1 | 66.67 |
| OPA-04 | 5 | 2 | 3 | 1 | 60 |
| OPA-07 | 7 | 1 | 6 | 1 | 85.71 |
| OPB-07 | 6 | 1 | 5 | 1 | 83.33 |
| OPB-10 | 6 | 2 | 4 | 1 | 66.67 |
| OPO-10 | 7 | 2 | 5 | 0 | 71.42 |
| OPO-13 | 7 | 3 | 4 | 1 | 57.14 |
| OPO-14 | 5 | 2 | 3 | 0 | 60 |
| OPO-19 | 7 | 2 | 5 | 1 | 71.42 |
| Total bands | 56(100%) | 17(30.36%) | 39(69.64%) | 7(12.5%) | 69.64% |

Table (4): Proximity matrix between two females (Zaghoul and Samani) with three males from Rashied, ElNobarria and New Valley.

| Case | Matrix File Input | | | | |
|-------------|-------------------|-------------|------------|---------|--------|
| | Rashied | El Nobarria | New Valley | Zaghoul | Samani |
| Rashied | 1.000 | | | | |
| El Nobarria | 0.632 | 1.000 | | | |
| New Valley | 0.526 | 1.000 | 1.000 | | |
| Zaghoul | 0.402 | 0.447 | 0.731 | 1.000 | |
| Samani | 0.667 | 0.282 | 1.000 | 0.211 | 1.000 |

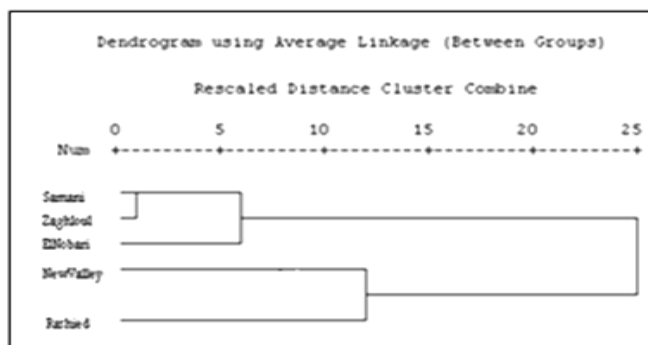


Figure (2): Dendrogram using average linkage (between groups) of date palm cultivars two females Zaghoul and Samani with three males Rashied, El Nobaria and New Valley.

Using RAPD-PCR technique of nine primers revealed 56 different bands, of them seventeen monomorphic bands (30.36%) and showed thirty nine were polymorphic bands (69.64%) polymorphism in Table (3). The genetic relationships among the five accessions were estimated in terms of similarity using Dice coefficient. As show in Table (4) the phylogenetic tree revealed high values between New Valley and both Zaghoul and Samani (0.731 and 1.00, respectively), while it revealed medium relations between Rashied pollinizer and both Zaghoul and Samani (0.402 and 0.667, respectively). Also the pollinizer from El Nobaria revealed medium value for relationships (0.447) with Zaghoul whereas El Nobaria with Samani recorded the lowest value (0.282).

As shown in Figure (1) and Table (3) the products of nine RAPD primers represented that the number of total bands for each primer was varied from 5 fragments of primers (OPA-04) and (OPO-14) to 7 fragments for (OPA-07, OPO-10, OPO-13 and OP O-19), with an average of 6.22 bands/ primer with polymorphism 85.71, 71.42 and 57.14 decreasingly. While, the primers (OPA-02 and OPB-10) gives six bands with percentage 66.67% polymorphism, also primer OPB-07 detected six bands were one of them monomorphic and five were polymorphic bands, with percentage 83.33% polymorphism. RAPD markers were used to describe five date palm genotypes grown in different regions from Egypt and more assays with regard to polymorphic detection analysis by UPGMA of the dendrogram in (Fig. 2). This phylogenetic tree revealed divided these varieties to two sub clusters include the first Rashid, New Valley and Samani while, the other cluster contains El Nobaria and Zaghoul varieties. On the other hand, the phylogenetic tree revealed high values between New Valley and both Zaghoul and Samani (0.731 and 1.00, respectively), the phylogenetic tree revealed medal related between Rashied pollinizer and both Zaghoul and Samani (0.402 and 0.667, respectively), whenever the pollinizer from El Nobaria revealed medal value for relationships (0.447) with Zaghoul. On the other hand, El Nobaria with Samani recorded the lowest value (0.282). The obtained result scored 69.64% polymorphism with SPSS analysis. Also, in the Proximity matrix (Table 4) distinguished the

relationships between five date palms; the variety New Valley was the best male with both Zaghoul and Samani 0.731 and 1.00, respectively, and El Nobaria with Samani recorded the lowest male with (0.282). These relationships were also shown in dendrogram using average linkage (between groups) Figure (2).

The horticultural study

Zaghoul female date palm

Results recorded in Table (5) show that the three pollinizers sources (New Valley, Rashied and El Nobaria) had an effect on fruit set of Zaghoul female date palm. In this respect, Rashied pollinizer recorded the highest fruit set percentage in both studied seasons. The same trend was observed concerning fruit retention percentage, bunch weight and palm yield, since Rashied pollinizer gave highest values in the two seasons. In this concern, bunch weight reached 17.60 and 17.93 kg, while palm yield recorded 140.8 and 143.4 kg in the first and second seasons, respectively. However, El Nobaria pollinizer was the second after Rashied concerning the previous parameters. As for fruit physical properties, results in Table (6) cleared that Rashied pollinizer gave the highest values concerning weight, length, diameter and volume of the fruits compared with the two other pollinizers. This was true in both studied seasons.

On the other side, El Nobaria pollinizer followed Rashied one in improving fruit physical properties. In respect to pulp and seed percentages, it's observed that no significant differences were detected concerning the pulp percentage in the first season, while in the second, El Nobaria pollinizer recorded the highest percentage of the pulp. The same pollinizer gave the lowest seed percentage in the second season followed without significance by Rashied, while there was no significant differences among the pollinizers in the first season. Concerning fruit chemical properties of Zaghoul date palm, it's observed from the results in Table (7) that Rashied pollinizer recorded the highest TSS percentage in the first season, while El Nobaria recorded the highest value in the second one.

On the other hand, acidity percentage was the lowest with New Valley pollinizer in the first season, while in the second one the lowest value was obtained with El

Nobaria pollinizer. Concerning reducing, non-reducing and total sugars content, it's clear that Rashied pollinizer was the most effective one, since it recorded the highest values of the three parameters in the first season, also the same pollinizer gave the highest values concerning reducing and total sugars in the second season, while as for non-reducing sugars this pollinizer came the second without significance after New Valley pollinizer.

Samani female date palm

Table (8) showed that results concerning fruit set and fruit retention were differed in the first season than that in the second one. In this respect, New Valley

pollinizer recorded the highest fruit set followed without significance by El Nobaria pollinizer in the first season, while in the second one Rashied pollinizer gave the highest significant value. On the other hand, Rashied pollinizer recorded the highest value of fruit retention in the first season, while in the second one the highest significant value was recorded by El Nobaria pollinizer.

Regarding bunch weight and palm yield, both parameters took the same trend, since El Nobaria pollinizer was the most effective and recorded the highest bunch weight (18.2 and 15.7 kg) and the highest palm yield (145.6 and 125.6 kg) in the first and second seasons, respectively.

Table (5): Effect of different pollinizer sources on fruit set, fruit retention, bunch weight and yield of Zaghoul date palm during 2012 and 2013 seasons.

| Pollinizer source | Fruit set % | | Fruit retention % | | Bunch weight (kg) | | Palm yield (kg) | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season |
| New Valley | 60.0 | 61.0 | 30.33 | 32.00 | 14.83 | 15.53 | 118.6 | 124.26 |
| Rashied | 63.0 | 62.0 | 34.66 | 36.00 | 17.60 | 17.93 | 140.8 | 143.46 |
| El Nobaria | 60.0 | 61.0 | 32.66 | 33.33 | 17.13 | 16.16 | 137.0 | 129.33 |
| LSD 5% | 2.17 | 2.97 | 1.25 | 2.26 | 0.47 | 0.52 | 8.22 | 4.20 |

Table (6): Effect of different pollinizer sources on physical properties of Zaghoul date palm during 2012 and 2013 seasons.

| Pollinizer source | Fruit weight (gm) | | Fruit length (cm) | | Fruit diameter (cm) | | Fruit volume (cm ³) | | Pulp % | | Seed % | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season |
| | New Valley | 25.23 | 25.90 | 4.76 | 4.90 | 2.40 | 2.33 | 24.73 | 26.00 | 79.0 | 79.33 | 21.0 |
| Rashied | 28.53 | 28.00 | 5.53 | 5.40 | 2.60 | 2.56 | 28.50 | 28.33 | 80.0 | 80.33 | 20.0 | 19.66 |
| El Nobaria | 26.76 | 26.76 | 5.16 | 5.06 | 2.40 | 2.40 | 26.66 | 27.00 | 80.0 | 81.33 | 20.0 | 18.66 |
| LSD 5% | 1.74 | 0.46 | 0.21 | 0.22 | 0.10 | 0.08 | 1.07 | 0.62 | 1.08 | 1.08 | 1.08 | 1.08 |

Table (7): Effect of different pollinizer sources on chemical properties of Zaghoul date palm during 2012 and 2013 seasons.

| Pollinizer source | TSS % | | Acidity % | | Non-reducing sugars% | | Reducing sugars % | | Total sugars % | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season |
| | New Valley | 32.33 | 34.00 | 0.23 | 0.25 | 7.50 | 7.06 | 73.66 | 71.00 | 81.16 |
| Rashied | 38.8 | 32.76 | 0.26 | 0.22 | 7.90 | 6.93 | 77.66 | 73.33 | 85.56 | 80.26 |
| El Nobaria | 35.86 | 35.83 | 0.26 | 0.21 | 6.93 | 6.50 | 73.00 | 67.00 | 79.60 | 73.50 |
| LSD 5% | 0.85 | 1.49 | 0.059 | 0.06 | 0.59 | 0.15 | 0.62 | 1.98 | 1.94 | 1.94 |

As for fruit physical properties, it's clear from the results in Table (9) that El Nobaria pollinizer gave the highest values concerning weight, length, diameter and volume of the fruits. This was true in both studied seasons. Concerning pulp percentage, it's observed that no significant differences were detected among the three pollinizers in the two studied seasons. Regarding seed percentage, El Nobaria pollinizer increased this parameter in the two seasons.

This increment was significant in the second season only. As for chemical fruit properties, results presented in Table (10) showed that TSS percentage was

significantly increased due to El Nobaria pollinizer comparing with the other ones.

This observation was detected in both studied seasons. On the other hand, the same pollinizer (El Nobaria) recorded the lowest acidity percentage in the two seasons of the study, but the obtained values lacked significance. As for reducing, non-reducing and total sugars, it's clear that El Nobaria pollinizer gave the highest values in the two studied seasons for the three parameters. The differences among the pollinizers were significant in the two seasons except that of reducing sugars I the second season.

Table (8): Effect of different pollinizer sources on fruit set, fruit retention, bunch weight and yield of Samani date palm during 2012 and 2013 seasons.

| Pollinizer source | Fruit set % | | Fruit retention % | | Bunch weight (kg) | | Palm yield (kg) | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season |
| New Valley | 79.66 | 37.50 | 39.00 | 70.66 | 16.0 | 14.33 | 128.0 | 114.66 |
| Rashied | 72.00 | 39.00 | 40.00 | 69.33 | 17.7 | 14.70 | 141.6 | 117.60 |
| El Nobaria | 79.00 | 35.80 | 37.33 | 80.33 | 18.2 | 15.70 | 145.6 | 125.63 |
| LSD 5% | 2.944 | 1.474 | 1.255 | 1.472 | 0.103 | 0.296 | 0.869 | 2.277 |

Table (9): Effect of different pollinizer sources on physical properties of Samani date palm during 2012 and 2013 seasons.

| Pollinizer source | Fruit weight (gm) | | Fruit length (cm) | | Fruit diameter (cm) | | Fruit volume (cm ³) | | Pulp % | | Seed % | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 2 nd season |
| New Valley | 26.43 | 27.16 | 5.30 | 5.06 | 2.63 | 2.66 | 27.00 | 27.33 | 93.13 | 89.66 | 6.86 | 14.33 |
| Rashied | 27.16 | 28.66 | 5.53 | 5.36 | 2.83 | 2.83 | 27.00 | 28.66 | 93.26 | 89.66 | 6.73 | 14.70 |
| El Nobaria | 29.40 | 30.86 | 5.76 | 5.66 | 3.06 | 3.16 | 29.33 | 30.00 | 92.70 | 89.66 | 7.30 | 15.70 |
| LSD 5% | 0.473 | 0.783 | 0.8724 | 0.133 | 0.06 | 0.146 | 0.627 | 0.769 | 1.171 | 1.037 | 1.171 | 0.286 |

Table (10): Effect of different pollinizer sources on chemical properties of Samani date palm during 2012 and 2013 seasons.

| Pollinizer source | TSS % | | Acidity % | | Non-reducing sugars% | | Reducing sugars% | | Total sugars% | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season | 1 st season | 2 nd season | 1 st season | 2 nd season | 1 st season |
| New Valley | 26.96 | 29.16 | 0.25 | 0.25 | 7.50 | 7.7 | 17.13 | 17.83 | 24.63 | 25.53 |
| Rashied | 27.16 | 30.16 | 0.25 | 0.25 | 7.73 | 7.76 | 18.16 | 18.33 | 25.90 | 26.10 |
| El Nobaria | 28.16 | 32.56 | 0.22 | 0.24 | 8.26 | 8.30 | 19.50 | 18.90 | 27.76 | 27.20 |
| LSD 5% | 0.565 | 0.827 | 0.060 | 0.060 | 0.433 | 0.243 | 0.8164 | 0.702 | 0.984 | 0.871 |

DISCUSSION

The obtained results concerning total number, monomorphic, polymorphic of bands and the percentage of polymorphism as revealed by nine RAPD primers on the five date palm genotypes are in agreement with those obtained by Sedra *et al.* (1998), Motawei *et al.* (1975) and Adawy *et al.* (2002) who found in date palm that the average number of polymorphic bands/primer was 1.9, 2.4, and 1.2, respectively. Moreover, the size of the amplified fragments varied with different primers, ranging from 100 to 1700bp. Hela *et al.* (2000) reported that fragment sizes ranged from 200 to 1600 bp, while, Adawy *et al.* (2002) stated that "In the RAPD analysis of five date palm cultivars using 10 primers, the fragment sizes ranged from 310 to 2800 bp". These discrepancies could be attributed to the use of different primers and different reaction conditions, in the present study, the RAPD results revealed a very low intra-varietal polymorphism. However, Hussein *et al.* (1979) pointed out the presence of intra-varietal polymorphism among five Egyptian date palm cultivars from the Delta

region. Our result in respect to the phylogenetic tree in date palm cultivars is confirmed with Cipriani *et al.* (1996) and Hormaza (2001) who stated that RAPD technique provides genetic markers which have been used extensively in many different applications.

The obtained result regarding was more confirmed than those of Moghaieb *et al.* (2010) who detected 60.23% polymorphism among the six genotypes in date palm by RAPD. However, Hussein *et al.* (2004) found a high polymorphism (92.4%) among their Egyptian date palm cultivars. Concerning the Proximity matrix, the latter results obtained by Elshibli, *et al.* (2011) showed a complex genetic relationships between some of the tested populations especially when isolation by distance was considered. They also reported that large amount of diversity exists among date palm germplasm, suggesting that the role of biological nature of the tree and their results about isolation by distance and environmental effects agreed with recorded results in our studies May throw the high polymorphism could be enhancing the

possibility of using these five date palm varieties in the upcoming breeding programs.

The horticultural study

The obtained results concerning fruit set and fruit retention could be due to the effect of metaxenic of pollen grain sources on fruit set percentage which are differed among the male sources (Aly, 2001; Al-Hamoudi *et al.*, 2006; Mustafa *et al.*, 2014). Regarding bunch weight, the previous results are in agreement with those reported by Ghalib *et al.* (1987), El-Kassas *et al.* (1996) and Mustafa *et al.* (2014) who reported that there was a positive correlation between fruit set percentage and bunch weight obtained at the harvest.

As for fruit properties, the obtained results are in agreement with those obtained by El-Makhtoun and Abdel-Kader (1990), El-Kosary (1993), El-Salhy *et al.* (1997), Soliman (1999) and Moustafa *et al.* (2014) since they reported that fruit quality varied according to type of pollen sources used in pollination of date palm.

The effect pollinizer source on fruit sugar contents might be due to the activities of the enzyme systems initiated by the metaxenia effect and later on that passed into extra cellular sites get dissolved readily into water and invert the sugars. Similarly, the hydrolytic enzymes like polygalactouronase and cellulose may also be involved in these biochemical changes by solubilizing the pectin and cellulose in date palm fruit (Hasegaw and Smolensky, 1971).

In this respect, Hussein *et al.* (1979) and Nour and Jasim (1984) stated that “specific pollens may possibly affected cell number in early fruit development response of the female tissue to hormones or ouxins introduced in the ovary by pollen source”.

CONCLUSION AND RECOMMENDATION

Molecular markers as modern breeding approaches were successfully used to estimate the genetic relationships between the studied date palms for improving the fruit indispensable to achieve date palm genetic improvement considering the lengthy and dioeciously nature of date palm and use of molecular tools. In this concern, RAPD is successfully and very important to explain genetic diversity. Thus, the tools should be increasing the relationships between molecular markers and fruit characteristics. This could increase the selection efficiency of date palm cultivars derived by sexual reproduction. The result by ANOVA analysis supported genetic relationships between the five genotypes to two clusters. This study has provided an efficient procedure that can be used routinely to identify date palm cultivars.

However, from the abovementioned results, it could be concluded that Rashied pollinizer was the most effective for using as a pollinizing Zaghoul female date palm, while El Nobarria pollinizer was the most suitable for Samani female date palm under this experiment conditions.

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