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Productivity, fruit properties and genetic diversity by molecular markers of four Egyptian female date palm cultivars and five different pollinizers

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Four female date palm cultivars namely Sewi, Amhat, Samany and Zaghloul were pollinized using pollen grains collected from five different locations (Nobaria, Rasheed, New Valley, Aswan and Giza) to study the effect of different pollinizer sources on productivity and fruit properties, also to determine the phylogenetic tree and the relationship between each pollinizer and the four female cultivars by using RAPD molecular markers. The obtained results showed that bunch weight character of each female cultivar was affected by the different sources of pollen grains. In the concern, Rasheed pollinizer followed by Nobaria recorded maximum bunch weight for Sewi female cultivar, while Nobaria pollinizer followed by Giza pollinizer gave the heaviest bunch for Amhat, on the other hand, Giza pollinizer followed by Nobaria was the best for Samany date palm; finally Rasheed pollen grain source followed by Giza gave the heaviest bunch of Zaghloul female date palm. RAPD markers detected the phylogenetic tree for the four date palms and five pollinizers; the polymorphism percentage was 76.08%, also total alleles using six primers were 46 and they ranged between170 to 2005bp. The proximity matrix between Nobaria pollinizer and Samany was 9.5% revealed the lowest relationship, while the highest relationships were 98.5%, and 81% between Giza pollinizer with Sewi and Zaghloul, respectively.

Keywords: Date palm, pollinizer, Fruit properties, RAPD- PCR markers and Genetic diversity.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) has long been one of the most important fruit crops in the arid regions also one of the oldest known fruit crops and has been cultivated in North Africa and the Middle East for at least 5000 years (Zohary and Hopf, 2000). Date culture had apparently spread into Egypt by the middle of the second millennium BCE. The spread of date cultivation later accompanied the expansion of Islam and reached southern Spain and Pakistan. The Spanish were the first to introduce date palms outside the Arabian Peninsula, North Africa, the Middle East and South Asia, carrying them to America (Nixon, 1934).

Dates can grow in very hot and dry climates, and are relatively tolerant for salty and alkaline soils. Date palms require a long, intensely hot summer with little rain and very low humidity during the period from pollination to harvest, but with abundant underground water near the surface of irrigation.

Dates are widely grown in the arid regions between 15ºN and 35ºN, from Morocco in the west to India in the east (Zaid and de Wet, 2002a).

Date palm is a dioeciously plant since the male and female flowers are borne by separate

palms. 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).

Recently, **(**Moghaieb et al., 2010) could identify an unknown date palm cultivar grown at Matrouh Governorate (Egypt) compared with the other known cultivars grown in the same area based on RAPD and ISSR analyses.

The main goal of this study was to investigate the effect of different pollinizer sources on productivity and fruit properties of four local female date palm cultivars, also to determine the phylogenetic tree and the relationship between each pollinizer and the four female cultivars using RAPD molecular markers.

MATERIALS AND METHODS

Horticultural study:

This study was carried out during two successive seasons on 20 years old four female date palm cultivars namely Sewi, Amhat, Samany and Zaghloul 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 grain source were selected from five different locations namely Nobaria, Rasheed, New Valley, Aswan and Giza. Pollination was achieved by inserting male strands into each of the female bunch. Five bunches on each female palm were received pollens from the five 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 following characters were determined for each female cultivar under this investigation.

- Bunch weight (kg)
- Fruit size (cm^3)
- Fruit length (cm)
- Fruit diameter (cm)
- Fruit shape index
- Total sugars content in the fruit (%) as the method described in A.O.A.C (A.O.A.C, 1995).

The experiment was arranged in a complete randomized design including three replications for each treatment.

Statistical analysis

The obtained data of the five pollinizers were subjected to analysis of variance individually for each female date palm and the method of Duncan's was used to differentiate means (Duncan 1955).

Molecular Studies

Extraction of DNA: The genomic DNA was extracted from leaf of nine date palm genotypes Table (1) according to the protocol of Biospin plant genomic DNA extraction Kit (Bio basic).

Table (1) Code name for nine genotypes from Egyptian date palms

No.	Name and Source	Pollinizers and females
1	Giza	Pollinizer
2	Aswan	Pollinizer
3	New Valley (El Wadi)	Pollinizer
4	Nobaria	Pollinizer
5	Rasheed	Pollinizer
6	Sewi	Female
7	Amhat	Female
8	Zaghloul	Female
9	Samany	Female

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 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-trans illuminator 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 rice according to gel analyzer protocol.

RAPD analysis:

A set of six random 10-mer primers, was used in the detection of polymorphism among nine genotypes of date palm. These primers were synthesized at RAPD-PCR and carried out according to the procedure given by (Williams et al, 1990) with minor modifications

RESULTS

a. Horticultural study:

The six agronomic characters which were determined for the four female cultivars were presented in the first six Figures (1-6) as the following Fig (1): Bunch weight (kg), Fig (2): Fruit size (cm^3), Fig (3): Fruit length (cm), Fig (4): Fruit diameter (cm), Fig (5): Fruit shape index and Fig (6): Total sugars content in the fruit (%).

In Fig (1) we can observe the effect of the five pollen grain sources on bunch weight of the commercial female date palms (Sewi, Amhat, Samany and Zaghloul). In this concern, it's clear that the variation of pollen grain sources showed a varied effect on bunch weight of each female cultivar. However, Sewi date palm gave the highest bunch weight due to pollination with Rasheed pollinizer followed by Nobaria. As for Amhat, it's clear that Nobaria pollinizer followed by Giza recorded heavier bunches. On the other hand, for Samany cultivar, Giza followed by Nobaria pollen grains gave higher value of bunch weight. Regarding Zaghloul cultivar, Rasheed pollinizer gave the maximum bunch weight followed by Giza pollinizer; this was true in both studied seasons.

Fig (2) reveals that different pollen grain sources affected the fruit size of female date palms. In this respect, Rasheed pollinizer gave the biggest fruit size followed by Nobaria in the first season and Giza in the second season when pollinized Sewi female. While Rasheed gave the highest value of fruit size followed by Nobaria pollinizer when used as pollinizer for Amhat cultivar. As for Samany female date palm, the obtained results show that both Nobaria and Giza gave more or less the same high value of fruit size comparing with the other pollinizer sources. Regarding Zaghloul female palm, Rasheed showed the highest fruit size followed closely by Giza pollinizer, the above results were observed in the two studied seasons for Amhat, Samany and Zaghloul cultivars.

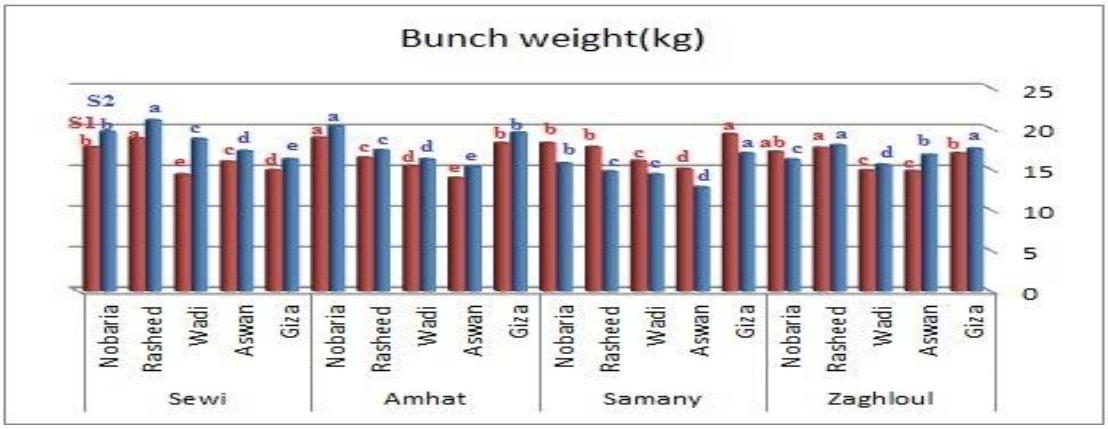

Fig (1): Bunch weight of female date palm cultivars as affected by different pollen grain sources in two seasons.

Means having the same letter(s) within a column are not significantly different at 5% level.

S1: First season S2: Second season

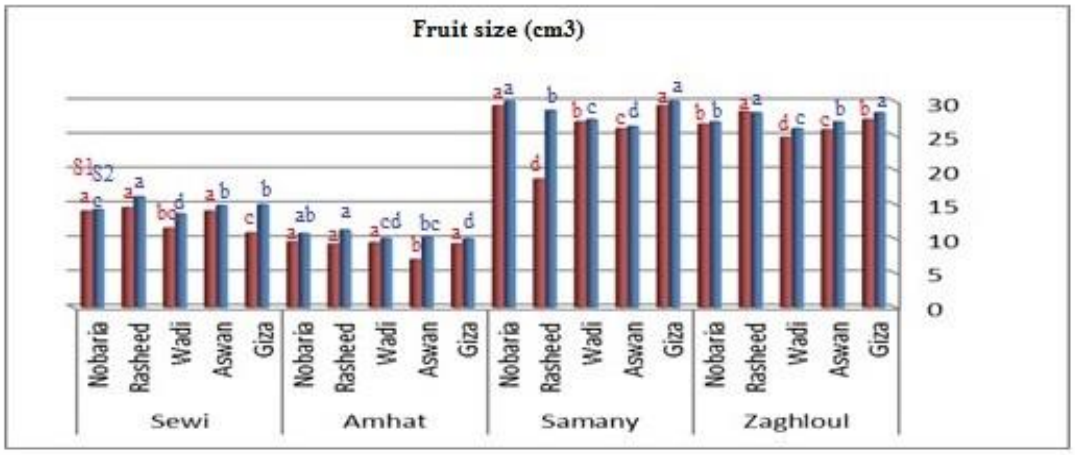

Fig (2): Fruit size of female date palm cultivars as affected by different pollen grain sources in two seasons.

*Means having the same letter(s) within a column are not significantly different at 5% level.

S1: First season S2: Second season

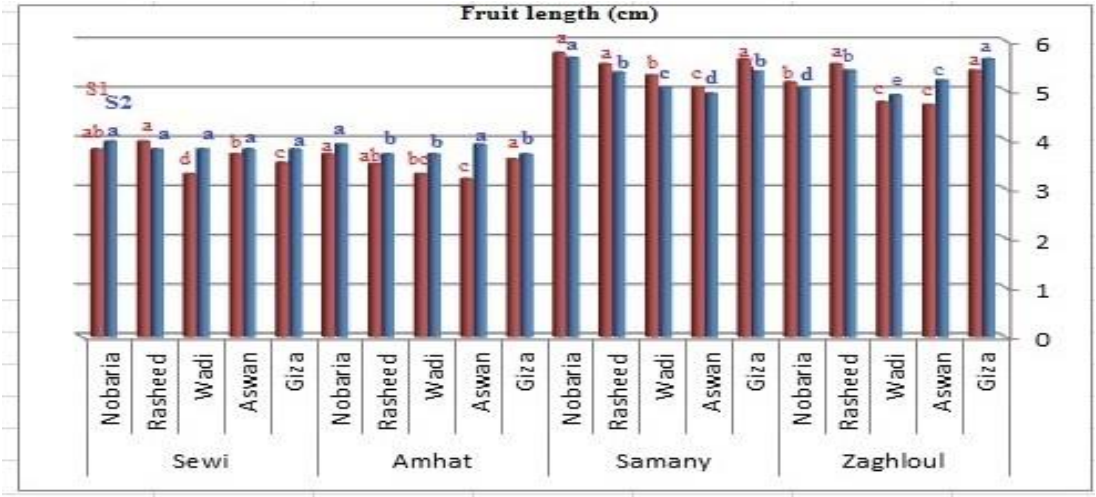

Fig (3): Fruit length of female date palm cultivars as affected by different pollen grain sources in two seasons.

Means having the same letter(s) within a column are not significantly different at 5% level.

S1: First season S2: Second season

Fig (3) showed that the fruit length was affected by different pollen grain sources. Generally, it's observed that Nobaria pollinizer was the most effective one for Amhat and Samany cultivars in the first season of the study, also the same pollinizer recorded the tallest fruit for Sewi, Amhat and Samany cultivars in the second season. As for Zaghloul female, Rasheed pollen grains followed by Giza pollinizer recorded higher values for fruit length in the first season while the opposite was in the second seasons.

On the other hand, Fig (4) clears that the fruit diameter was affected by different pollinizer sources. In general, similar trend for that obtained with fruit length, Nobaria pollinizer was the most effective one especially in the second season for Sewi, Amhat and Samany cultivars, while Rasheed gave the highest value of fruit diameter when pollinated Zaghloul female palms in both studied seasons.

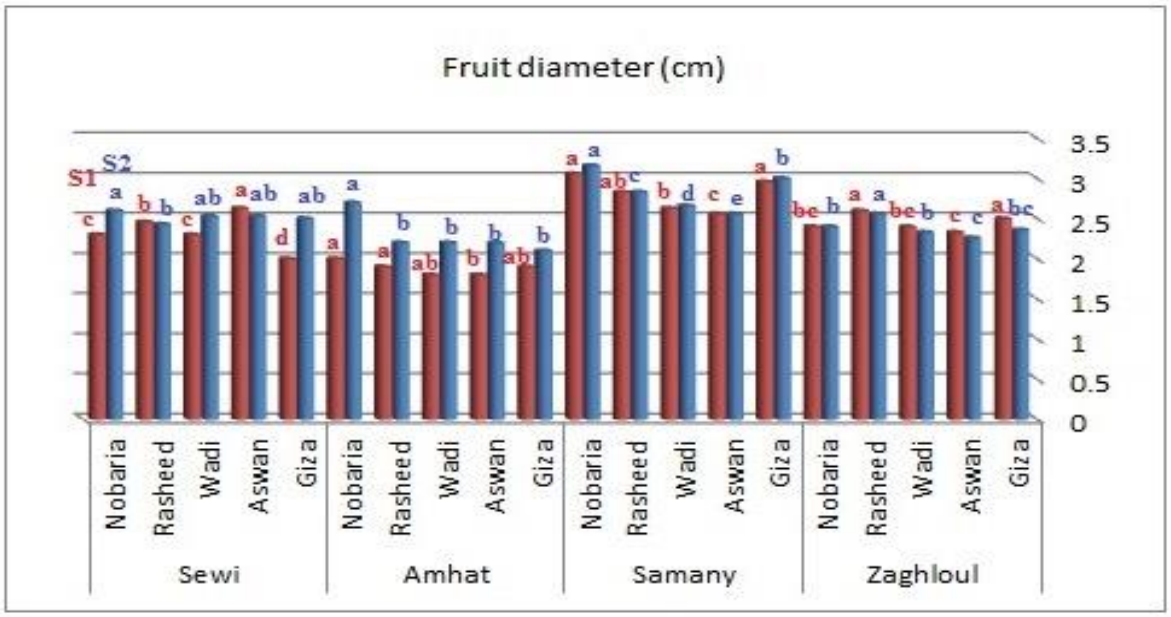

Fig (4): Fruit diameter of female date palm cultivars as affected by different pollen grain sources in two seasons.

Means having the same letter(s) within a column are not significantly different at 5% level.

S1: First season S2: Second season

Fig (5) shows the effect of different pollinizer sources on fruit shape index of the four female date palms. Sewi cultivar was affected positively by Nobaria pollinizer, since it gave the highest value of shape index in the first season followed closely by Rasheed, while in the second season; there were no differences among the pollinizers. As for Amhat, in the first season, Giza followed by the other pollinizer sources except Aswan gave high values of shape index, while in the second season Giza and Aswan gave higher and similar values. Concerning Samany cultivar, El Wadi pollinizer followed closely by Aswan recorded higher values in the first season, while in the second one, Aswan pollinizer followed by Rasheed and El Wadi gave higher shape index of Samany cultivar. Regarding Zaghloul female palms, it's clear that Giza pollen grains source was the superior comparing with the other pollen grain sources especially in the second seasons.

Fig (6) shows the effect of the five pollen grain sources on total sugars content of the four female cultivars. In this respect, the five pollinizers show more or less than the same trend of total sugars content in Sewi date palm fruits. This was true in the two seasons. While as for Amhat cultivar, it's observed that Nobaria followed closely by Giza pollinizers gave higher content of total sugars in both seasons of the study. Regarding Samany, it's clear that Giza pollinizer followed closely by Nobaria one recorded higher values of total sugars in both seasons. Concerning Zaghloul cultivar, the obtained results showed that Rasheed was the superior for recording the highest sugars content in the fruits also in the two studied seasons.

The obtained results concerning the effect of different pollen grain sources on bunch weight, fruit size, fruit length, fruit diameter, fruit shape index and total sugars are in agreement with those recorded by (Mustafa et al., 2014), (Merwad et al., 2015), (Samy et al. 2015) and (Mostafa et al., 2016), since they found that the differences between pollinizer source had a great effect on bunch weight and both weight, size, length, diameter and shape index of date palm fruits, also the total sugars content in the fruits.

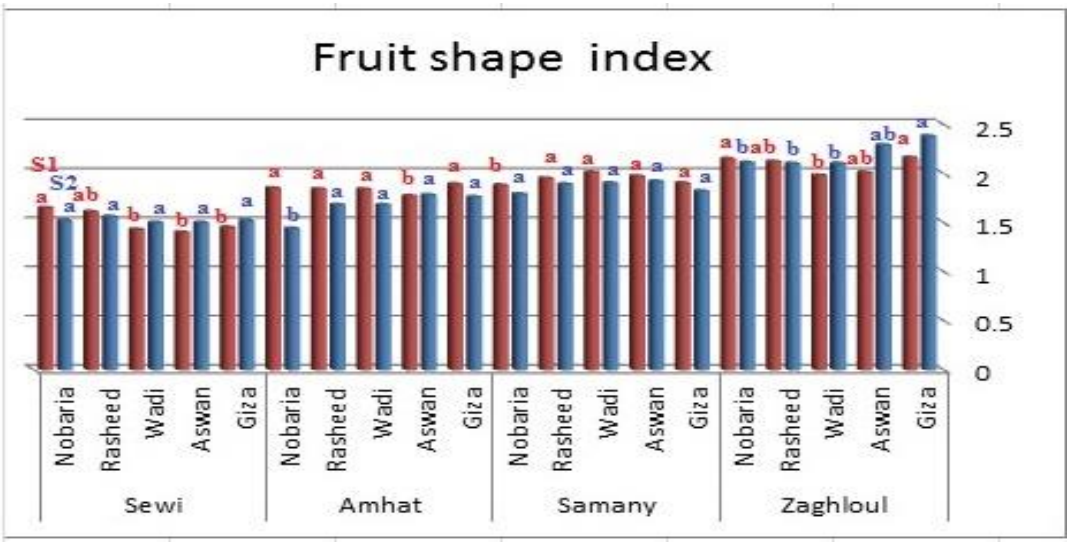

Fig (5): Fruit shape index of female date palm cultivars as affected by different pollen grain sources in two seasons.

Means having the same letter(s) within a column are not significantly different at 5% level.

S1: First season S2: Second season

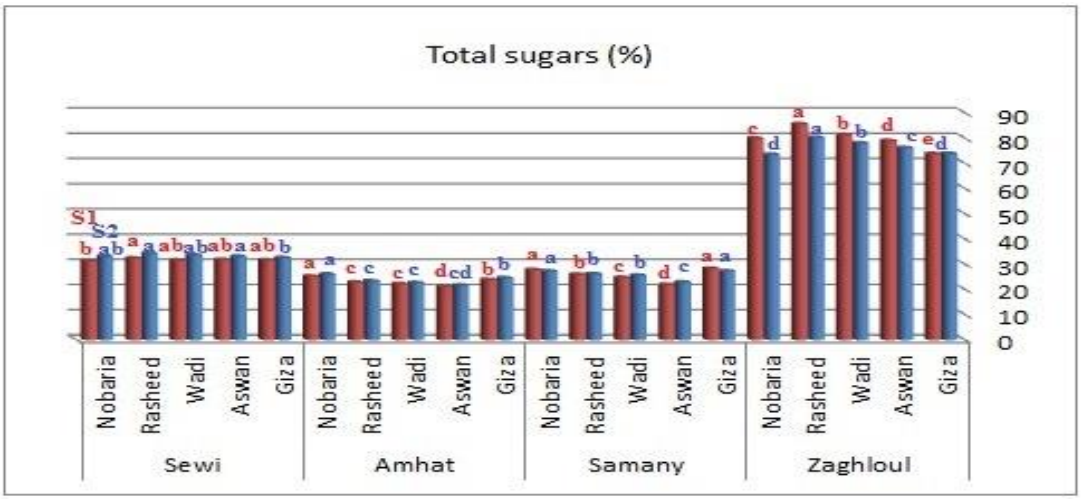

Fig (6): Total sugars content in the fruits of female date palm cultivars as affected by different pollen grain sources in two seasons.

Means having the same letter(s) within a column are not significantly different at 5% level.

S1: First season S2: Second season

b. Molecular Studies based on RAPD investigation:

The results in Table (2) showed that recorded six primers (Primers code and sequence) have given a total number of 46 alleles; 11 of them were monomorphic and 35 polymorphic alleles with polymorphism percentage of 76.08%, and the molecular weight ranged between 170 to 2005bp, these results were detected in nine genotypes of Egyptian date palm which have been studied using RAPD-PCR technique for detected variability Fig. (7).

On the primers levels we notes that: Primer OP-A02 revealed six different alleles, four were polymorphic and two monomorphic alleles while, primer OP-A04 gave ten alleles one of them was monomorphic and nine bands were polymorphic bands with percentage 90% and the bands ranged from 2005bp to 224bp.

Moreover the third primer (OP-B07) gave seven different alleles, ranged from 970bp to 350bp with 71.43% polymorphism.

Primer OP-O10 revealed nine bands, three monomorphic and six polymorphic alleles with ratio 66.67% polymorphism and these bands ranged between1765bp to 170bp.

Also, OP-O14 and OP-O19 primers were revealed six and eight different bands, with polymorphism percentage 50% and 100% respectively. These results listed in Table (2) aide for detected phylogenetic tree in Fig. (8).

About the similarity and dissimilarity between the five pollinizers and the four female Egyptian date palm cultivars we can observe that Giza pollinizer was genetically closer with Sewi cv. 98.5%, and 81% with Zaghloul cv. but the lowest value between Giza pollinizer was with Amhat cv. 42.9%. While in generally, Nobaria pollinizer revealed the lowest value with Samany cv. (9.5%). On the other hand the middle values of similarity was observed between Sewi female and New Valley and Rasheed pollinizers, also between Amhat female and Nobaria pollinizer by ratio (52.4%), (57.1%) and (52.4%) respectively, Table (3).

The dendrogram between five pollinizers and four females from Egyptian date palm divided to two clusters, the first one cluster included on Rasheed, Amhat, Aswan, Nobaria and Samany, while the second cluster included Sewi, Zaghloul, New Valley and Giza (Fig. 8).

Research in molecular markers is taking the lead in this context (Aitchitt et al., 1995, Corniquel & Mercier 1997, Lewis et al., 2000). While, these methods are at a preliminary stage with regard to date-palm research, and they have been tested on a limited number of cultivars (Benabdellah et al., 2000 and Trifi et al., 2000). Despite using RAPD markers for a large number of cultivars were evaluated by (Sedra et al., 1998) and (Ahmed et al., 2016).

Elshibli and Korpelainen (2011) showed a complex genetic relationship between some of the tested populations, especially when isolated by distance was considered and they also found that a large amount of diversity exists among date palm germplasm; the findings suggest that, there is a role of biological nature to phylogenetic tree by distance and environmental effects. These results agree with recorded results in our studies. Finally, this study has provided an efficient procedure that can be used routinely to identify palm cultivars successfully used in modern breeding.

Table (2): Total alleles, monomorphic alleles, polymorphic alleles, and polymorphism of the nine date palm types for six primers using RAPD-PCR techniques.

No	Primer code	Sequence(5`→3`)	Range of alleles	Total alleles	Monomorphic alleles	Polymorphic alleles	% polymorphism
a.	OP-A02	CAGGCCCTTC	801:242	6	2	4	66.67
b.	OP-A04	AATCGGGCTG	2005:224	10	1	9	90
c.	OP-B07	GGTGACGCAG	970:350	7	2	5	71.43
d.	OP-O10	TCAGAGCGCC	1765:170	9	3	6	66.67
e.	OP-O14	AGCATGGCTC	1059:278	6	3	3	50
f.	OP-O19	CAATCGCCGT	958:230	8	0	8	100
Total			2005:170	46	11	35	76.08%

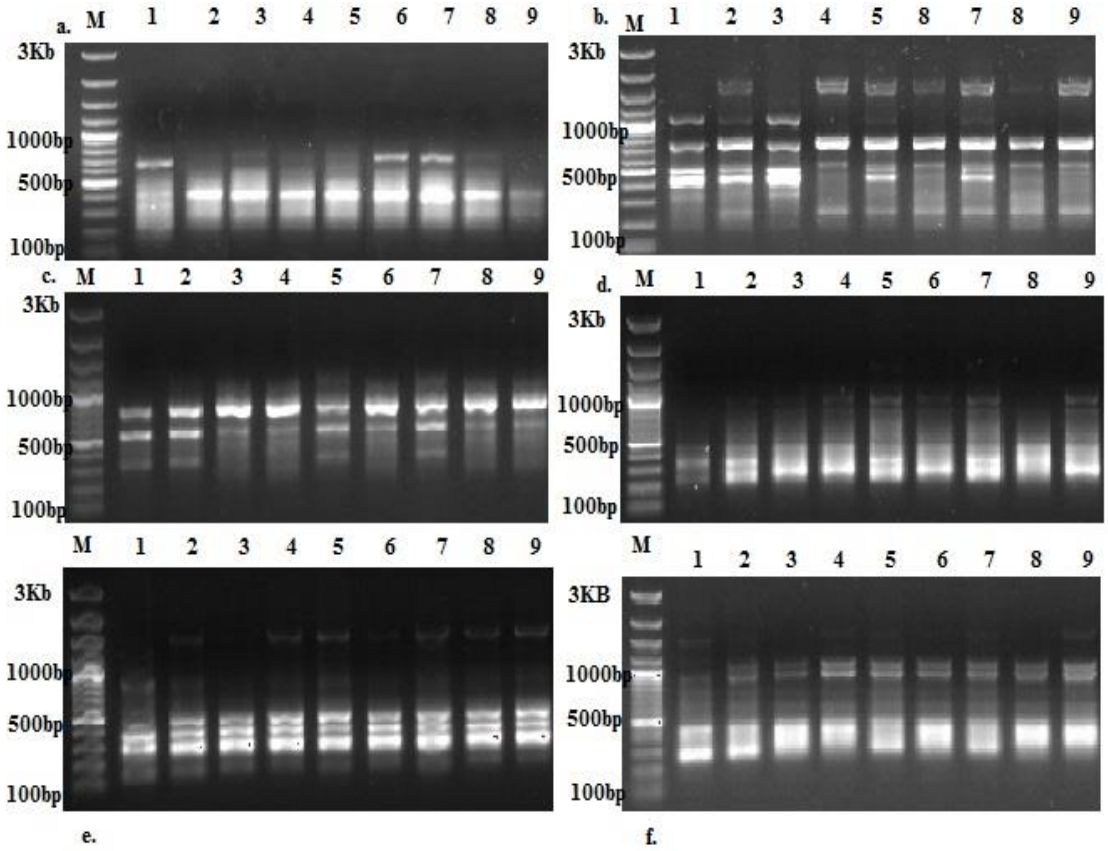

Fig.(7) : Banding patterns of nine date palm genotypes using six RAPD primers, five pollinizers(1 Giza; 2 Aswan; 3 New Valley; 4 Nobaria and 5 Rasheed) and four females(6 Sewi; 7Am hat; 8 Zaghloul and 9 Samany); M= DNA ladder 100bp.

Table (3): Similarity and dissimilarity between five pollinizers and four female Egyptian date palm cultivars.

Case	Matrix File Input								
	Giza	Aswan	NewValley	Nobaria	Rasheed	Sewi	Amhat	Zaghloul	Samany
Giza	1.000								
Aswan	.619	1.000							
NewValley	.667	.429	1.000						
Nobaria	.952	.333	.571	1.000					
Rasheed	.619	.195	.619	.429	1.000				
Sewi	.985	.286	.524	.143	.571	1.000			
Amhat	.429	.190	.714	.524	.122	.571	1.000		
Zaghloul	.810	.381	.429	.238	.762	.190	.762	1.000	
Samany	.667	.143	.762	.095	.143	.429	.143	.524	1.000

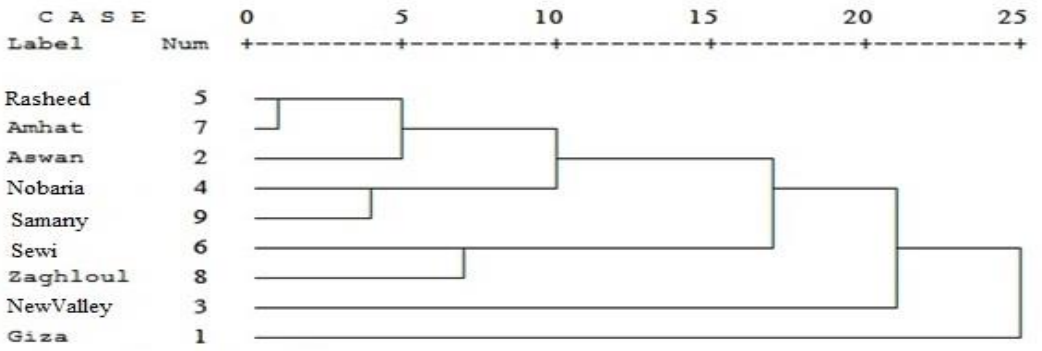

Figure (8): Dendrogram of five pollinizers and four female Egyptian date palm cultivars.

CONCLUSION

From the abovementioned results, it's clear that using different pollen grain sources (Nobaria, Rasheed, New Valley, Aswan and Giza) had a varied effects on productivity (bunch weight) and fruit properties (size, length, diameter and shape index) of the fruits and total sugar contents of the four female date palm cultivars under investigation (Sewi, Amhat, Samany and Zaghloul). RAPD markers detected the phylogenetic tree for the four date palms and five pollinizers; the polymorphism percentage was 76.08%. Total alleles using six primers were 46 and they ranged between 170bp to 2005bp. The proximity matrix between Nobaria pollinizer and Samany was 9.5% revealed the lowest relationship, while the highest relationship were 98.5and 81.0% between Giza pollinizer with Sewi and Zaghloul, respectively.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The team work appreciate the role of each one helped to produce this work in the final form.

AUTHOR CONTRIBUTIONS

M.E.A.M. designed and helped in implementation, written, also the final version of the horticultural part of the study. M.M.S.S. helped in determination of the physical and chemical fruit properties, written also the final version of the horticultural part. N.E.A. helped in implementation of the field experiment and determination of the physical and chemical fruit properties, also the data statistical analysis of the horticultural part. On the other hand A.A.A.H. and A.M.F.A. performed the practical part, analysis data of DNA-RAPD also written the final version of the molecular part.

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