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Research Article

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Selection, Evaluation and DNA Fingerprinting of Some Superior Female Seeded Date Palms

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Abstract The aim of this experiment was to evaluate two seeded semi-dry female date palms grown under El Wadi El Gedeed governorate comparing with the commercial cultivar namely Sewi. The obtained results reveal that, Sewi date palm recorded the highest value of vegetative parameters such as height and girth of trunk and leaf length. Also, the same cultivar produced the highest value of fruit chemical properties such as TSS%, non-reducing sugars % and total sugars content in both studied seasons. The highest bunch weight (20.3 and 20.0 kg) was recorded from the selected date palm El Wadi (1). The highest yield/palm was recorded with seeded date palm El Wadi (2) since it recorded 200 and 203.3 kg in the first and second seasons, respectively. From the other site, fingerprinting analysis using RAPD has been performed to investigate the phylogenetic relationships among the two seeded female genotypes date-palms grown at El Wadi El Gedeed region with Sewi cultivar. The RAPD-PCR with nine primes detected a total number of 57 fragments, 38 bands of them were polymorphic with a ratio of 33.33%. The genetic variability and relationships among the three accessions of the Egyptian date palms were estimated in the term of similarity using Dice coefficient. The phylogenetic tree revealed high similarity value between El Wadi (2) and Sewi (0.826) while, revealed little value between El Wadi (1) and Sewi cultivar (0.556).

Keywords date palm, yield, fruit quality, female seeded, DNA fingerprinting

Introduction

Date palm is one of the ancient domestic fruits in the Middle East countries and their fruits play an important role in the nutrition patterns of many people as well as a strategic crop in food and biochemical industries [1]. Date palm cultivars are divided into three main groups according to their fruit moisture content, *i.e.* soft: more than 30% humidity, semi-dry: from 20-30% and dry cultivars: less than 20%.

Some of date palm orchards under El Wadi El Gedeed governorate were planted with seeded palms. For successful improvement program of date palms, it could evaluate the unknown seeded date palms that already grown in a big population [2].

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 [3]. RAPD markers have been used for germplasm characterization in date palm [4-7]. The aim of this study is to select the superior palms from these seeded plantations that achieved by studying the morphological characteristics of palms and their fruit properties as a start point for developing date palm orchards and to obtain the promising date palms for producing semi-dry dates as an initial date palm cultivars under El Wadi El Gedeed governorate and at molecular level used RAPD tools for detect DNA fingerprinting.

Materials and Methods

The present investigation was conducted during two successive seasons (2015 and 2016) to evaluate two semidry female seeded date palms comparing with Sewi cultivar as commercial semi-dry one. The selected seeded date palms were 15-20 years old.

Palms were grown in orchard located in Shark EL-Oinat conditions, El Wadi El Gedeed governorate and treated with the same horticultural practices such as irrigation, fertilization, artificial pollination and pest control.

So, this study included two seeded female date palms in comparison with Sewi cultivar arranged as follows

- 1- El Wadi (1)
- 2- El Wadi (2)
- 3- Sewi cultivar.

This experiment was arranged in a randomized completely block design with three replicates as one palm per each.

Vegetative parameters

Trunk height and girth

The palm height and girth were measured in meters at the end of October every season.

Leaf characteristics

Leaf length (cm), number of leaflet/leaf, leaflet length (cm), number of spins, and spine length (cm) were determined and recorded in the end of each season.

Each spathe was pollinated within two days after opening. Also the date of harvesting was recorded when fruits reached the ripe stage (Rutab stage).

On the other side, number of bunches was counted for each palm during the two studied seasons. At harvest time, bunch weight (kg) of each palm was recorded individually. Yield (kg/ palm) was calculated.

Fruit quality

1- Fruit physical characteristics

Samples of 30 fruits were randomly collected from each bunch to determine the fruit physical characteristics such as fruit weight (gm), fruit length and diameter (cm).

After peeling, both seed weight and flesh weight were determined and recorded.

2- Fruit chemical characteristics

Total soluble solids (TSS) were determined by hand refractometer, total acidity was determined as Malic acid, total soluble sugars, reducing and non-reducing sugars content were determined according to the methods of A.O.A.C. [8].

Statistical analysis

The data were subjected to the statistical analysis of variance and means separation were done according to Duncan [9] at 5% level.

A) Plant Material

The study included three date palm collected from different locations in Egypt. These accessions represent five date- palm genotypes (Sewi cultivar as female and two genotypes of El Wadi (1) and El Wadi (2). Samples were collected from leaves surrounding the palm meristem of 3 to 5 palms.

B) Molecular Studies

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

a) DNA extraction

DNA was extracted from three 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.*, [10].

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.

RAPD analysis

A set of nine random 10-mer primers Table1 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.* [3] with minor modifications.

No.	Sequence $(5 \rightarrow 3)$	Primer code
1	CAGGCCCTTC	OPA-02
2	AATCGGGGCTG	OPA-04
3	GAAACGGGTG	OPA-07
4	GGTGACGCAG	OPB-07
5	CTGCTGGGAC	OPB-10
6	TCAGAGCGCC	OPO-10
7	GTCAGAGTCC	OPO-13
8	AGCATGGCTC	OPO-14
9	CAATCGCCGT	OPO-19

Table 1: Code and sequences of nine RAPD primers

Results and Discussion Vegetative Characteristics Height and Girth of Trunk:

Results presented in Table (2) show that height and girth of trunk were differed in vegetative trails of various seeded date palms. In this regard, Sewi cultivar was the tallest one (11.50 and 11.7 m) in the first and second seasons. While, the seeded date palm namely El Wadi (1) was the shortest (9.33 and 9.5 m) in both seasons of the study. Referring to girth of trunk, Sewi cultivar recorded the highest value (1.90 and 1.93 m) in both seasons. Trunk girth of the seeded date palm El Wadi (1) recorded the lowest values (1.18 and 1.35 m) in the first and second seasons, respectively. These results are in harmony with the presented results by many researchers [11-14] who mentioned that there was a wide and great variation on vegetative growth of most date palm strains or cultivars.

Leaf length (m)

Results in Table (2) clear that the longest leaf length (3.10 and 15m) was obtained from Sewi cultivar in the first and second seasons of the study. The shortest leaf length (2.26 and 2.25m) was recorded from the seeded date palm namely El Wadi (2) in both seasons of the study.

Number of leaflets per leaf and leaflet length

Results presented in Table (2) indicate that the highest number of leaflets per leaf in the first season (220) was recorded from the seeded date palm namely El Wadi (1), followed by Sewi cultivar (214.67). While in the second season, the highest number of leaflets per leaf (215.3) was found in Sewi cultivar, followed by the seeded date palm El Wadi (1) since it recorded 210 leaflets. The lowest number of leaflets per leaf (208 and 200) was obtained from seeded date palm El Wadi (2) in the first and second seasons of the study.

In case of leaflets length, results show that the highest leaflets length 30.3 and 32.6cm was recorded from seeded date palm El Wadi (2) in the two seasons of the study. While the lowest leaflets length (19.50 and 20.1 cm) was found with Sewi cultivar in the first and second seasons, respectively.



These results are in agreement with the researchers [11-14] who found that, Leaflets length were ranged between 16.33 and 63.1 cm.

Number of spines per leaf and spine length:

Results in Table (2) clear that the highest number of spines per leaf (30 and 31.3) was recorded from seeded date palm namely El Wadi (2) which recorded 29 and 29.6 in both seasons of the study. Meanwhile, the lowest number of spines per leaf (23 and 26) was obtained from Sewi cultivar in the first and second seasons.

Results in Table (2) show that the highest spine length (11.5 and 10.8 cm) was found with the seeded date palm namely El Wadi (1) followed by seeded date palm El Wadi (2) since it recorded 10.6 and 10.7 cm in the first and second seasons of the study.

Meanwhile, the lowest spine length (8.8 and 9.0cm) was found by Sewi cultivar in the first and second seasons, respectively.

Such results emphasized the fact not vegetative and fruiting trails depend on cultivar. The differences between cultivars or seeded types of date palm may be due to either cytological difference between them or to the genotypes that are transmitted via seeds.

These results are in line with these obtained by [2, 13-16] who mentioned that there was a wide and great variation on vegetative growth and fruiting of most date palm strains or cultivars.

Gedeed governorate									
Cultivars	Trunk length (m)	Trunk girth (m)	Leaf length (m)	No. of leaflets	Leaflet length (cm)	No. of spines/leaf	Spine length (cm)		
			The first	st season					
El Wadi(1)	9.33 ^b	1.18 ^c	2.43 ^b	220 ^b	30.3 ^a	30.0 ^a	11.5 ^a		
El Wadi(2)	10.0^{b}	1.30 ^b	2.26 ^c	208°	30.6 ^a	29.0^{ab}	10.66 ^b		
SewiCv.	11.5 ^a	1.90 ^a	3.10 ^a	214.7	14.5 ^b	23.0 ^c	8.80°		
LSD at 0.5%	0.67	0.13	0.16	5.2	2.8	1.45	0.73		
	The second season								
El Wadi(1)	9.50 ^b	1.35 ^b	2.50 ^b	210 ^b	32.3 ^a	31.3 ^a	10.8^{a}		
El Wadi(2)	10.20 ^b	1.40^{b}	2.35 ^b	200°	32.6 ^a	24.6 ^b	10.7^{a}		
SewiCv.	11.7^{a}	1.93 ^a	3.15 ^a	215.3 ^a	20.1 ^b	26.0 ^c	9.0 ^b		
LSD at 0.5%	1.30	0.16	0.14	2.68	1.19	2.29	0.70		

 Table 2: Vegetative characteristics of some semi-dry seeded date palms and SewiCv. grown in El Wadi El

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Number of bunches/palm, bunch weight and yield/palm

Number of bunches/palm

Results in Table (3) indicate that the two seeded date palms were significantly varied in bunches number per palm. The highest number of bunches per palm (11 and 11.1 bunches/palm) was recorded from Sewicultivar followed by seeded date palm El Wadi (2) that recorded 10 and 10.2 in both seasons of the study. The lowest number of bunches per palm (4.3 and 9.5) was found with the seeded date palm namely El Wadi (1).

Bunch weight (kg)

Concerning bunch weight, results in Table (3) show that the seeded date palm namely El Wadi (1) recorded the highest significant bunch weight (20.3 and 20 kg) in the first and second seasons, respectively. The lowest bunch weight was obtained from Sewi cultivar since it was 11.9 and 12.1 kg in the first and second seasons, respectively.

Yield/ palm (kg)

Results in Table (3) clear that the highest yield/ palm was found with the seeded date palm El Wadi (2) since it was 200 and 203.3 kg followed by seeded date palm El Wadi (1) since it recorded 188.1 and 190 kg in the first and second seasons, respectively.



The lowest yield kg/ palm was recorded with Sewi cultivar (130.5 and 135.5kg) in both seasons. Similar results were obtained by [12-14, 18-21] who indicated that there was a wide and great variation on fruiting behavior of the most cultivars or seeded date palms.

 Table 3: Number of bunches/palm, bunch weight and yield / palm of some semi-dry seeded date palms and Sewicv. grown in El Wadi El Gedeed governorate

Cultivars	Cultivars Bunch number		Yield/palm (kg)
	The first seas	son	
El Wadi (1)	9.3 ^c	20.3 ^a	188.1 ^b
El Wadi (2)	10.0 ^b	20.0^{a}	200^{a}
Sewi cv.	11.0^{a}	11.9 ^b	130.5 ^c
LSD at 0.5%	0.17	0.16	3.14
	The second se	ason	
El Wadi (1)	9.5 [°]	20.0^{a}	190 ^b
El Wadi (2)	10.2 ^b	20.0^{a}	203.3 ^a
Sewi cv.	11.2^{a}	12.1 ^b	135.5 ^c
LSD at 0.5%	0.43	0.98	11.08

Fruit quality

1- Fruit physical characteristics

Results in Table (4) show that the weight of fruit, flesh and seed were significantly differed among all the seeded date palm and Sewi cultivar under this study conditions in the two seasons.

Sewi cultivar produced the heaviest fruit weight (15.9 and 16.2gm) in both seasons, followed by seeded date palm namely El Wadi (1) which recorded 15.5 and 16.2gm in the two seasons. The lowest fruit weight was obtained from seeded date palm namely El Wadi (2) since it was 10.6 and 10.2 gm in the first and second seasons, respectively.

Flesh weight parameter show the same trend of fruit weight with significant differences between values in both seasons of the study.

Concerning seed weight, the seeded date palms namely El Wadi (1) recorded the highest seed weight (2.1 and 2.2 gm) in the first and second seasons, respectively. While the lowest seed weight (1.3 and 1.4 gm) was obtained from seeded date palm El Wadi (2) in both seasons of the study.

Fruit dimensions

Results in Table (4) clear that fruit length and diameter were significantly differed among all seeded date palms and Sewi cultivar in the two seasons of the study.

The seeded date palm namely El Wadi (2) produced the longest fruit (8.2 and 6.8 cm in the first and second seasons, respectively), while the shortest fruit (4.0 and 4.2 cm) was obtained from Sewi cultivar in both seasons of the study.

Regarding fruit diameter, the seeded date palm El Wadi (2) had the highest value of fruit diameter (3.6 and 3.4 cm) in the first and second seasons.

The lowest fruit diameter (2.3 cm) was recorded from Sewi cultivar in the two seasons of the study.

 Table 4: Fruit physical characteristics of some semi-dry seeded date palms and Sewi cultivar grown in El Wadi

 El Gedeed governorate

CultivarsFruit weight (gm)Flesh weight (gm)Seed weight (gm)Fruit length (cm)Fruit diam (cm)The first seasonEl Wadi(1) 15.5^a 13.4^b 2.1^a 4.3^b 3.2^b El Wadi(2) 10.6^b 9.3^c 1.3^b 8.2^a 3.6^a Sewicv. 15.9^a 14.3^a 1.6^b 4.0^b 2.3^c LSD at 0.5% 0.93 0.7 0.5 0.2 0.09 The second seasonEl Wadi(1) 16.2^a 14.8^a 2.2^a 4.5^b 3.0^b			Li Ocuccu go	veniorate		
(gm)(gm)(gm)(cm)(cm)The first seasonEl Wadi(1) 15.5^{a} 13.4^{b} 2.1^{a} 4.3^{b} 3.2^{b} El Wadi(2) 10.6^{b} 9.3^{c} 1.3^{b} 8.2^{a} 3.6^{a} Sewicv. 15.9^{a} 14.3^{a} 1.6^{b} 4.0^{b} 2.3^{c} LSD at 0.5% 0.93 0.7 0.5 0.2 0.09 The second season	Cultivars	Fruit weight	Flesh weight	Seed weight	Fruit length	Fruit diameter
El Wadi(1) 15.5^{a} 13.4^{b} 2.1^{a} 4.3^{b} 3.2^{b} El Wadi(2) 10.6^{b} 9.3^{c} 1.3^{b} 8.2^{a} 3.6^{a} Sewicv. 15.9^{a} 14.3^{a} 1.6^{b} 4.0^{b} 2.3^{c} LSD at 0.5% 0.93 0.7 0.5 0.2 0.09 The second season		(gm)	(gm)	(gm)	(cm)	(cm)
El Wadi(2) 10.6^{b} 9.3^{c} 1.3^{b} 8.2^{a} 3.6^{a} Sewicv. 15.9^{a} 14.3^{a} 1.6^{b} 4.0^{b} 2.3^{c} LSD at 0.5% 0.93 0.7 0.5 0.2 0.09			The first s	season		
Sewicv. 15.9 ^a 14.3 ^a 1.6 ^b 4.0 ^b 2.3 ^c LSD at 0.5% 0.93 0.7 0.5 0.2 0.09 The second season	El Wadi(1)	15.5 ^a	13.4 ^b	2.1 ^a	4.3 ^b	3.2 ^b
LSD at 0.5% 0.93 0.7 0.5 0.2 0.09 The second season	El Wadi(2)	10.6 ^b	9.3 ^c	1.3 ^b	8.2^{a}	3.6 ^a
The second season	Sewicv.	15.9 ^a	14.3 ^a	1.6 ^b	4.0^{b}	2.3 ^c
	LSD at 0.5%	0.93	0.7	0.5	0.2	0.09
El Wadi(1) 16.2^{a} 14.8^{a} 2.2^{a} 4.5^{b} 3.0^{b}			The second	l season		
	El Wadi(1)	16.2 ^a	14.8 ^a	2.2 ^a	4.5 ^b	3.0 ^b



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El Wadi(2)	10.2 ^b	8.8 ^c	1.4 ^b	6.8 ^a	3.4 ^a
Sewicv.	16.2 ^a	14.7 ^b	1.5 ^b	4.2 ^b	2.3 ^c
LSD at 0.5%	0.58	7.5	0.3	0.2	0.16

2- Fruit chemical characteristics:

Results in table (4) indicate that the fruit Juice TSS values were significantly differed among the seeded date palms and Sewi cultivar in both seasons. Sewi date palm produced the highest TSS values (27 and 27.8%) during both seasons of the study.

On the other hand, the seeded date palm namely El Wadi (1) had the least values of TSS (25 and 25.9%) in the first and second seasons, respectively.

Concerning acidity percentage, the highest value of acidity (0.30%) was obtained from the seeded date palm El Wadi (1) in the two seasons. While the lowest value (0.25 and 0.24 %) was recorded from the seeded date palm El Wadi (2) in both seasons.

Total sugars %

Total sugars percentage was not significantly affected with seeded date palms and Sewi cultivar in the second season. The obtained results clear that the seeded date palm El Wadi (2) recorded the highest percentage of total sugars 63.01% in the first season of the study. The lowest percentage of total sugars was obtained from seeded date palm El Wadi (1) which recorded 55.3 and 76% in both seasons.

Reducing sugars%

Results in table (4) show that the reducing sugars percentage were not affected significantly with the seeded date palm El Wadi (2) and Sewi cultivar in the first season. The highest reducing sugars percentage (36 and 37%) was found due to the seeded date palm (2) in the first and second seasons, respectively. The lowest reducing sugars values (33 and 35%) were obtained from the fruits of the seeded date palm El Wadi (1) in the first and second seasons.

Similar results were obtained by [12-14, 19-21].

Non reducing sugars %

Results in Table (5) clear that the highest non-reducing sugars value (28.3 and 33%) in the fruits was obtained from Sewi date palm cultivar in both seasons of the study, while, the seeded date palm El Wadi (1) gave the lowest non-reducing sugars percentage (22.3%) in the first season. Meanwhile, the lowest non-reducing sugars percentage in the second season was recorded from the seeded date palm of El Wadi (2) since it was 29%.

Similar results for the abovementioned ones in respect to the variation between different female cultivars or seeded types were reported by [13, 14, 17, 19-21.

Cultivars	TSS%	Acidity %	Total sugars %	Reducing sugars %	Non-reducing sugars%
		The first	t season		
El Wadi(1)	25 ^b	0.30 ^a	55.3 ^b	33. ^a	22.3 ^b
El Wadi(2)	26^{ab}	0.25 ^b	63.1 ^a	36 ^a	27.0^{a}
Sewicv.	$27^{\rm a}$	0.30 ^a	62.3 ^a	34 ^a	28.3 ^a
LSD at 0.5%	1.5	0.04	1.2	NS	3.64
		The secor	nd season		
El Wadi(1)	25.9 ^C	0.30 ^a	67.0 ^a	35.0 ^b	32.0 ^a
El Wadi(2)	26.8 ^b	0.24 ^{cb}	66.0^{a}	37.0 ^a	29 ^{ab}
Sewicv.	27.8^{a}	0.26 ^b	69 ^a	36.0 ^{ab}	33.0 ^a
LSD at 0.5%	0.42	0.06	NS	1.41	1.04

 Table 5: Fruit chemical characteristics of some semi dry seeded date palms and Sewi cultivar grown in El Wadi

 El Gedeed governorate

Using RAPD-PCR technique of nine primers (Table 6) revealed 47 fragments were of them 37 polymorphic bands with 80.43% polymorphism, 50f them were unique bands with 10.87% from total bands and 9 bands were monomorphic with 29.57% showed that, in Fig. 1 and the results listed in Table (6), while the relationships

revealed high value between Sewi and Wadi (2) was 0.826. On the other hand, the phylogenetic tree revealed middle value between Sewi with wadi (1) was 0.556, these results was the lowest value (0.230) found between Wadi (1) and Wadi (2) that showed in Fig. 2 and showed that, in Proximity Matrix Table (7).

In this respect, Sedra *et al.* [22] and Adawy *et al.* [23] found that in date palm 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. It has been reported that fragment sizes ranging from 200 to 1600bp, while, Adawy *et al.* [23] stated that in the RAPD analysis of five date palm cultivars using 10 primers, the fragment sizes ranged from 310 to 2800bp.

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.* [24] pointed out the presence of intra-varietal polymorphism among five Egyptian date palm cultivars from the Delta region.

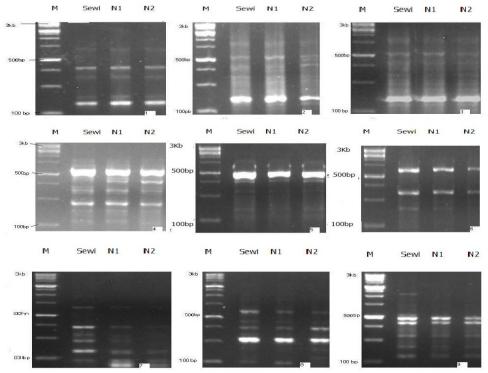


Figure 1: RAPD-PCR banding patterns of the three date palm genotypes amplified with the nine 10-mer random primers; = 100-bp ladder of nine primers

Table 6 : Total number, monomorphic, polymorphic of bands and percentage of polymorphism as revealed by
nine RAPD primers on Sewi date palm cultivar with two genotypes from El Wadi El Gedeed governorate

Primer	Total bands	Monomorphic bands			% polymorphism
			unique		
OPA-02	7	2	2	3	71.429
OPA-04	7	2	2	3	71.429
OPA-07	5	2	2	1	60
OPB-07	8	3	4	1	62.500
OPB-10	4	1	2	1	75
OPO-10	5	1	2	2	80
OPO-13	7	1	4	2	85.714
OPO-14	7	3	2	2	57.143
OPO-19	7	4	2	1	42.857
Total bands	57(100%)	19(33.33%)	22(38.60%)	16(28.07%)	66.67%

Using RAPD-PCR technique of nine primers revealed 57 different bands were of them 19 monomorphic bands (33.33%) and 38 were polymorphic bands (66.67% polymorphism) 16 of them were unique bands with ratio (28.07%) showed that, in Table (6).The genetic relationships among the three accessions were estimated in terms of similarity using Dice coefficient. The phylogenetic tree revealed high values between El Wadi (2) and Sewi, the phylogenetic tree revealed. On the other hand, El Wadi (1) with Sewi recorded the lowest value (0.282) in Table (7).

The products of nine RAPD primers represented as shown in Figure (1) and Table (6). The number of polymorphic bands for each primer was varied from 4 fragments of primers (OPB-10) to 8 fragments for (OPB-07), with an average of 6.33 bands/ primer, with range polymorphism in nine primers between 42.857 to 85.714% of polymorphism.

Five primers (OPA-02, OPA-04, OPO-13, OPO-14 and OPO-19) give seven bands with percentage 71.429, 71.429, 85.714, 57.143 and 42.857 % polymorphism, respectively. While, primer OPA-07 and OPO-10 revealed five bands with polymorphism 60 and 80 %. On the other hand, the highly frequency of bands was 8 using primer OPB-07 with percentage 62.5% polymorphism.

RAPD markers were used to describe three date palm genotypes grown in El Wadi region 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 clusters the first one include Sewi and El Wadi(2) while, the other cluster contains El Wadi (1).

On the other hand, the phylogenetic tree revealed high values between El Wadi (2) and Sewi (0.826), while, El Wadi (1) with Sewi recorded the lowest value (0.556). Our result in date palm cultivars is confirm with Cipriani *et al.* [25] and Hormaza [26] whom stated RAPD technique provides genetic markers which have been used extensively in many different applications.

While, the obtained result is scored 66.67% polymorphism with SPSS analysis more confirmed than Moghaieb *et al.* [27] whom detected 60.23% polymorphism among the six genotypes in date palm by RAPD.

Table 7: Proximity matrix between two genotypes from El Wadi with Sewi cultivar **Matrix File Input** Case El Wadi (1) Sewi El Wadi (2) 1.000 Sewi El Wadi(1) 0.556 1.000 1.000 El Wadi(2) 0.826 0.230

Whenever, Hussein et al. [24] showed high polymorphism (92.4%) among their Egyptian date palm cultivars.

Also, in the Proximity matrix Table (7) distinguish the relationships between three date palms, the variety El Wadi (2) was the high relations with Sewi and the lowest relationship was between El Wadi (1) with Sewi showed in dendrogram using average linkage (between groups) Figure (2). The last results may be affecting in these of date palm varieties in date palm breeding programs.

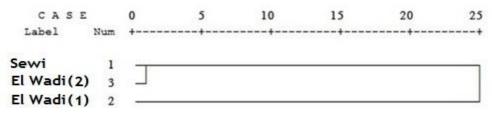


Figure 2: Dendrogram using average linkage between Sewi cultivar and two date palm genotypes from El Wadi El Gedeed governorate.

Elshibli, *et al.* [28] showed a complex genetic relationships between some of the tested populations especially when isolation by distance was considered and they also found although a large amount of diversity exists among date palm germplasm, the findings suggest that the role of biological nature of the tree, isolation by distance and environmental effects these results agree with recorded results in our studies.



Conclusion

Generally, the obtained results are important from the economical and horticultural point of view to select the superior and promising female seeded palms for propagation in order to obtain new colons with high yield and good fruit quality and it is important to keep them in gene bank.

Molecular markers as modern approaches were successfully used to estimate the genetic relationships between the studied date palm cultivars for improving the fruit indispensible to achieve date palm genetic improvement considering the lengthy and dioeciously nature of date palm and use of molecular tools, RAPD, is successfully and very important to explain genetic diversity. Thus, the tools should be increasing the relationships between molecular markers and fruit characteristics. The obtained result by AMOVA analysis supported genetic relationships between three genotypes to two clusters. Finally, this study has provided an efficient procedure that can be used routinely to identify date palm cultivars.

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