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## The Effect of Metaxenia on Fruit Yield and the Relation between Some Date Palm Pollinizers and Two Female Cultivars Using RAPD Molecular Markers.

<sup>1</sup>Mostafa, E.A.M, <sup>1</sup>M.M.S. Saleh\*, <sup>1</sup>N.E. Ashour, <sup>2</sup>S.A.A. Heiba, and <sup>2</sup>Sara E.I. El Dessouky.

<sup>1</sup>Pomology Dept., National Research Centre, Giza, Egypt.

<sup>2</sup>Genetics and Cytology Dept., National Research Centre, Giza, Egypt.

### ABSTRACT1

This investigation was carried out to study the effect of metaxenia and the relation between two female date palm cultivars (Sewi as semi dry date and Amhat as moist date) and three pollinizers. The pollen grains were selected from male pollinizers located at Giza, Aswan and El-Wadi (New Valley) governorates, Egypt. Also, RAPD technique was used to compare among the five date palm genotypes. Many traits such as fruit set, bunch weight, yield per palm and both physical and chemical fruit properties were studied. The results showed that Giza and Aswan pollinizers were the most suitable for pollinizing Sewi and Amhat female, respectively. At the molecular level, 9 primers were used for RAPD analysis. These primers gave a total of 55 different alleles for all genotypes. The most Polymorphic Information Content (PIC) value and polymorphism percentage that detected by OPB-07 and OPO-14 markers showed the high score (8 bands) with polymorphism (87.5% and 50%), respectively. Primers OPB-10 and OPO-19 revealed low level from bands (4bands) with 75%. Also, OPA-02 and OPO-13 and revealed six fragments with 50 and 83.33 polymorphism, respectively. The relationships among the genotypes divided into two clusters. The first cluster included two sub clusters. The first sub cluster included Amhat and Giza, while the second one includes Aswan. The second cluster included Sewi and El-Wadi (New Valley).

**Keywords:** Sewi and Amhat, Date palm, Metaxenia, Pollinizers, RAPD markers.

*\*Corresponding author*

## INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is considered one of the oldest cultivated plants in the world. In Egypt, it is one of the most important fruit crops that widely distributed in different districts. Date palm is a dioecious plant in which artificial pollination is essential for economic crop. Metaxenia is a phenomenon that known as the effect of pollen grains on yield and fruit quality of the plant (Nixon, 1934).

Therefore, this phenomena was studied by many researchers, Mustafa *et al.* (2014), Samy *et al.* (2015), Merwad *et al.* (2015) since they proved that fruit set, yield and fruit quality were affected by different sources of pollen grains.

The newly developed techniques, based on the polymerase chain reaction (PCR), give a new tool for genetic analysis and construction of linkage maps. The random amplified polymorphic DNA (RAPD) technique using arbitrary primers is used to amplify a template of DNA (Welsh and McClelland, 1990).

Halward *et al.* (1992) and Carlson *et al.* (1991) found that the use of RAPD technique was effective for evolution studies and linkage analysis of several plant species.

RAPD analyses have been used to identify and assist the genetic diversity of date palm germplasm in Saudi Arabia. Most of these findings indicated that the RAPD technique is reliable, but other reports have suggested that RAPD markers have significant difficulties because of low polymorphism, irreproducibility and the construction of weak grouping associations (Yang and Quiros, 1993; Billotte *et al.*, 2004 and Trifi *et al.*, 2000).

The aim of this study was to maximize the fruit retained and yield as well as to improve yield and fruit quality of both Sewi (semi dry date) and Amhat (moist date) grown under Giza governorate conditions by detecting the most suitable pollen grain sources selected from Giza, Aswan and New Valley governorates 2.to determine the genetic similarity between the two female date palms based on RAPD markers and identify the unknown Egyptian male date palms through known female cultivars.

## MATERIALS AND METHODS

**Table (1): The average temperature degrees of the three pollinizer’s locations in the two studied seasons (2013 and 2014).**

Location	Giza						Aswan						New Valley (El-Wadi)					
	2013			2014			2013			2014			2013			2014		
Season	Max	Avg.	Min	Max	Avg.	Min	Max	Avg.	Min	Max	Avg.	Min	Max	Avg.	Min	Max	Avg.	Min
Max	112	82	46	109	82	59	117	94	59	117	94	59	108	77	54	109	77	58
Mean	93	73	44	94	73	55	102	81	52	102	81	52	90	69	50	90	69	54
Min	79	63	41	86	64	36	88	68	42	88	68	42	78	61	41	77	61	43

[https://www.wunderground.com/history/airport/HEMM/2015/1/22/CustomHistory.html?dayend=22&monthend=2&yearend=2016&req\\_city=&req\\_state=&req\\_statename=&reqdb.zip=&reqdb.magic=&reqdb.wmo=&MR=1](https://www.wunderground.com/history/airport/HEMM/2015/1/22/CustomHistory.html?dayend=22&monthend=2&yearend=2016&req_city=&req_state=&req_statename=&reqdb.zip=&reqdb.magic=&reqdb.wmo=&MR=1)

This study was carried out during 2013 and 2014 seasons on 40 years old female date palms (Sewi as semi dry date) and (Amhat as moist date) grown on loamy soil at a private orchard located in Al-Badrashin district, Giza governorate, Egypt. The selected palms were healthy, nearly uniform in growth vigor and fruiting and received regular horticultural practices. Moreover, date palms were thinned at one bunch per eight leaves (8:1 leaf/bunch ratio). In addition, male palms as a pollen source were selected from three locations namely, Giza, Aswan and New valley (El-Wadi) governorates which characterized with a variation in temperature that may affect the pollen grains properties. So, the accessions represent two female date palm cultivars (Sewi and Amhat) and three males as pollen grain sources selected from Giza, Aswan and El-Wadi governorates. The average temperatures of the three governorates of the pollen grain sources in the two studied seasons are shown in Table (1).

Samples were collected from the leaves surrounding the palm meristem of 3 to 5 palms from each location.

Pollination was achieved by inserting male strands into each 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 female bunch was bagged just after pollination process with news paper bags which were removed after three weeks. The experiment was arranged in a complete randomized design including three replications for each treatment.

This investigation included two main studies.

- Horticultural study.
- Molecular studies.

**The horticultural study:**

This study included the following determinations:-

- Fruit set percentage (one month after pollination).
- Fruit retention percentage (at harvest time).
- Fruit set % =  $N_s/N_t \times 100$
- Fruit retention % =  $N_r/N_r - N_f \times 100$

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 as average bunch weight (kg) for each treatment crossing the number of bunches per palm. All bunches were harvested at the second week of September in each season.

**Fruit quality**

Bunch weight was recorded and 30 fruits were picked randomly from each bunch to determine fruit physical and chemical properties, since the physical properties were fruit weight (g), fruit size (cm<sup>3</sup>), fruit dimensions (cm), flesh and seed percentages, also specific gravity, while the chemical properties were total soluble solids percentage, acidity % and total sugars percentage were measured as outlined in A.O.A.C. methods (A.O.A.C., 1995).

**Molecular studies**

The five date palms under this study were listed in Table (2).

**Table 2: The kinds and traits for five Egyptian genotypes of date palm**

No.	Genotypes	Kind
1	Sewi Cv.	Female
2	Amhat Cv.	Female
3	Giza	Male
4	Aswan	Male
5	El-Wadi	Male

**Molecular Studies and DNA extraction**

Molecular studies aimed to determine phylogenetic tree and relationships between different five date palms in steps; DNA was extracted from five date palms by Bio basic kits protocol.

**PCR- Amplification and RAPD analysis**

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 thermo cycler (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 according to gel analyzer protocol. A set of nine random 10-mer primers Table 3 was used in the detection of polymorphism among the nine broad wheat 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 genetic similarity was calculated for each primer separately and average for all primers was carried out with each comparison. The genetic similarity was calculated for each primer separately and average for all primers was carried out with each comparison. Dendrogram was constructed using the average linkage between groups (Sneath and Sokal, 1973).

**Table (3): Names and sequences of nine RAPD primers.**

No.	Primer name	Sequence (5`→3`)
1	OPA-02	CAGGCCCTTC
2	OPA-04	AATCGGGCTG
3	OPA-07	GAAACGGGTG
4	OPB-07	GGTGACGCAG
5	OPB-10	CTGCTGGGAC
6	OPO-10	TCAGAGCGCC
7	OPO-13	GTCAGAGTCC
8	OPO-14	AGCATGGCTC
9	OPO-19	CAATCGCCGT

**Statistical analysis:**

The experiment was designed as completely Randomized Design (CRD) and the results were analyzed a one way and Duncan's multiple range tests was used to differentiate means (Duncan, 1955).

**RESULTS AND DISCUSSION**

**The horticultural study**

**Fruit set and fruit retention**

It's clear from Fig (1) that pollen grain source have a significant effect on fruit set percentage, In this concern, Giza pollen grains had a superior effect on fruit set of Sewi date palm, while Aswan pollinizer gave the best effect with Amhat date comparing with the other pollinizers.

As for fruit retention percentage, it's observed from Fig (2) that the results of Sewi date palm are differ from season to another and did not take the same trend in both studied seasons, since Aswan pollinizer recorded the highest fruit retention percentage in the first season, while in the second one, El-Wadi pollen

grains gave the best results comparing with the other pollinizers. Regarding Amhat date palm, it is notice that Aswan pollinizer recorded the highest percentage of fruit retention in both seasons.

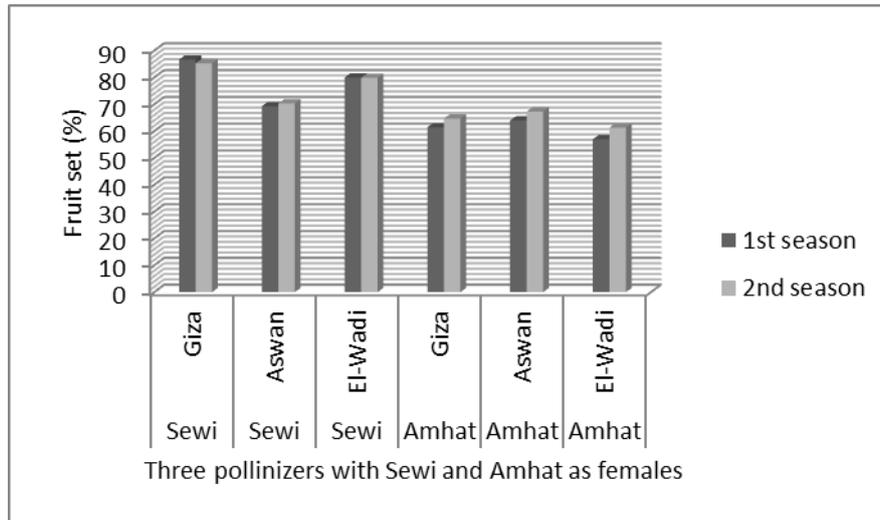


Fig (1): Effect of different pollinizer sources on fruit set of Sewi and Amhat cultivars in the two seasons.

The obtained results respecting fruit set and fruit retention could be explained due to the effect different pollen grain sources (effect of metaxenia) on fruit set percentage that are differed among the male sources (Aly, 2001; Al-Hamoudi *et al.*, 2006; Mustafa *et al.*, 2014; Samy *et al.*, 2015; Merwad *et al.* 2015). In this concern, Hussein *et al.* (1979); 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 auxins introduced in the ovary by pollen source”.

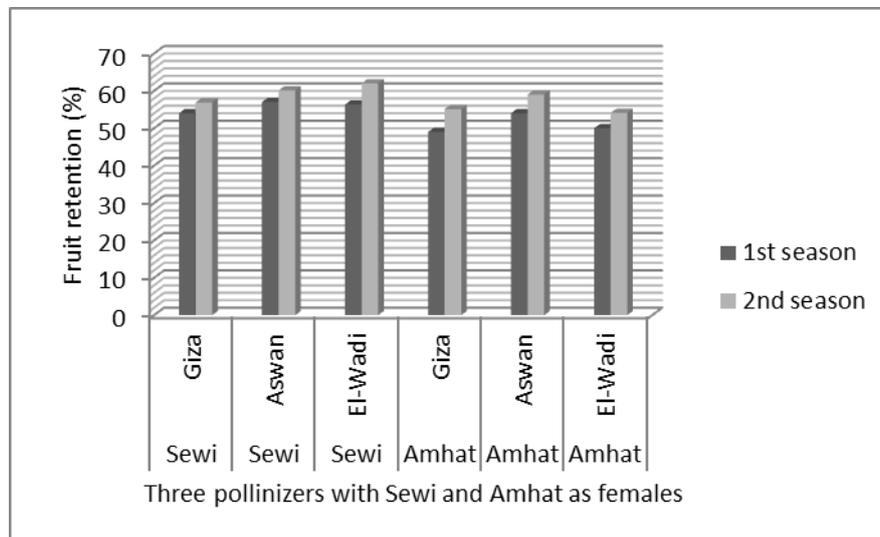


Fig (2): Effect of different pollinizer sources on fruit retention of Sewi and Amhat cultivars in the two seasons.

**Bunch weight and yield per palm**

As for bunch weight Fig (3) and yield per palm (kg) Fig (4), it is clear from the obtained results that there is a correlation between fruit set percentage and both bunch weight and yield weight per palm, since the results of bunch weight and palm yield show the similar trend of fruit set. In this concern, Giza pollen grains gave the best results and recorded the highest values comparing with the other pollen grain sources. This was true with Sewi date in the two studied seasons. While, as for Amhat date palm, it is clear that the results of bunch weight and yield per palm are in the same line of fruit set and fruit retention, since the results Aswan

pollinizer recorded the highest bunch weight and yield (kg) per palm when compared with the other pollinizers in the first and second seasons.

The obtained results concerning bunch weight are in agreement with those obtained by Ghalib *et al.*, (1987); El-Kassas *et al.*, (1996); Mustafa *et al.* (2014); Samy *et al.*, (2015) and Merwad *et al.*, (2015) who reported that bunch weight is corresponding with fruit set percentage at harvest time.

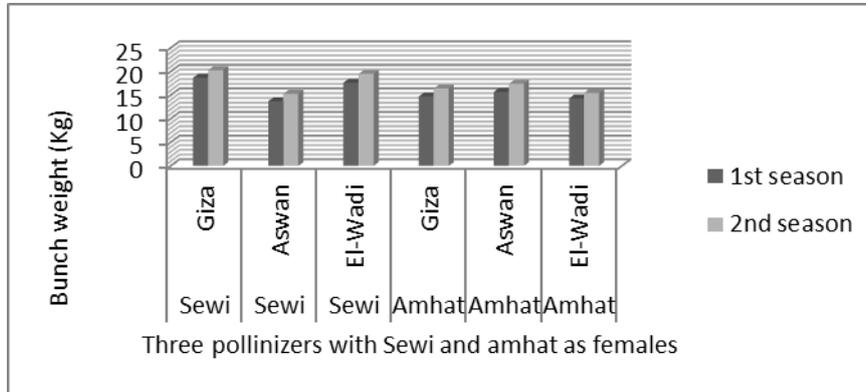


Fig (3): Effect of different pollinizer sources on bunch weight of Sewi and Amhat cultivars in the two seasons.

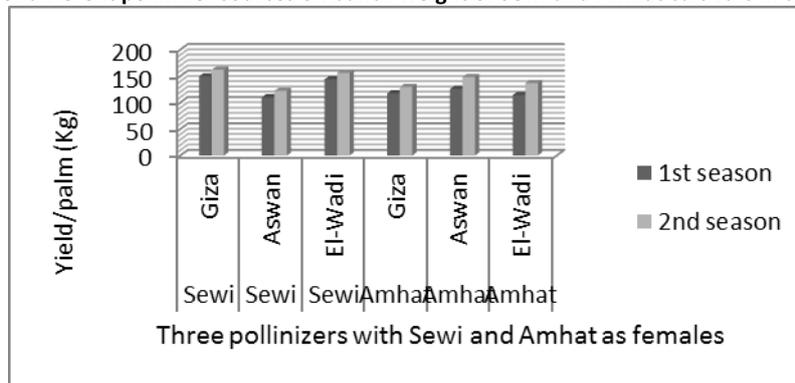


Fig (4): Effect of different pollinizer sources on yield per palm of Sewi and Amhat cultivars in the two seasons.

**Fruit characteristics:**

**Physical properties**

Figs (5, 6, 7, and 8) clearly show that pollen grain source has significant effects on fruit physical properties (length, diameter, weight and size). In this respect, fruit length, weight, diameter and size of Sewi date palm were significantly affected by Giza pollen grains in the two studied seasons, except for fruit size in the first season. Amhat date palm also show a significantly affect by Aswan pollen grains than the other pollen grain sources. The obtained results were detected in the two studied seasons.

Concerning flesh and seed percentage, results in Figs (9 and 10) show that Giza and El-Wadi pollen grains increased flush value of Sewi and gave higher percentage without differences between them. The same pollinizers gave lower values concerning seed percentage. On the other hand, Aswan pollinizer gave the highest flesh % of Amhat date palm and recorded the lowest seed value comparing with the other pollinizers (Giza and El-Wadi). This was true in both studied seasons.

As for specific gravity Fig (11), all pollinizer sources (Giza, Aswan and El-Wadi) had no observed effect on specific gravity of the two date palm cultivars under investigation (Sewi and Amhat).

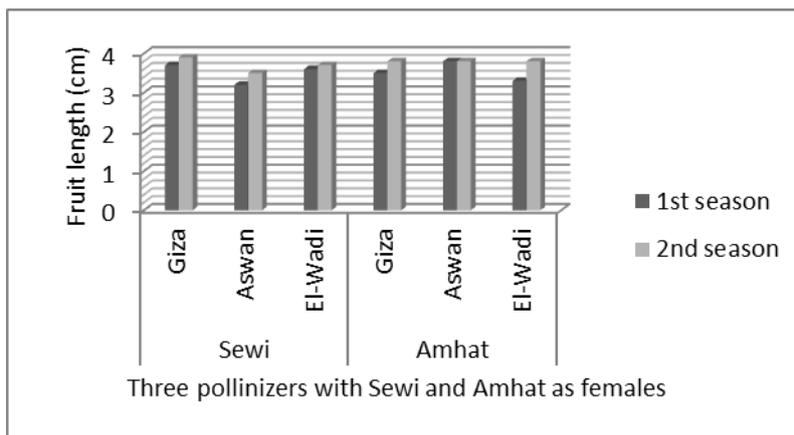


Fig (5): Effect of different pollinizer sources on fruit length of Sewi and Amhat cultivars in the two seasons.

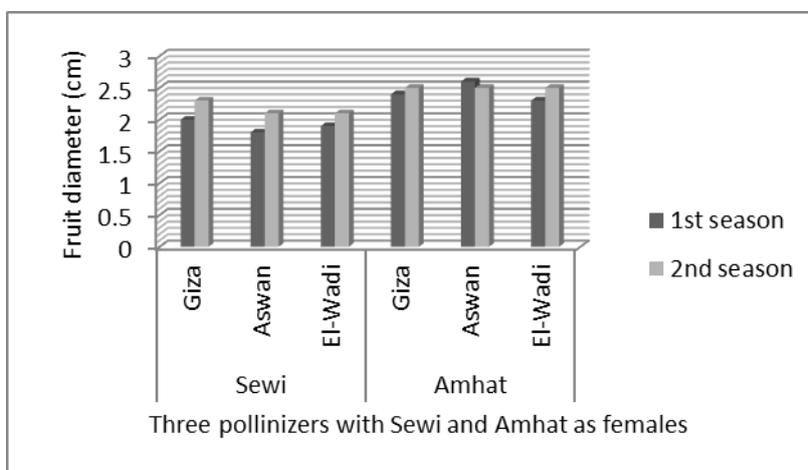


Fig (6): Effect of different pollinizer sources on fruit diameter of Sewi and Amhat cultivars in the two seasons.

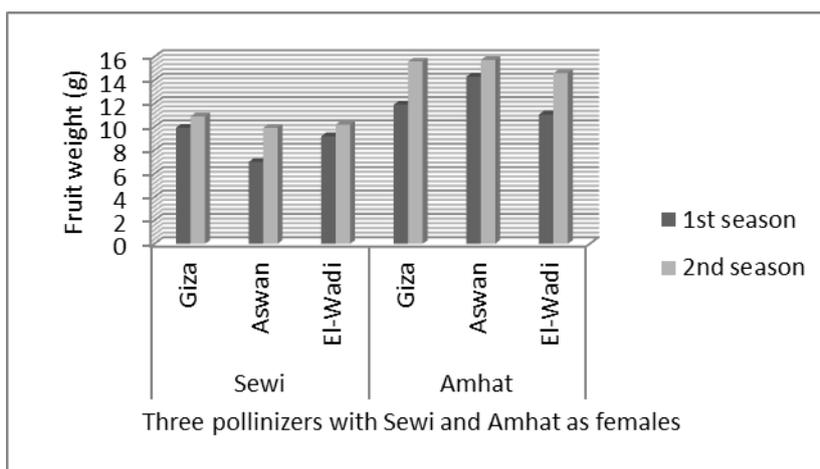


Fig (7): Effect of different pollinizer sources on fruit weight of Sewi and Amhat cultivars in the two seasons.

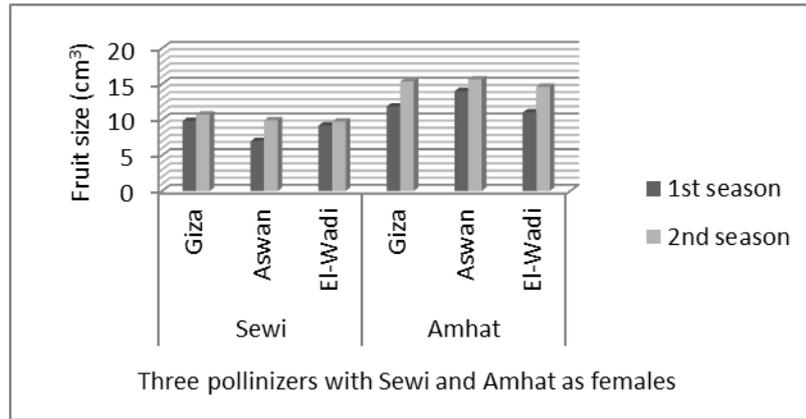


Fig (8): Effect of different pollinizer sources on fruit size of Sewi and Amhat cultivars in the two seasons.

The obtained results respecting fruit physical properties are in agreement with those obtained by El-Makhtoun and Abdel-Kader (1990); El-Kosary (1993); El-Salhy *et al.*, (1997); Soliman (1999); Mustafa *et al.*, (2014); Samy *et al.*, (2015) and Merwad *et al.*, (2015) since they reported that the different type of pollen sources used in pollination had different effects on fruit physical quality of date palm.

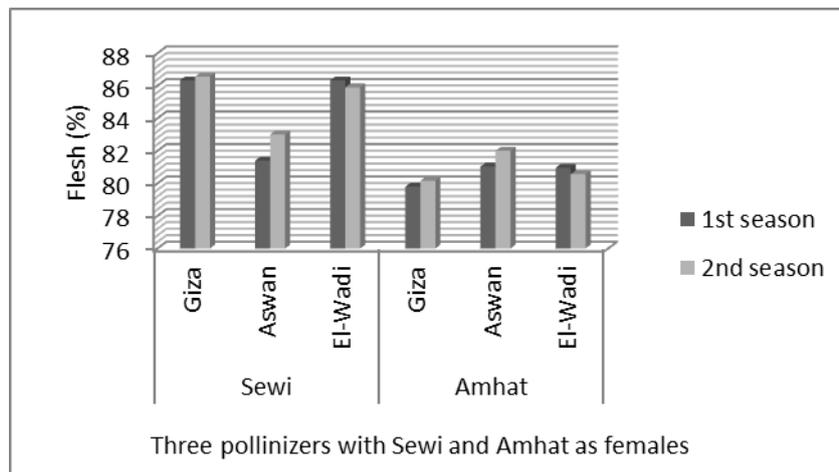


Fig (9): Effect of different pollinizer sources on flesh percentage of Sewi and Amhat cultivars in the two seasons.

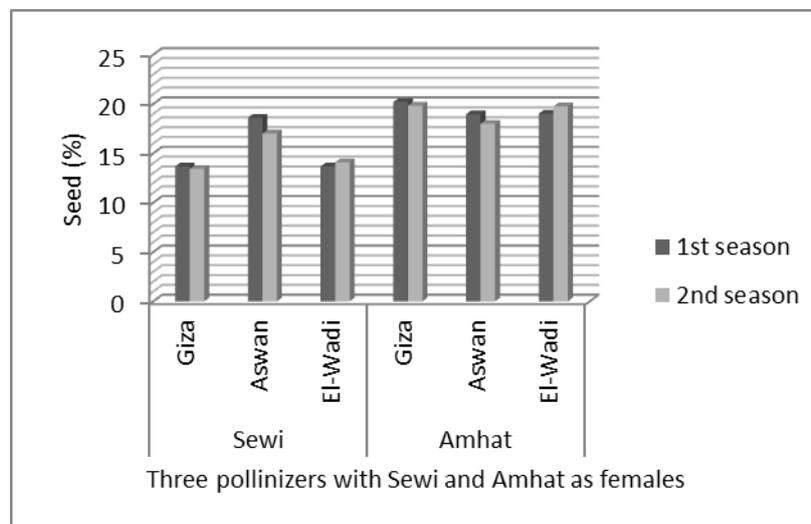


Fig (10): Effect of different pollinizer sources on seed percentage of Sewi and Amhat cultivars in the two seasons.

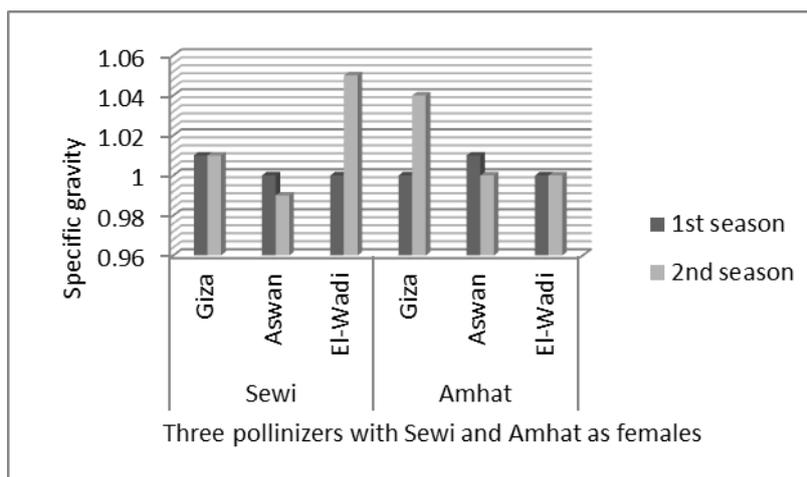


Fig (11): Effect of different pollinizer sources on specific gravity of Sewi and Amhat Cultivars in the two seasons.

### Chemical properties

Results in Figs (12, 13 and 14) show the effect of pollinizer source on chemical properties of both Sewi and Amhat date palm fruits. In this concern, Giza pollen grains recorded the highest TSS value when pollinated Sewi date palms, although, the same pollinizer gave the lowest TSS value when used as a pollinizer for Amhat palms. In contrast, while Aswan pollen grains recorded the highest TSS percentage with Amhat palms, it showed the lowest value with Sewi date palms.

As for acidity percentage, it's clear that Aswan pollinizer recorded the highest acidity value with Sewi palms, while the other pollinizers gave lower values. On the other side, while Giza pollinizer gave the lowest acidity percentage when pollinated Amhat palms, the other pollinizers recorded lower values. This was true in both studied seasons.

Regarding total sugars in dates of both studied cultivars, results of Sewi date palms show that Giza pollinizer recorded the highest value of total sugars in the first season, while in the second one the same pollinizer (Giza) and El-Wadi recorded the same value. As for Amhat cultivar, it's clear that Aswan pollinizer recorded the highest value in the first season, while in the second one no differences were detected between the three pollinizers on the total sugars value.

Hasegawa and Smolensky (1971) reported that the effect of pollinizer source on chemical properties could be due to as 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. Also, the hydrolytic enzymes like polygalactouronase and cellulose may be involved in these biochemical changes by solubilizing the pectin and cellulose in date palm fruit. Our results are confirmed with those obtained by Mustafa *et al.* (2014); Samy *et al.* (2015) and Merwad *et al.* (2015).

### Molecular studies

RAPD-PCR technique of nine primers was used in Fig. 15 and Table 4. They revealed 55 different bands were of them 18 monomorphic bands (32.73%) and fifty eight were polymorphic bands (67.27% polymorphism) showed that in Table (3) while, Relationships revealed high value between two cultivars Sewi Cv. (female) and New Valley were 0.454 and lowest value between Sewi Cv. (female) and Aswan (male) 0.135 showed that, in Proximity matrix Table (5). Moreover, the relationships between Sewi Cv. (female) and Giza (male) were 0.195. On the other hand, the tree revealed high relationship between Amhat Cv. (female) and Giza (male) was a value 1 While, the low relationship between Amhat Cv. (female) and New Valley (male) was the value 0.250 Moreover, the ratio was 0.979 between Amhat Cv. (female) and Aswan (male). The relationships divided to two clusters the first one was included two sub clusters the first included Amhat and

Giza while, the second sub cluster include only Aswan. On the other hand, the second cluster included Sewi cv. and New Valley (El-Wadi). These results are shown in Fig. 16.

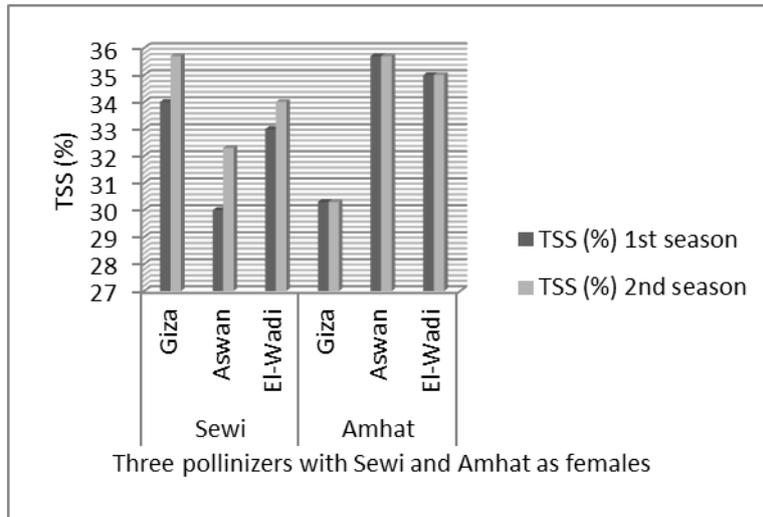


Fig (12): Effect of different pollinizer sources on TSS percentage of Sewi and Amhat cultivars in the two seasons.

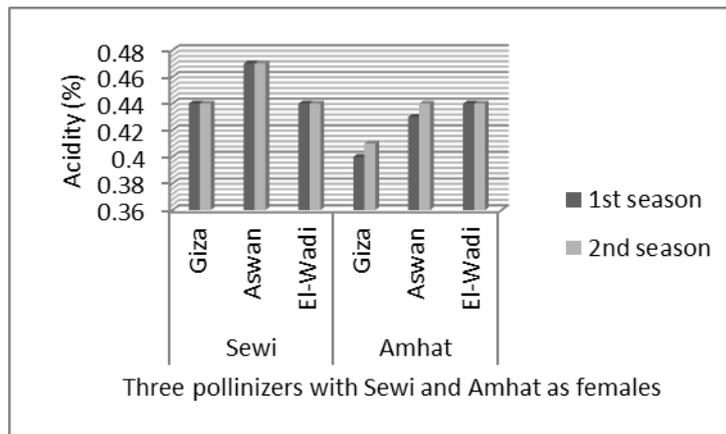


Fig (13): Effect of different pollinizer sources on fruit acidity of Sewi and Amhat cultivars in the two seasons.

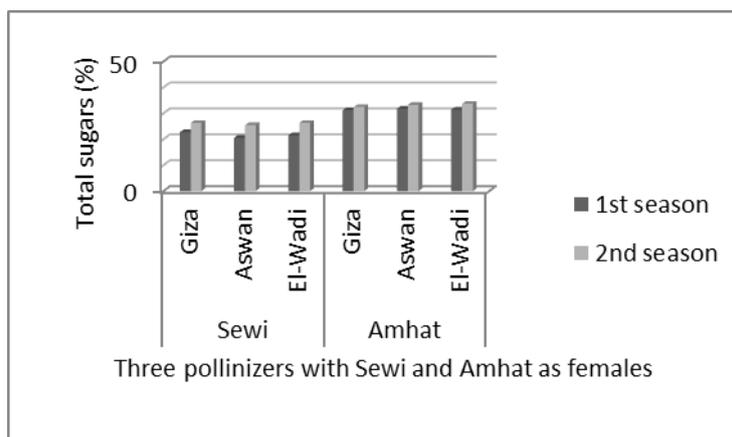


Fig (14): Effect of different pollinizer sources on fruit total sugars of Sewi and Amhat cultivars in the two seasons.

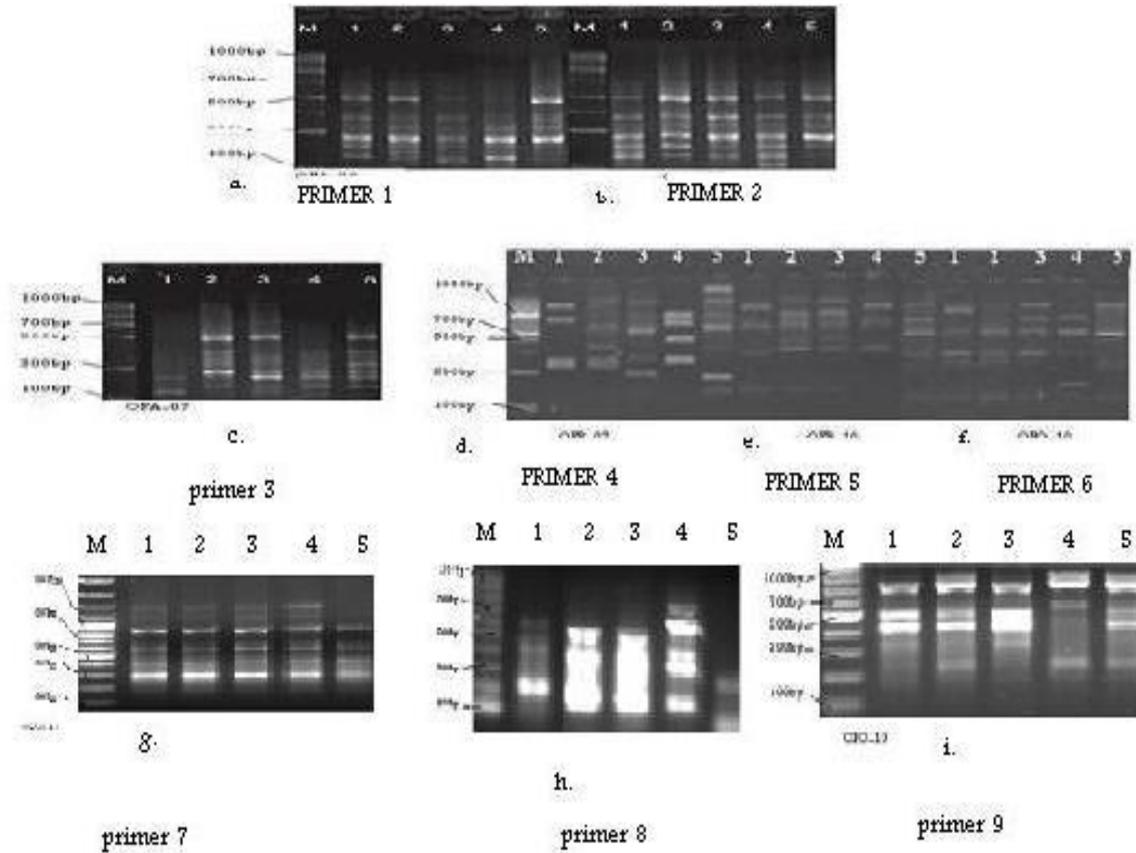


Fig.15: DNA amplification fragments with RAPD-PCR technique for five date palm genotypes two females {Sewi Cv.(1) and Amhat Cv. (2)}and three males {Giza (3), Aswan (4) and New Valley (5)} using(a) OPA-02,(b) OPA-04, (c) OPA-07, (d) OPB-07, (e) OPB-10, (f) OPO-13,(h) OPB-14 and (i) OPO-19 primers, M= 100bp ladder.

Table (4): Total number, monomorphic, polymorphic of alleles and percentage of polymorphism as revealed using nine RAPD primers of five date palms.

Primer	Total alleles	Monomorphic alleles	Polymorphic alleles	% polymorphism
OPA-02	6	3	3	50%
OPA-04	5	2	3	60%
OPA-07	7	3	4	57.14%
OPB-07	8	1	7	87.5%
OPB-10	4	1	3	75%
OPO-10	7	2	5	71.43%
OPO-13	6	1	5	83.33%
OPO-14	8	4	4	50%
OPO-19	4	1	3	75%
Total bands	55(100%)	18(32.73%)	37(67.27%)	(67.27%)

Table (5): Proximity matrix among five Egyptian date palm genotypes.

Case	Sewi	Amhat	Giza	Aswan	New Valley
Sewi	1.000				
Amhat	.000	1.000			
Giza	.195	1.000	1.000		
Aswan	.135	.979	.596	1.000	
New Valley (El-Wadi)	.454	.250	.149	.244	1.000

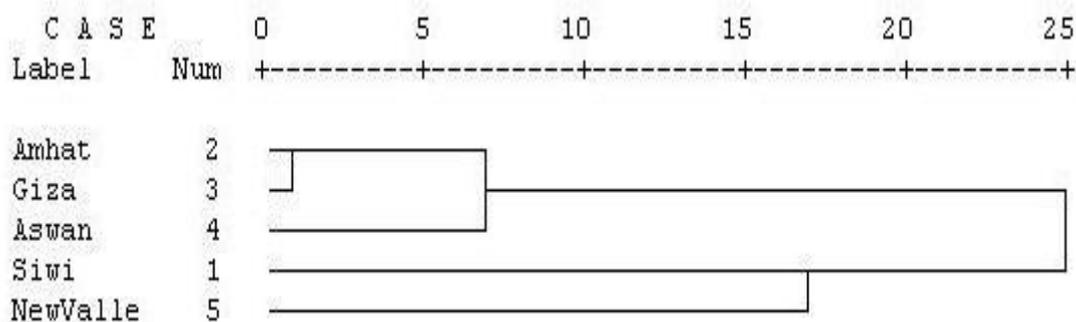


Fig.16: Dendrogram using average linkage (between groups) for five Egyptian date palm genotypes (1 Sewi Cv., 2 Amhat Cv., 3 Giza, 4 Aswan and 5 New Valley).

**RAPD Analysis:**

Nine RAPD primers were used to discriminate among five date palm accessions. They produced multiple band profiles with a number of bands ranging from 4 to 8 bands (Table 4 and Fig. 15). Sedra *et al.* (1998) showed that 19 out of 123 prescreened random primers revealed polymorphic and reproducible results. However, Hussein *et al.* (2002) stated that the presence of intra-varietal polymorphism among five Egyptian date palm cultivars from the Delta area.

In the present study, the total number of fragments produced by the nine primers was 55 with an average of 6.11 fragments/primer (Table 4). While, the number of polymorphic fragments ranged from 4 to 8. A maximum number of 8 bands were amplified with OPB-07 and OP-O14 primers, while the minimum number of fragments (4) was amplified with primer OPB-10 and OPO-19. The highest polymorphism ratio (87.5%) was detected using OPB-07 primer.

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 and comparing between two females and three males. However, Hussein *et al.* (2002) pointed out the presence of intra-varietal polymorphism among five Egyptian date palm cultivars from the Delta region.

To determine the genetic relationships among the 14 accessions, the scored data (1 for presence and 0 for absence) resulting from the 16 primer combinations were used to compute the similarity matrices through Dice coefficient. The genetic similarity estimates ranged from 80.4% to 99%. This confirmed the high level of genetic similarity among the studied cultivars.

The highest genetic similarity (99%) was between Sewi/El-Kharga and Sewi/El-Dakhla. While, the lowest genetic similarity value (80.4%) was detected between Malkaby/Aakab and Sewi/Tamazough (Adawy *et al.*, 2005) since they were estimated the genetic relationships among the six cultivars using the common bands between the different accessions representing each cultivar, the genetic similarity estimates ranged from 96.1% to 99.9%. The highest genetic similarity (99.9%) was between Sakkoty and Bertmoda, while the lowest genetic similarity (96.1%) was detected between Fraihy and Gandila. While, in this study the similarity values were 13.5% to 45.4% between Sewi/ Aswan and New Valley (El-Wadi). On the other hand, the values ranged from 25% to 97.9 between Amhat/ New Valley and Aswan. In addition, the efficiency of AFLP markers in the date palm genome analysis has been compared with RAPD and ISSR markers used in a previous study (Hussein *et al.*, 2005).

The results were in agreement with Hamza *et al.* (2012) that revealed confirmed with a large genotypes and primers. Some results of this study showed clear and high yields of amplified DNA fragments. Finally, RAPD method appears to be a powerful technique for analysis of genetic diversity of date palm germplasm and many oligonucleotide primers are now known for future genetic diversity analysis of date palm and for identification of variable varieties.

## CONCLUSION

From the abovementioned results, it could be concluded that the effect of the three pollen grains sources selected from male pollinizers located at Giza, Aswan and El-Wadi (New Valley) governorates, Egypt and the relation between them and two female date palm cultivars (Sewi as semi dry date and Amhat as moist date), were studied as horticultural parameters and at molecular level, since RAPD technique was used to compare among the five date palm genotypes. The obtained results concerning fruit set, bunch weight, yield per palm and both physical and chemical fruit properties showed that Giza and Aswan pollinizers were the most suitable for pollinizing Sewi and Amhat female, respectively. At the molecular level, 9 primers were used for RAPD analysis. These primers gave a total of 55 different alleles for all genotypes. The most Polymorphic Information Content (PIC) value and polymorphism percentage that detected by OPB-07 and OPO-14 primers showed the highest score 8 bands. RAPD markers succeeded in detecting relationships among these genotypes and showed the correlation between yield traits and molecular traits.

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