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Effect of Different Pollinizer Sources on Yield, Fruit Characteristics and Phylogenetic Relationships with Amhat Cv. Date Palm (*Phoenix dactylifera* L.) in Egypt Using RAPD Markers

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ABSTRACT

The present study was carried out during two successive seasons (2012 and 2013) on mature date palm (Amhat cv.) cultivated at a private orchard in El-Badrashin district, Giza governorate, Egypt. Pollen grains from different regions (Noubaria, Aswan, Giza, Rashid and New Valley) were used to pollinate female (Amhat cv.) date palms. All studied characters were significantly affected with different pollen grain sources used in this trial. Noubaria pollen grain gave the highest fruit set, retention, bunch weight and yield per palm in both seasons comparing with the other pollen grain sources. In addition, the same pollen grain source recorded the highest fruit characteristics (fruit weight, fruit dimensions and fruit volume) and chemical composition of fruit (total soluble solids, reducing sugars, non reducing sugars and total sugars). Fingerprinting analysis has been performed to investigate phylogenetic relationships among six Egyptian date-palm varieties (one female and five males). The RAPD-PCR with six successful primers out of twelve primers detected forty five fragments where eight were monomorphic bands with 17.78% and 37 fragments were polymorphic with 82.22% polymorphism. The relationships revealed a high value between Amhat with Noubaria (63.2%) and the lowest value between Amhat and Aswan (0.368%) in proximity matrix. On the other hand, six primers revealed the medium values between Amhat as female and both New Valley, Rashid and Giza with values of 52.6, 47.4 and 42.1, respectively. The phenetic analyses among some of the good fruit quality varieties were conducted using appropriate programs. Thus, clusters including the tested varieties are apparently related according to their date quality. However, Rashid and Noubaria varieties, characterized by their opposite fruit qualities, seem to be dissimilarly related to the others. Data provides evidence of RAPDs as a powerful technique which may be used to get phenetic information within Egyptian date-palm varieties.

Key words: Date palm, pollinizers, fruit set, RAPD-PCR, genetic diversity, molecular breeding

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), $2n = 36$, is a dioecious long-lived monocotyledonous plant which belongs to the family Arecaceae. It is one of the excellent candidate crops in arid and semiarid regions of the world with high tolerance to environmental stresses. In addition to its valuable fruit, the palm is cultivated for fuel, fiber and as shelter for ground crops. The annual

world production of dates has reached 6-8 million mt (metric tons), representing a market exchange value of over 1 billion USD. The fruit is nutritionally rich; several products are made that generate employment and thus benefit the socioeconomic status of local people (El Hadrami and Al-Khayri, 2012). Moreover, this crop has great potential as a source of renewable energy due to the high carbohydrate content, 44-88%, of date palm fruit (Jain, 2012).

Date palm is one of the most important fruit crops in Egypt and Arab countries. It is dioecious with female and male flowers occurring on separate plants. Artificial pollination is essential for economical crop. It is generally known that pollen grains from different male palms affect the yield and fruit quality, it is known as phenomenon "Metexenia" (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 "xinia" but also has a direct effect on fruit set, yield and fruit physical and chemical characteristics of "metexenia". Mothew *et al.* (1975), El-Hammady *et al.* (1977), Shaheen *et al.* (1989a, b) and El-Salhy *et al.* (2010) reported that pollen sources affected fruit and seed characteristics and exhibited metaxenic effect depending on female cultivar used.

Usually an indirect approach for detecting genomic variation, DNA fingerprinting in plants is primarily used for identification of genetic diversity, protection of biodiversity or germplasm conservation and identifying markers associated with specific traits (Khanam *et al.*, 2012).

The RAPD technique provides genetic markers which have been used extensively in many different applications and in different plant species because of their simplicity (Cabrita *et al.*, 2001; Cipriani *et al.*, 1996; Goulao *et al.*, 2001; Hormaza, 2001; McGregor *et al.*, 2000; Salimath *et al.*, 1995; Samaee *et al.*, 2003; Ulanovsky *et al.*, 2002). Moreover, RAPDs have been successfully used to study the genetic relationships among various date palm accessions and cultivars in Egypt, Morocco, Tunisia, Iraq and Saudi Arabia (Adawy *et al.*, 2002; Motawei *et al.*, 2003; Hela *et al.*, 2000; Sedra *et al.*, 1998; Soliman *et al.*, 2003; Trifi *et al.*, 2000).

DNA fingerprinting in plants is primarily used for identification of genetic diversity, protection of biodiversity or germplasm conservation and identifying markers associated with specific traits (Khanam *et al.*, 2012). Molecular markers based on RAPD (Random Amplified Polymorphic DNA) are powerful techniques which can be used to identify and determine plant genomes or to estimate the phylogenetic relationship among genotypes of date palm (Cullis, 2011; ElShibli and Korpelainen, 2011). The RAPD technique has been used for cultivar genotyping (Ben Abdallah *et al.*, 2000; Trifi *et al.*, 2000) and for analyses of phylogenetic relationships and genetic diversity, estimating the genetic distance assists in studying genetic (Al-Khalifah and Askari, 2003; El-Tarras *et al.*, 2007; Al-Khalifah *et al.*, 2012).

The objective of this study was to further reevaluate the effect of using different pollinizer sources on fruit set, yield and fruit quality of Amhat dates grown under Giza governorate conditions. Molecular studies aimed to detect phylogenetic palm and relationships between different varieties of date palms.

MATERIALS AND METHODS

This study was carried during 2012 and 2013 seasons on 30 years old Amhat date palm cv. grown on loamy soil at private orchard located in EL-Badrashin district, Giza Governorate, Egypt.

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 five locations namely Noubaria, New Valley, Giza, Aswan and Rashid.

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

Horticulture study: Initial fruit set and fruit retention percentages were evaluated one month after pollination and at harvest time. Five female strands/bunch were randomly selected from each replicate. Number of fruit set was recorded and then fruit set and fruit retention percentage was calculated using the following equations:

$$\text{Fruit set (\%)} = \frac{N_s}{N_t} \times 100$$

$$\text{Fruit retention (\%)} = \frac{N_r}{N_r + N_f} \times 100$$

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 characteristics such as fruit weight (g), fruit volume (cm³), fruit dimensions (cm), fruit shape index, fruit pulp and seed (%), also chemical characteristics as total soluble solids, acidity, reducing sugars (%), non reducing sugars (%), total sugars and tannins as out lined in A.O.A.C. methods (AOAC, 1995).

Phylogenetic study: Molecular studies aimed to detect phylogenetic relationships among different varieties of date palm. Fresh leaf samples were collected from six date palm varieties included one female: Amhat and five males: Giza, Aswan, Rashid, New Valley and Noubaria (Table 1).

Plant material: The study included six date palm (*Phoenix dactylifera* L.) accessions collected from different locations in Egypt. These accessions represent six date palm cultivars: Amhat as female and five males Giza, Noubaria, Aswan, Rashid and New Valley. Samples were collected from the young leaves surrounding the palm meristem of 3 to 5 palms from each location. The accessions, cultivars and the respective locations are represented in Table 1.

RAPD analysis: A set of six random 10-mer primers from twelve experiment primers in Table 2, were used in the detection of polymorphism among the six date palm accessions. These primers synthesized on RAPD-PCR were carried out according to the procedure given by Williams *et al.* (1990) with minor modifications.

The six failed primers are: OPA-14, OPB-03, OPB-06, OPB-08, OPC-03 and OPZ-08 and the other primers listed in Table 2.

The amplification reaction was carried out in 25 mL reaction volume containing 1X PCR buffer, 1.5 mM MgCl₂, 2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 25 ng templates DNA. PCR amplification was performed in a Perkin-Elmer/GeneAmp® 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 36°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

PCR-amplification of RAPD: Amplification reaction was carried out in 25 µL reaction mixture contained 2 µL of genomic DNA, 3 µL of the primer, 2.5 µL 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 sec, 42°C for 90 sec for annealing temperature 72°C for 90 sec and final extension at 72°C for 2 min.

Amplification products for 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 100 bp for RAPD was used.

PCR products: PCR products were visualized by UV-transilluminator and photographed by gel documentation system, Biometra-Bio Doc. 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.

Table 1: Code numbers, cultivars names and type of six date palms

Code No.	Cultivar name	Type
1	Amhat	Female
2	Giza	Male
3	Noubaria	Male
4	Aswan	Male
5	Rashid	Male
6	New Valley	Male

Table 2: Code and sequences of six RAPD primers

Primer code	Sequence (5'-3')
OP-A02	CAGGCCCTTC
OP-B07	GGTGACGCAG
OP-B10	CTGCTGGGAC
OP-O10	TCAGAGCGCC
OP-O13	GTCAGAGTCC
OP-O14	AGCATGGCTC

Statistical analysis: The obtained data was tabulated and statistically analyzed using MSTAT program (MSTAT, 1998) and the significant differences among the probability at 0.05 were performed according to Duncan (1955).

RESULTS AND DISCUSSION

Horticultural study

Fruit set and fruit retentions percentage: Fruit set percentage as an important indicator for exist of compatibility or in compatibility phenomena was studied and illustrated in Table 3.

- It was evident from the data in Table 3 that the highest fruit set of Amhat date palm was obtained from Noubaria male pollen (86 and 85.3%) in the first and second seasons, respectively. Meanwhile, the pollinizer from Aswan recorded the lowest fruit set percentage (69.2 and 70.3%) in the two seasons, respectively

Data illustrated in Table 3 indicated that the least retained fruit at harvest was obtained from Aswan pollinizer used on Amhat date palm in both seasons of the study. In the same trend, fruit retention of Amhat date palm was varied according to the pollen grain source. The highest percentage of fruit retention was recorded from Noubaria pollinizer source (57 and 62% in both seasons, respectively) with non-significant differences than Giza pollinizer in the first season only.

These results are in agreement with those obtained by Aly (2001) and Al-Hamoudi *et al.* (2006) who reported that pollen grain sources had metaxenic effect on fruit set percentage but these effects were differed among the male sources.

Bunch weight: It is quite clear from Table 3 that Amhat date palm pollinated with Noubaria pollen grains gave the heaviest bunches (18.9 and 20.3 kg/bunch) as compared with the analogous ones pollinated with Aswan pollen grains (13.7 and 15.3 kg/bunch) in 2012 and 2013 seasons, respectively.

The obtained results regarding the effect of pollen grain sources on palm fruiting are in agreement with the findings of Shaheen *et al.* (1989a) since they mentioned the source of pollen grains.

Yield/palm (kg): Table 3 shows that pollen grains sources markedly affected palm productivity, hence, Amhat date palm pollinated with Noubaria pollen grains produced comparatively the highest yield (151.2 and 162.1 kg/palm) in the first and second seasons, respectively, followed by

Table 3: Effect of different pollinizer sources on fruit set, fruit retention, bunch weight and yield of Amhat date palm during 2012 and 2013 seasons

Pollinizer	Fruit set (%)		Fruit retention (%)		Bunch weight (kg)		Yield/palm (kg)	
	2012	2013	2012	2013	2012	2013	2012	2013
Noubaria	86.0 ^a	85.3 ^a	57.0 ^a	62.0 ^a	18.9 ^a	20.3 ^a	151.2 ^a	162.1 ^a
Aswan	69.2 ^c	70.3 ^d	50.0 ^d	52.3 ^d	13.7 ^e	15.3 ^e	109.9 ^e	122.3 ^e
Giza	80.3 ^b	79.7 ^b	55.7 ^{ab}	60.3 ^b	18.2 ^b	19.5 ^b	145.3 ^b	155.7 ^b
Rashid	80.0 ^b	80.3 ^b	52.7 ^c	57.0 ^c	16.4 ^d	17.3 ^c	131.5 ^e	137.7 ^e
New valley	69.6 ^c	73.0 ^c	54.0 ^{bc}	56.7 ^c	15.4 ^d	16.2 ^d	123.5 ^d	129.6 ^d
Significance	S	S	S	S	S	S	S	S

Giza pollen grain that produced 145.3 and 155.7 kg/palm in both seasons, respectively. Whereas, the lowest yield/palm was obtained from Aswan pollen grains, since it was 109.9 kg/palm in the first seasons and 122.3 kg/palm in the second season. These results are in agreement with those reported by Ghalib *et al.* (1987), Shaheen *et al.* (1989b) and El-Kassas *et al.* (1996), who reported that there was a positive correlation between fruit set percentage and bunch weight obtained at the harvest.

Physical fruit characteristics: Data presented in Table 4 indicated the effect of different pollen grain sources on fruit physical properties of Amhat date palm.

- **Fruit weight (g):** Table 4 revealed that Amhat date pollinated by Noubaria pollinizer recorded the highest fruit weight compared with the other pollinizer sources, since it recorded 9.8 g in the first season. Whereas, in the second season, Rashid pollinizer source recorded the highest fruit weight 11.5 g, followed by Noubaria pollinizer source (10.9 g)

Also fruit volume took the same trend of fruit weight in both seasons.

- **Pulp (%):** Pollen grain source from Rashid and New Valley pollinizer recorded the lowest pulp (%) in the first season, since it recorded 85.5 and 85.8%, respectively. Whereas, Noubaria pollinizer recorded the highest pulp (%) in the first season, meanwhile, the highest pulp (%) in the second season was recorded with Rashid pollinizer
- **Seed (%):** Pollen grain sources from Rashid and New Valley pollinizer recorded the highest portion of fruits occupied by seed (%) in the first season. Meanwhile, in the second season, higher seed (%) were recorded from New Valley, Giza and Rashid pollinizer, respectively
- **Fruit dimensions:** Data in Table 4 cleared that the highest fruit length was obtained from Noubaria pollinizer source when recorded 3.7 and 3.9 cm in the first and second seasons,

Table 4: Effect of different pollinizer sources on fruit physical characteristics of Amhat date palm during 2012 and 2013 seasons

Pollinizer	Fruit weight (g)		Fruit volume (cm ³)		Pulp (%)		Seed (%)	
	2012	2013	2012	2013	2012	2013	2012	2013
Noubaria	9.8 ^a	10.9 ^{ab}	9.5 ^a	10.7 ^b	86.2 ^a	86.2 ^b	12.8 ^b	11.2 ^b
Aswan	6.9 ^c	10.4 ^b	6.9 ^c	9.9 ^b	87.3 ^a	86.3 ^b	12.7 ^b	13.7 ^{ab}
Giza	9.2 ^b	10.2 ^b	9.0 ^b	9.7 ^b	86.8 ^a	85.7 ^b	13.2 ^b	14.5 ^a
Rashid	9.2 ^b	11.5 ^a	9.0 ^b	11.0 ^a	85.5 ^b	88.8 ^a	15.5 ^a	13.8 ^a
New Valley	9.0 ^b	10.5 ^b	10.2 ^b	85.8 ^b	84.8 ^b	14.2 ^a	14.2 ^a	15.2 ^a
Significance	S	S	S	S	S	S	S	S

Pollinizer	Fruit length (cm)		Fruit diameter (cm)		Fruit shape index (L/D)	
	2012	2013	2012	2013	2012	2013
Noubaria	3.7 ^a	3.9 ^a	2.0 ^a	2.7 ^a	1.9 ^a	1.7 ^a
Aswan	3.2 ^d	3.9 ^a	1.8 ^c	2.2 ^{ab}	1.8 ^a	1.8 ^a
Giza	3.6 ^a	3.7 ^a	1.9 ^b	2.1 ^b	1.9 ^a	1.8 ^b
Rashid	3.5 ^b	3.7 ^a	1.9 ^b	2.2 ^{ab}	1.8 ^a	1.7 ^a
New Valley	3.3 ^c	3.7 ^a	1.8 ^c	2.2 ^{ab}	1.8 ^a	1.7 ^a
Significant	S	NS	S	S	NS	NS

S: Significant, NS: Not significant

respectively with no significant differences than the other pollinizer sources in the second season. The lowest fruit length was recorded by Aswan pollinizer in the first season while in the second one, fruit length was not affected significantly by different pollen grain sources. As for fruit diameter 2 and 2.7 cm was obtained from Noubaria pollinizer source in both seasons, respectively

- **Fruit shape index:** Data in Table 4 cleared that fruit shape index was not affected significantly by different pollen grain sources in both seasons. Fruit quality (fruit weight, fruit dimensions, fruit volume, pulp and seed (%)) was affected significantly by different pollen grain sources used in this study. It was clearly noticed that Noubaria pollen sources used in this trail to increase the fruit quality. These results are in agreement with those obtained by Shaheen *et al.* (1989a), El-Makhtoun and Abdel-Kader (1990), El-Kosary (1993), El-Salhy *et al.* (1997) and Soliman (1999) since they reported that fruit quality varied according to type of pollen sources used in pollination of date palm

Fruit chemical characteristics: Data in Table 5 showed that total soluble solids (%) was affected significantly by different pollen grain sources in both seasons of study.

Fruits produced from palms pollinated with Noubaria pollen source had the highest TSS percentage in both seasons (34 and 35.7%, respectively). Meanwhile, Aswan pollen grain source had the lowest TSS percentage (31 and 32.3%) in the first and second seasons, respectively.

- **Acidity (%):** Data in Table 5 cleared that the effect of pollen grain from Noubaria did not differ in two seasons of the study while the highest acidity (%) was recorded from Aswan pollen grain sources (0.49%) in the first season and 0.47% in the second season
- **Non-reducing sugars (%):** It is clear that pollen grain from Noubaria gave the highest reducing sugars (%) (1.83 and 2%) in the two seasons, respectively
- **Reducing sugars (%):** Data in Table 5 showed that the highest amount of reducing sugars was obtained from Noubaria pollen grain (23.4 and 23.9%) in the first and second seasons, respectively

The lowest amount of reducing sugars was obtained from Aswan pollen grain (19.5 and 19.7%) in both seasons, respectively.

Table 5: Effect of different pollinizer sources on chemical characteristics of Amhat date palm during 2012 and 2013 seasons

Pollinizer	TSS (%)		Acidity (%)		Non-reducing sugars (%)		Reducing sugars (%)		Total sugars (%)		Tannins (%)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Noubaria	34.0 ^a	35.7 ^a	0.44 ^d	0.44 ^c	1.8 ^a	2.0 ^a	23.4 ^a	23.9 ^a	25.2 ^a	25.9 ^a	0.70 ^a	0.68 ^a
Aswan	31.0 ^f	32.3 ^e	0.49 ^a	0.47 ^a	1.6 ^b	1.8 ^b	19.5 ^e	19.7 ^d	21.1 ^d	21.5 ^e	0.71 ^a	0.68 ^a
Giza	33.0 ^{ab}	34.0 ^b	0.47 ^e	0.44 ^c	1.5 ^c	1.7 ^c	22.3 ^b	22.8 ^b	23.8 ^b	24.5 ^a	0.70 ^a	0.67 ^a
Rashid	32.0 ^{bc}	33.7 ^b	0.45 ^e	0.46 ^b	1.7 ^b	1.8 ^b	21.1 ^c	21.5 ^e	22.8 ^c	23.3 ^b	0.70 ^a	0.68 ^a
New valley	31.5 ^{bc}	32.3 ^e	0.46 ^b	0.46 ^b	1.7 ^b	1.8 ^b	20.5 ^d	20.8 ^c	22.2 ^c	22.6 ^b	0.69 ^a	0.69 ^a
Significance	S	S	S	S	S	S	S	S	S	S	NS	NS

S: Significant. NS: Not significant, TSS: Total soluble solids

- **Total sugars (%):** It could be observed that five pollinizer sources significantly affected amount of total sugars in Amhat date fruits during the two seasons

Table 5 showed that Noubaria pollen grain source produced the highest total sugars (25.2 and 25.9%) in the first and second seasons, respectively. Whereas, the lowest amount of total sugar was obtained from Aswan pollinizer in the first season (21.1%) and in the second season (21.5%).

- **Tannins (%):** Results in Table 5 revealed that the percentage of tannins in fruits was affected by pollen grain sources and the differences were not affected significantly in both seasons

Sugars content of the fruit that affected by pollen grain source might be due to the activities of enzymes system 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).

Regarding the effect of pollen grains on reducing sugars contents, it took a similar trend of total sugars contents. These findings are in harmony with those reported by Hussein *et al.* (1979), Nour and Jasim (1984) and Shaheen *et al.* (1989a, b) who 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.

Phylogenetic study: Fresh leaf samples of date palm germplasm were collected from different Egyptian regions. This included six date palm genotypes comprising one female, Amhat and five selected males.

These results showed in Fig. 1 and Table 6.

- **Randomly Amplified Polymorphic DNA (RAPD) analysis:** Twelve RAPD primers were screened with the DNA of the six date palm accessions, these produced multiple band profiles with a number of amplified DNA fragments ranging from 6-10 (Table 6 and Fig. 2). Sedra *et al.* (1998) reported that 19 out of 123 prescreened arbitrary decamer primers revealed polymorphic and reproducible results. In the present study, the total number of fragments produced by the six primers was 45 with an average of 7.5 fragments per primer (Table 6). While, the number of polymorphic fragments ranged from 4-9. A maximum number of 10 amplicons were amplified with primer OP-O14 while the minimum number of fragments (4) was amplified with primer OP-O13. The highest number of polymorphic bands (9) was obtained with primers OP-O14 and exhibited the highest percentage (90%) of polymorphism

In Table 6, by six successful primers polymorphic revealed thirty seven polymorphic from forty five bands with 82.22% polymorphism.

In this respect, Sedra *et al.* (1998), Motawei *et al.* (2003) and Adawy *et al.* (2004) found in date palm that the average number of polymorphic bands per primer was 1.9, 2.4 and 1.2, respectively. Moreover, the size of the amplified fragments varied with different primers, ranging from 100-1700 bp. Hela *et al.* (2000) reported fragment sizes ranging from 200-1600 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 310-2800 bp.

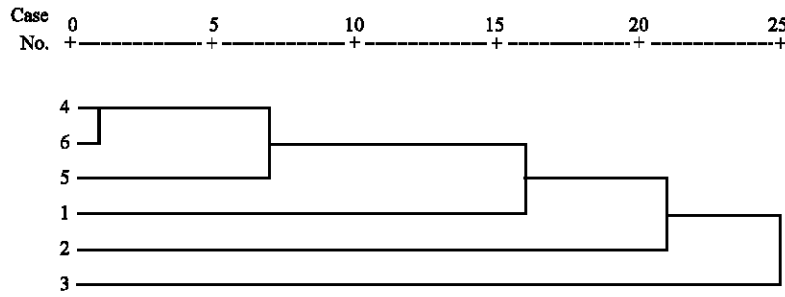


Fig. 1: Dendrogram and hierarchical cluster analysis using average linkage (between groups) of date palm cultivars one female and five males (2, 3, 4, 5 and 6)

Table 6: Total number of bands, monomorphic bands, polymorphic bands, mean of band frequency and polymorphism of the six date palm cultivars using RAPD-PCR

Primer name	Total No. of bands	No. of monomorphic bands	No. of polymorphic bands	Mean of band frequency (No.)	Polymorphism (%)
OP-A02	8	1	7	0.542	87.500
OP-B07	8	2	6	0.667	75.000
OP-B10	6	2	4	0.639	66.667
OP-O10	7	1	6	0.524	85.714
OP-O13	6	1	5	0.667	83.333
OP-O14	10	1	9	0.567	90.000
Total	45	8	37	0.601	82.222
Average	7.5	1.33	6.17	-	-

These discrepancies could be attributed to the use of different primers and different reaction conditions. In the present study, the RAPD results revealed 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.

- **Proximity matrix analysis:** The dendrogram showed the highest relationships between Amhat with Noubaria and New Valley moreover, showed the lowest relationships between Aswan with Amhat but found medium relationships between Amhat with Rashid and Giza

To estimate the genetic relationships among the six date palm cultivars based on RAPD results, only the common bands between different accessions representing each cultivar were scored. This strategy produced some loci with missing values for some cultivars, such loci were disregarded in the analysis. The estimated genetic similarities ranged from 66.5-85.3% in Table 7 the highest genetic similarity (85.3%) was between the cultivar Amhat and the cultivars Noubaria and New Valley while, the lowest genetic similarity (66.5%) between Amhat and Aswan.

The UPGMA cluster analysis was carried out to graphically represent the genetic distances among the six date palm accessions (Fig. 1). The obtained dendrogram was divided into two main clusters, one cluster included the three Giza, Aswan and Rashid accessions of cultivar Amhat while the other main cluster included two sub clusters New Valley and Noubaria.

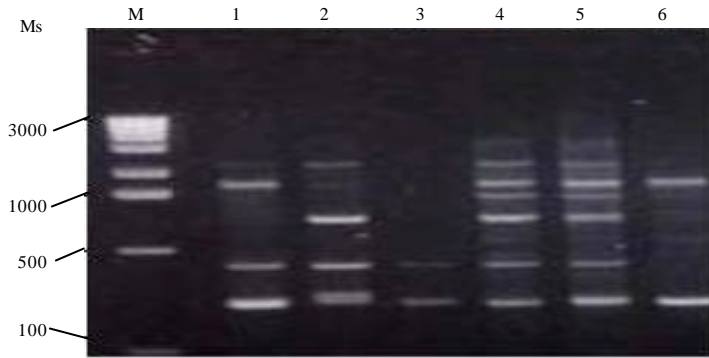


Fig. 2: Example of RAPD-PCR banding patterns of six date palm cultivars amplified with six 10-mer random primers OP-B07, Ms: 100 bp ladder

Table 7: Proximity matrix between female Amhat and five males

Case	Matrix file input					
	Amhat	Noubaria	New Valley	Giza	Rashid	Aswan
Amhat	1.000					
Noubaria	0.632	1.000				
New Valley	0.526	1.000	1.000			
Giza	0.421	0.474	0.579	1.000		
Rashid	0.474	0.632	0.737	0.211	1.000	
Aswan	0.368	0.526	0.632	0.000	0.158	1.000

CONCLUSION

From the above-mentioned results, it could be concluded that all pollen grains sources had a significant effect on different studied characteristic. However, Noubaria pollen grain sources give the highest fruit set, retention. The genetic markers and polymorphism revealed the highest relationships between Amhat and Noubaria while, the lowest relationships between Amhat and Aswan date palm varieties. The RAPD technique provides genetic markers which have been used extensively in many different applications and in different plant species because of its simplicity. Moreover, RAPDs have been successfully used to study the genetic relationships among various date palm accessions and cultivars.

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