(Original Article)

Molecular Characterization of some Egyptian Date Palm (*Phoenix dactylifera L.*) Cultivars Using RAPD-PCR

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Abstract

Thirty Egyptian date palm cultivars were collected from different locations in three governorates: Assiut, New Valley, and Aswan. Physical characteristics of the Fruit were studied, such as fruit dimensions, fruit weight, seed weight, and flesh weight. Moreover, genetic diversity among cultivars was studied using RAPD-PCR molecular marker. Physical characteristics results of the fruit showed significant differences among the tested cultivars. Samani and Magdool showed the highest values for both fruit dimensions and weight characteristics. All 5 tested RAPD primers were able to differentiate among the tested date palm cultivars and showed high polymorphism percentage ranging from 75-100%. Thirteen different specific positive and negative markers associated with some cultivars were obtained. The genetic similarity among cultivars ranged from 40% to 100% and the highest genetic similarity value was observed between Nawashf Red and Nawashf White. The results showed that the cultivars with the same name from different geographical locations are genetically different and showed significant differences in fruit physical characteristics.

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most excellent crops of economic and ecological value for oasis agriculture in arid and semi-arid areas. It is widely distributed in Mediterranean countries, Africa, part of Asia and North America and Australia (Chevalier, 1952). *P. dactylifera* is a diploid ($2n = 2x = 36$) perennial monocotyledonous dioecious plant (Barrow, 1998, Chao and Krueger, 2007).

There are about 79 cultivars grown all over Egypt. The diversity in climatic conditions in the different areas of Egypt allows of cultivation of different date types such as dry, semi-dry and soft (El-Sharabasy and Rizk, 2019).

Snice 2020, Egypt has ranked as the first place among the top five date – producing countries in the world. Annually Egypt produces 1.7 million tons which

represents 17.7 % of the world's dates production and 24.4% of all dates production of Arab countries (FAO, 2020).

Although Egypt is the world's leading producer of dates, it faces some problems which negatively affect the production including dispersal of date palm trees which propagated with seeds throughout Egypt which grow up to be male tree or female that produce dates with poor qualities and don't have the same desirable traits of established clonal tree (Zaid and De Wet, 2002). Transplantation of varieties over years from the original regions to other areas, and they have been adapted and cultivated with different names. As a result, a variety may have a different name in different plantation areas or even two genetically different varieties may have the same name (Jain *et al.*, 2011; Bekheet and El-Sharabasy, 2015). This leads farmers to focus on cultivating certain varieties and neglecting others, even though they could contain important genes such as those resist biotic and abiotic stresses. Therefore, several researchers aimed to differentiate between different date palm cultivars.

Basically, the morphological characteristics were used to differentiate date palm cultivars. The most common phenotypic characteristics of date palm are the morphology of leaves, spines and fruits. These morphological features are sensitive to environmental factors and can be observed only in mature trees (Elshibli and Korpelainen, 2009). Physical and chemical characteristics of the fruits can influence their mechanical and physiological properties, which in turn indicate the quality. Also, they reveal essential information for better understanding of date fruit to enhance industrialization and propagation of the best date palm varieties in order to satisfy producers and consumers demands (Ismail *et al.*, 2006).

Nowadays, DNA molecular markers are used to differentiate genotypes. Molecular markers are techniques that enable specific DNA sequences to be particularly amplified from genomic DNA sections using specific or arbitrary oligonucleotide primers. Molecular markers constitute a very useful tool currently available for research in plant differentiation and improvement (Collard *et al.*, 2005).

DNA markers have several advantages; they are unlimited and not affected by environmental factors, unlike morphological and biochemical markers (Winter and Kahl, 1995). Additionally, they have many applications in plant breeding as an assessment of genetic diversity among genotypes. Marker technology based on polymorphisms in DNA has catalyzed research in different disciplines such as phylogeny, taxonomy, ecology, genetics and plant breeding (Weising, 1995; Baird *et al.*, 1997; Henry, 1997; Jahufer *et al.*, 2003; Weising, 2005; Leijman, 2011). Random Amplified Polymorphic DNAs (RAPDs) is an important dominant marker (Hartl and Lozovsky, 2005; Williams *et al.*, 1990). RAPD was applied to assess the genetic diversity in date palm (Al-Khalifah and Askari, 2003; Atia *et al.*, 2017; Haider, 2017)

Based on this approach, the aim of this investigation was to collect different cultivars from different locations in Egypt and molecularly characterize them accurately using RAPD-PCR technique, and to assess the genetic diversity and genetic relationships among the cultivars, trying to find out if cultivars with the same names and different locations could be genetically similar.

Materials and Methods

This study was carried out in Genetics Department, Faculty of Agriculture, Assiut University, during 2020-2022 to investigate the physical, and molecular differences among the candidate date palm cultivars collected from three different geographical locations in Egypt using RAPD-PCR technique.

Plant Material

As shown in Figure 1, thirty samples were collected from three governorates in southern Egypt (Assiut, New Valley and Aswan) as leaves and fruits, and then stored at −20°C. Each sample was collected from three different palm trees of the same cultivar. The leaf samples were taken from unopened newly grown greenish white leaves close to terminal bud. Fruit samples were collected at the appropriate harvest time for each cultivar.
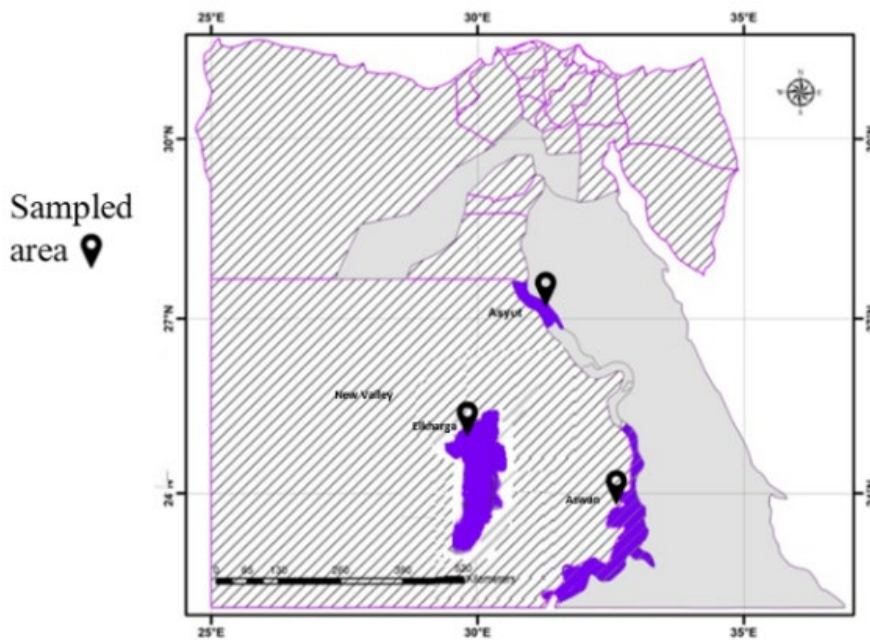
Figure 1. The samples collecting locations.

Thirty date palm cultivars were collected from 3 governates and 4 different locations. Figure (2) and Table (2) shows that 10 cultivars were collected from Assiut 1 (Pomology department field station), 6 from Assiut 2 (Assiut valley), 8 from New Valley (New Valley Agricultural Research Station of Elkharga), and 6 from Aswan (The central laboratory for palm research and development, Aswan).

Fruit physical characterization

Fruit length (L) and Fruit diameter (D) were measured by Vernier caliper (in cm) then the shape index was calculated using the following equation:

Shape index = Fruit length /Fruit diameter

Fruit weight and Seed weight were measured by balance (in gm) then Flesh weight was calculated by subtracting the Seed weight from the Fruit weight.

Molecular characterization

DNA extraction

Total genomic DNA was isolated from leaves using CTAB protocol for plants (Murray and Thompson, 1980) with some modifications. Then the DNA concentration and purity were estimated by Nano-drop. DNA dilutions were made to detect the optimum concentration for PCR analysis.

PCR amplification

Five RAPD-PCR primers Table (1) were used to discriminate the 30 tested cultivars and detect their DNA polymorphisms.

Table 1. RAPD primers names, their sequences and annealing temperature

Primer	Primer sequences (5`-3`)	Annealing temperature
OPA-01	CAGGCCCTTC	34
OPA-12	GGACCTCTTG	34
OPM-13	GGTGGTcAAG	34
OPAB-4	GGCACGCGTT	34
OPN-03	AAGCGGCCTC	34

PCR Master Mix reactions (GeneDirex) were conducted in a 20μL total volume, containing 1x PCR master mix, 1 μL of primer (100 ng/ μL) and 2 μL of DNA template, and 7 μL dH2O. The PCR program was as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, 30 s of annealing at 34°C, 60 s of extension at 72°C, and a final extension for 5 min at 72°C. PCR products were electrophoresed onto a submerged 1% agarose gel and the results were compared with a 100 bp ladder marker. The gel documentation system was used to visualize the banding patterns.

Statistical Analysis

All statistical analysis of fruit characteristics was performed using GraphPad Prism 9 software. Agarose gel photos were scanned by 1DscanEX software for the detection of the presence of banding patterns and calibrating them for size and intensity. A binary data matrix recording the presence (1) or the absence (0) of bands was made. The software package MVSP (Multi Variate Statistical Package) was used to calculate the genetic similarities using the Dice (Dice, 1945) coefficient of similarity of Nei and Li (Nei and Li,1979)

Marker parameters were estimated according to Ghislain *et al*. (1999), Powell *et al*. (1996), and Prevost and Wilkinson (1999).

Results and Discussion

Plant Material

As shown in Table (2), the thirty collected cultivars had three types of fruit moisture (11 soft cultivars, 7 dry cultivars, and 12 semi-dry cultivars) and different fruit colors.

Table 2. Summarizes the details of collected date palm cultivars and their locations used in the present study

| Sample no.	Cultivar name	Collecting location	Fruit	
			Type	Color
1	Zaghlol	Assiut 1	Soft	Shiny-Red
2	Samani		Soft	Orange
3	Hayani		Soft	Shiny-Red
4	Sewi		Semi-dry	Pale -Yellow
5	Halawi		Soft	Pale -Red
6	Bent Eisha		Soft	Shiny-Red
7	Amri		Semi-dry	Yellowish-Red
8	Medjool (Magdool)		Semi-dry	Yellow
9	Sakaai		Semi-dry	Yellow
10	Khedri		Semi-dry	Pale -Yellow
11	Zaghlol*	Assiut 2	Soft	Red
12	Samani*		Soft	Orange
13	Nawashf_White		Dry	Pale -Yellow
14	Nawashf_Red		Dry	Pale -Red
15	Sewi*		Semi-dry	Pale -Yellow
16	Maghl(unknown)		Soft	Red
17	Barhi	New Valley	Soft	Yellow
18	Seadi		Semi-dry	Orange-Yellow
19	Tamar Elwadi		Semi-dry	Yellow
20	Hegazi		Soft	Red
21	Elfalk		Semi-dry	Yellow
22	Mantor_1		Semi-dry	Red
23	Mantor_2		Semi-dry	Red
24	Mantor_3		Semi-dry	Red
25	Elhag Heseen	Aswan	Soft	-
26	Shamia boni		Dry	Pale -Yellow
27	Bartamouda		Dry	Yellow-Orange
28	Sakkoti		Dry	Yellow
29	Gondila		Dry	Yellow
30	Malakabi		Dry	Shiny-Red
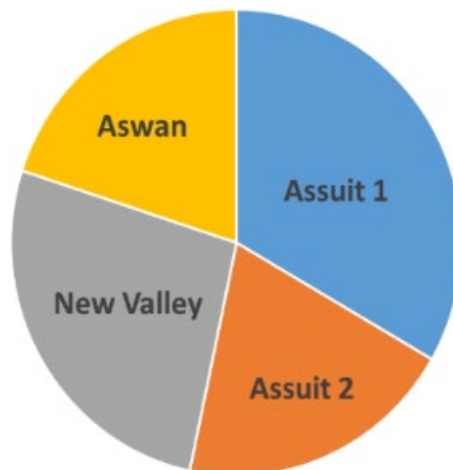
Figure 2. Quantity of the cultivars over collected locations.

Fruit physical characterization

Several research characterized dates based in different ways. date palm cultivars can be identified by physical and chemical characteristics (Selim *et al*., 1970; Hussein and Hussein, 1982). Both characteristics can impact the mechanical and physiological properties of dates, which also reveal their quality. Additionally, they provide crucial details for deeper comprehension of date fruit, enhancing industrialization and the propagation of the best date palm cultivars to meet the expectations of both producers and consumers (Ismail *et al*., 2006).

Thus, this investigation aimed to discriminate between Egyptian local cultivars, so several fruit samples of the different cultivars were collected according to harvest time. Only 20 cultivars from 3 locations (Assiut, Aswan, and New Valley) were selected for the Fruit physical characterization. Samples from the 2nd location of Assiut were neglected. Fruit dimensions, shape index, fruit weight, seed weight and flesh weight were studied. The results of all tested traits in figures (3 and 4) revealed significant differences among tested cultivars. Fruit length as shown in Figure (3A), ranged from (5.52 cm) with Samani to (3.31 cm) with Sakaai. While the highest value of fruit diameter (Figure 3B) was (3.06 cm) with Samani and the lowest was (1.9 cm) with Sakkoti. Shape index is one of the most important quality traits of date palm fruits. Results in Figure (3C) showed that it ranged from (2.44) with Sakkoti to (1.37) with Barhi.
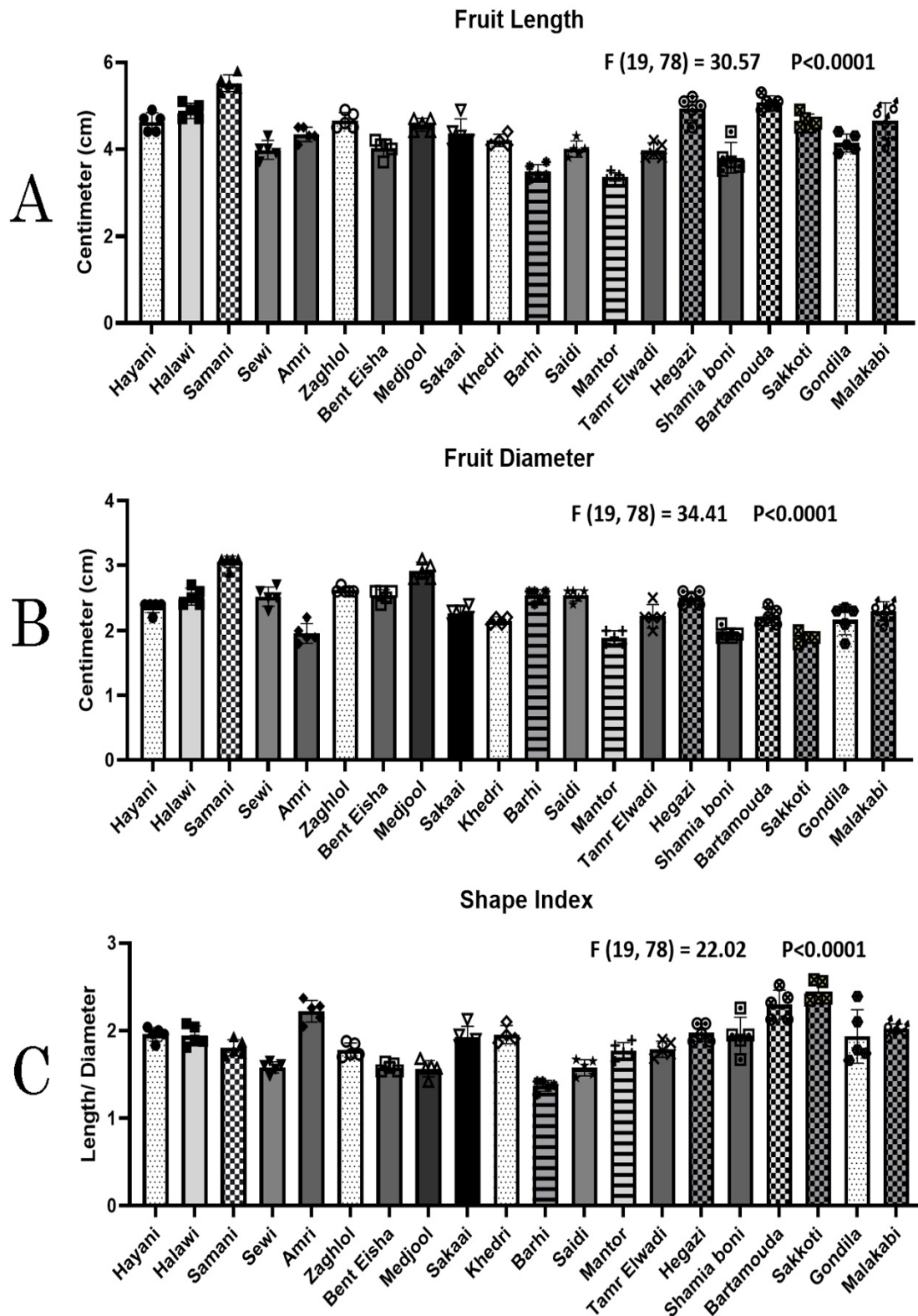
Figure 3. Fruit length, diameter and shape index of 20 Egyptian cultivars collected from Assiut, New valley and Aswan during 2021 season.

Fruit Weight

Seed Weight
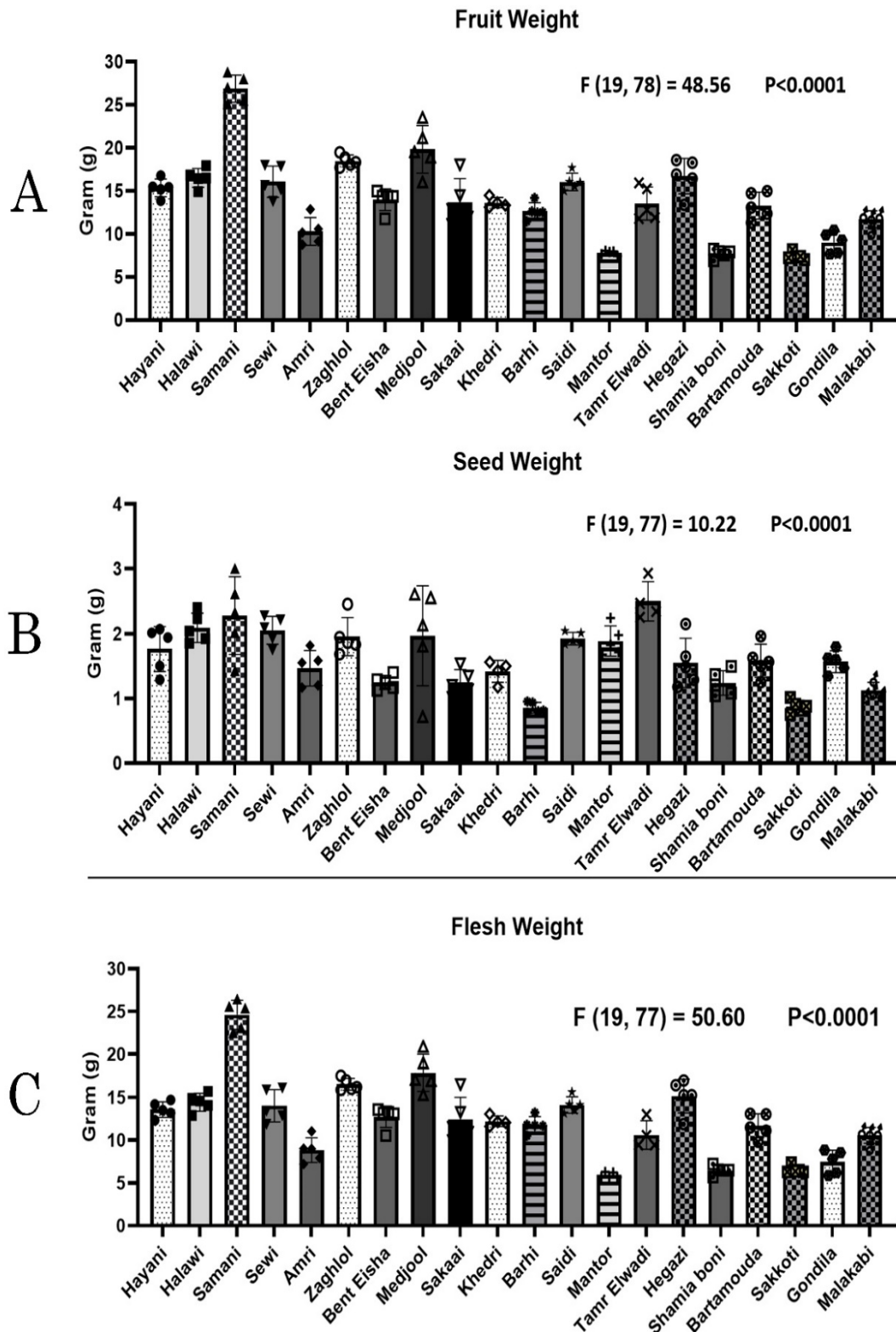
Flesh Weight

Figure 4. Fruit, Seed and Flesh weight of 20 Egyptian cultivars collected from Assiut, New valley and Aswan during 2021 season.

As shown in Figure (4A and 4C), Samani gave the highest fruit and flesh weight as a soft date cultivar (26.89 g, and 24.61 g) respectively, these results

agreed with Meligi *et al*., (1983). While Magdool was the heaviest semi-dry date which weighed (19.83 g) for fruit weight and (17.86 g) for flesh weight. In dry cultivars, Sakkoti showed the lowest weights (7.437g, and 6.56 g), while Bartamouda was superior in fruit weight (13.29 g), and flesh weight (11.70 g). Similar results were also found with El-Merghany *et al*., (2014).

Molecular characterization

In this study, RAPD-analysis was performed to detect the genetic diversity among 30 Egyptian date palm cultivars collected from three locations. RAPD-PCR uses short primers which need lower annealing temperatures and result in multiple random primer annealing with unique profile for each cultivar, which can be used in the discrimination between different cultivars. Five random primers which are shown in Table (1), were used to determine the genetic variation between the cultivars. Figures 5- 9 show the RAPD-PCR profile for the thirty tested Egyptian cultivars using different primers. Table (3) shows that the largest amplified band (2446 bp) was found with OPN-3 primer, while the smallest (296 bp) was with the OPAB-4 primer. OPA-12 primer gave the highest number of amplified bands (12 bands), while OPA-1 and OPM-13 gave the lowest number of amplified bands (8 bands).
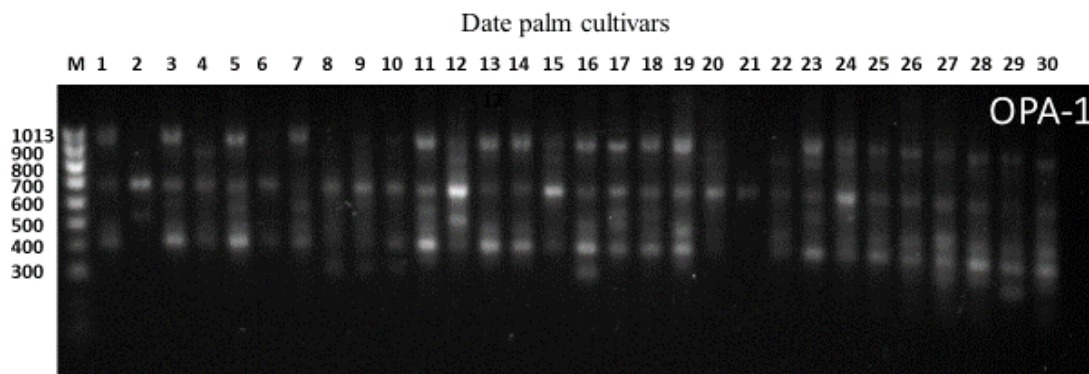
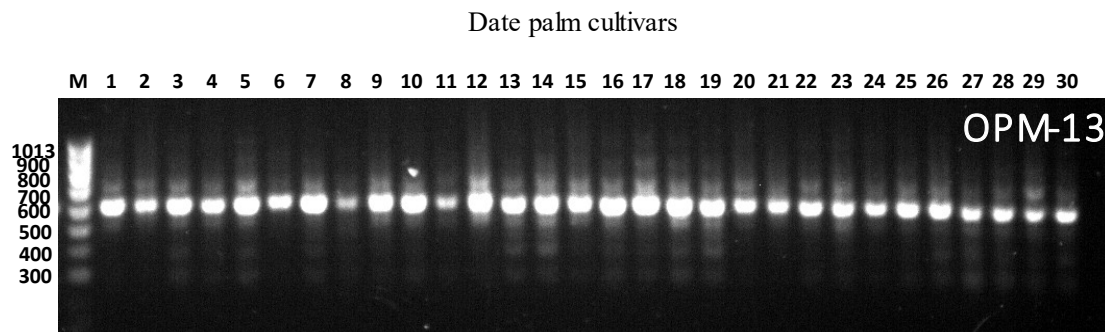**Figure 5. Agarose gel electrophoresis of RAPD products amplified by OPA-1 primer.**

As shown in Figure (5) and Table (3), OPA-1 primer amplified 8 bands ranged in size from 304 to 974 bp, they were all polymorphic bands. This primer gave the maximum polymorphism percentage 100% among all tested primers.
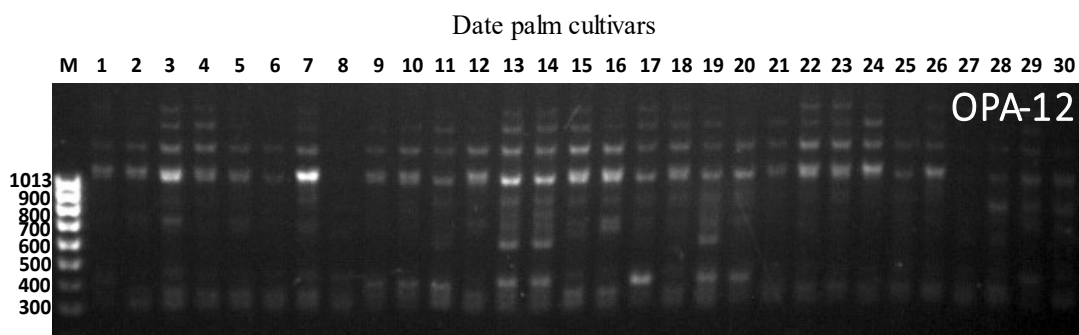
Figure (6). Agarose gel electrophoresis of RAPD products amplified by OPA-12 primer.

As shown in Figure (6), OPA-12 gave 13 amplified bands ranged in size from 333 to 1830 bp. Table (3), shows that number of the polymorphic bands for this primer was 12 which resulted a polymorphism percentage of 92.3%.

Date palm cultivars

Figure 7. Agarose gel electrophoresis of RAPD products amplified by OPM-13 primer.

Figure (7) shows that the OPM-13 gave 8 amplified bands ranged in molecular weight from 304 to 1131 bp. Table (3) shows that number of the polymorphic bands was 6 with a polymorphism percentage of 75%.

Date palm cultivars

Figure 8. Agarose gel electrophoresis of RAPD products were amplified by OPAB-4 primer.

Results in Figure (8) illustrated that the OPAB-4 gave 10 amplified bands ranged in molecular weight from 296 to 1733 bp. Table (3) shows that number of the polymorphic bands was 9 which resulted high polymorphism percentage 90%.
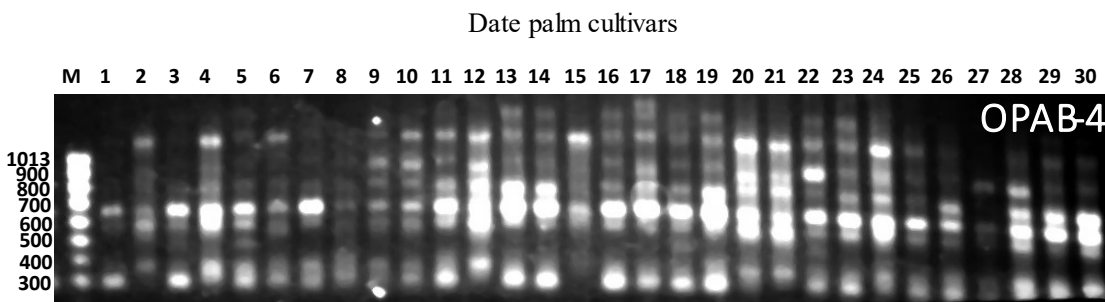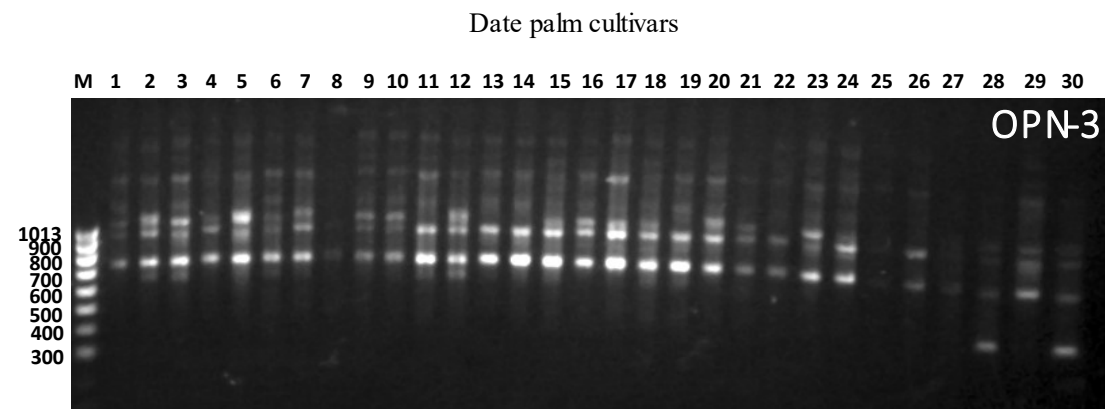
Date palm cultivars

Figure 9. Agarose gel electrophoresis of RAPD products were amplified by OPN-3 primer.

As shown in Figure (9) OPN-3 gave 9 amplified bands ranged in molecular weight from 327 to 2446 bp. Table (3) shows that number of the polymorphic bands was 8 which resulted an 88.8% polymorphism.

Table 3. Genetic marker information obtained by RAPD-analysis among thirty Egyptian date palm cultivars

Primer	The smallest band	The biggest band	Total number of bands (a)	Number of Poly-morphic bands (b)	Poly-morphism% b/a*100	PIC	RP
OPA-1	304	974	8	8	100	0.35	3.87
OPA-12	333	1830	13	12	92	0.30	5.27
OPM-13	304	1131	8	6	75	0.27	2.80
OPAB-4	296	1733	10	9	90	0.25	3.13
OPN-3	327	2446	9	8	89	0.28	3.73

Al-Khalifah and Askari, (2003) and Munshi and Osman, (2010) indicated that RAPD markers are useful for determining date palm molecular polymorphism. Table (3) shows that the number of polymorphic bands ranged from 8 (OPA-1 and OPN-3) to 12 (OPA-12) and the percentage of polymorphism across all primers ranged from 100% (OPA-1) to 75% (OP-M13). This high polymorphism was also found with Haider (2017) who also used RAPD-PCR marker to determine Phylogenetic relationship among date palm cultivars.

The PIC (Polymorphism Information Content) values ranged from 0.25 (OPAB-4) to 0.35 (OPA-1). High PIC value means that the chosen primers of RAPD can be used efficiently to study the genetic diversity in date palm. RP (Resolving Power) values ranged from 2.8 with the OPM-13 to 5.27 with the OPA-12 primer thus it was considered the best primer in the discrimination of the cultivars.

RAPD markers were used to identify date palm cultivars molecularly (Samy *et al.*, 2007; El Ameen, 2013). The present and absent bands of RAPD-PCR could be used as specific positive and negative DNA markers which are useful for genetic identification of date palm cultivars especially high-quality commercial cultivars.

Table 4. shows that, out of thirty tested cultivars, twenty cultivars (Samani, Hegazi, Elfalk, Gondila, Amri, Zaghlol, Nawashf_Red, Nawashf_white, Bent Eisha, Tamar Elwadi, Halawi, Seadi, Mantor_1, Barhi, Sewi*, Elhag Heseen, Bartamouda, Malakabi, Magdool and Sakkoti) showed specific markers.

Although the used primers could produce molecular markers with 20 different cultivars, they couldn't produce any positive or negative markers with 10 of the tested cultivars (Hayani, Sewi, Sakkai, Khedri, Zaghlol*, Samani, Maghl, Mantor_2, Mantor_3, Shamia boni). Therefore, we recommend using other RAPD primers or even another marker like ISSR, SRAP or SCoT to identify these date palm cultivars.

Table 4. Positive and negative markers and their molecular size detected by different RAPD primers

Primer	Cultivar	Positive marker (bp)	Negative marker (bp)
OPA-1	Samani	-	415
	Hegazi		
	Elfalk		
	Amri	-	678
	Gondila		
OPA-12	Zaghlol	-	381
	Nawashf_Red	665	-
	Nawashf_white		
	Tamar Elwadi		
	Magdool	-	1047
		-	1303
OPM-13	Bent Eisha	-	575
	Magdool		
OPAB-4	Halawi	484	-
	Seadi		
	Mantor_1		
	Zaghlol	-	594
	Magdool		
	Sewi*		
	Barhi	1733	-
OPN-3	Sakkoti	327	-
	Malakabi		
	Magdool	-	1069
	Elhag Heseen		
	Bartamouda		
	Magdool	-	1753
	Bartamouda		
	Sakkoti		

All the 5 tested RAPD primers gave specific positive and negative markers (13 markers). OPA-1 primer gave 2 negative markers (415bp for Samani-Hegazi-Elfalk and 678 bp Amri-Gondila). OPA-12 gave 1 positive marker (665bp for Nawashf_Red – Nawashf_white -Tamar Elwadi) and 3 negative markers (1047 bp and1303 bp for Magdool and 381bp for Zaghlol). OPM-13 gave 1 negative marker (575bp for Bent Eisha- Magdool). OPAB-4 gave 2 positive markers (484 bp for Halawi-Seadi-Mantor_1 and 1733bp for Barhi) and 1 negative marker (594 bp for Zaghlol-Magdool- Sewi*). OPN-3 gave 2 negative markers (1069 bp for Magdool-Elhag Heseen-Bartamouda and 1753bp for Magdool- Bartamouda-Sakkoti) and 1 positive maker (327bp for Malakabi- Sakkoti). Magdool cultivar showed the highest number of specific markers (5 markers).

Table 5. The genetic similarity scores of the thirty distinct cultivars derived from DNA amplification using five RAPD primers

| Cultivars	Zaghlol	Samani	Hayani	Sewi	Halawi	Bent Eisha	Amri	Medjool	Sakaai	Khedri	Zaghlol*	Samani*	Nawashf W.	Nawashf R.	Sewi*	Maghl	Barhi	Seadi	Tamar Elwadi	Hegazi	Elfalk	Mantor_1	Mantor_2	Mantor_3	Elhag Heseen	Shamia boni	Bartamouda	Sakkoti	Gondila	Malakabi
Zaghlol	1.0																													
Samani	0.7	1.0																												
Hayani	0.8	0.7	1.0																											
Sewi	0.7	0.8	0.8	1.0																										
Halawi	0.7	0.8	0.9	0.8	1.0																									
Bent Eisha	0.7	0.9	0.7	0.8	0.8	1.0																								
Amri	0.7	0.7	0.9	0.8	0.9	0.7	1.0																							
Medjool	0.5	0.5	0.6	0.6	0.7	0.6	0.7	1.0																						
Sakaai	0.7	0.7	0.8	0.9	0.8	0.8	0.8	0.7	1.0																					
Khedri	0.7	0.8	0.7	0.9	0.8	0.8	0.8	0.7	0.9	1.0																				
Zaghlol*	0.7	0.7	0.8	0.8	0.9	0.8	0.8	0.6	0.9	0.8	1.0																			
Samani*	0.7	0.8	0.8	0.8	0.9	0.8	0.8	0.6	0.8	0.8	0.8	1.0																		
Nawashf W.	0.6	0.6	0.8	0.8	0.7	0.6	0.8	0.5	0.8	0.7	0.8	0.7	1.0																	
Nawashf R.	0.6	0.6	0.8	0.8	0.7	0.6	0.8	0.5	0.8	0.7	0.8	0.7	1.0	1.0																
Sewi*	0.7	0.7	0.7	0.8	0.7	0.6	0.7	0.4	0.7	0.7	0.7	0.7	0.8	0.8	1.0															
Maghl	0.7	0.6	0.8	0.8	0.8	0.7	0.8	0.6	0.8	0.8	0.7	0.8	0.8	0.8	0.8	1.0														
Barhi	0.7	0.7	0.8	0.8	0.8	0.7	0.8	0.6	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.9	1.0													
Seadi	0.7	0.6	0.8	0.8	0.9	0.7	0.8	0.5	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.9	0.9	1.0												
Tamar Elwadi	0.6	0.6	0.8	0.7	0.8	0.7	0.8	0.6	0.8	0.8	0.8	0.7	0.9	0.9	0.7	0.9	0.9	0.9	1.0											
Hegazi	0.7	0.8	0.7	0.8	0.8	0.8	0.8	0.5	0.8	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.7	0.8	0.8	1.0										
Elfalk	0.6	0.8	0.6	0.8	0.7	0.8	0.7	0.5	0.7	0.8	0.7	0.8	0.6	0.6	0.7	0.7	0.7	0.7	0.6	0.9	1.0									
Mantor_1	0.7	0.7	0.7	0.9	0.8	0.7	0.7	0.6	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.7	0.7	1.0								
Mantor_2	0.7	0.7	0.9	0.8	0.8	0.7	0.9	0.6	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.8	0.9	0.8	0.7	0.7	0.8	1.0							
Mantor_3	0.7	0.7	0.8	0.9	0.8	0.8	0.8	0.6	0.9	0.8	0.9	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9	1.0						
Elhag Heseen	0.7	0.7	0.7	0.8	0.8	0.8	0.7	0.7	0.8	0.7	0.8	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.8	1.0					
Shamia boni	0.7	0.7	0.8	0.9	0.8	0.7	0.8	0.7	0.8	0.8	0.8	0.8	0.7	0.7	0.8	0.9	0.8	0.8	0.8	0.7	0.7	0.9	0.8	0.9	0.9	1.0				
Bartamouda	0.6	0.6	0.6	0.7	0.7	0.6	0.7	0.8	0.7	0.8	0.6	0.6	0.6	0.6	0.5	0.7	0.6	0.6	0.6	0.5	0.6	0.7	0.6	0.6	0.8	0.8	1.0			
Sakkoti	0.7	0.7	0.7	0.8	0.8	0.8	0.8	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.8	0.8	0.9	0.8	1.0		
Gondila	0.6	0.6	0.8	0.8	0.8	0.7	0.8	0.6	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.8	0.8	0.7	0.8	0.7	0.8	1.0	
Malakabi	0.7	0.7	0.7	0.8	0.8	0.7	0.8	0.7	0.8	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.7	0.9	0.9	1.0

The genetic similarity for 30 date palm cultivars was estimated, especially between the cultivars with same names from different locations.

As shown in Table (5) the genetic similarity ranged from 0.4 to 1 among the cultivars. Although, both Nawashf_W. and Nawashf_R were genetically identical and showed genetic similarity value of (1), they showed different morphological traits (Table 2) since they have different fruit color.

Nearly close genetic similarity was found between all of: Khedri and Sakaai (0.93), Sakaai and Malakabi (0.91) and Barhi and Seadi (0.91). On the other hand, the lowest genetic similarity (0.4) was recorded between Medjool and Sewi*, Medjool and Samani (0.46), Bartamouda and Sewi* (0.48) and Hegazi and Bartamouda (0.51).

Although there is an obvious difference between both Nawashf_W. and Nawashf_R in fruit color (Table 2), RAPD estimated their genetic similarity as 100%, which is clearly not true. Accordingly, RAPD is not the best used method to estimate genetic similarity between the cultivars. Similar results were found with (Van De Zande, and Bijlsma, 1995).

The genetic similarity between the different cultivars was used to draw a dendrogram (Figure 10) and to find out the relationships among the cultivars and their association with tested traits and locations.
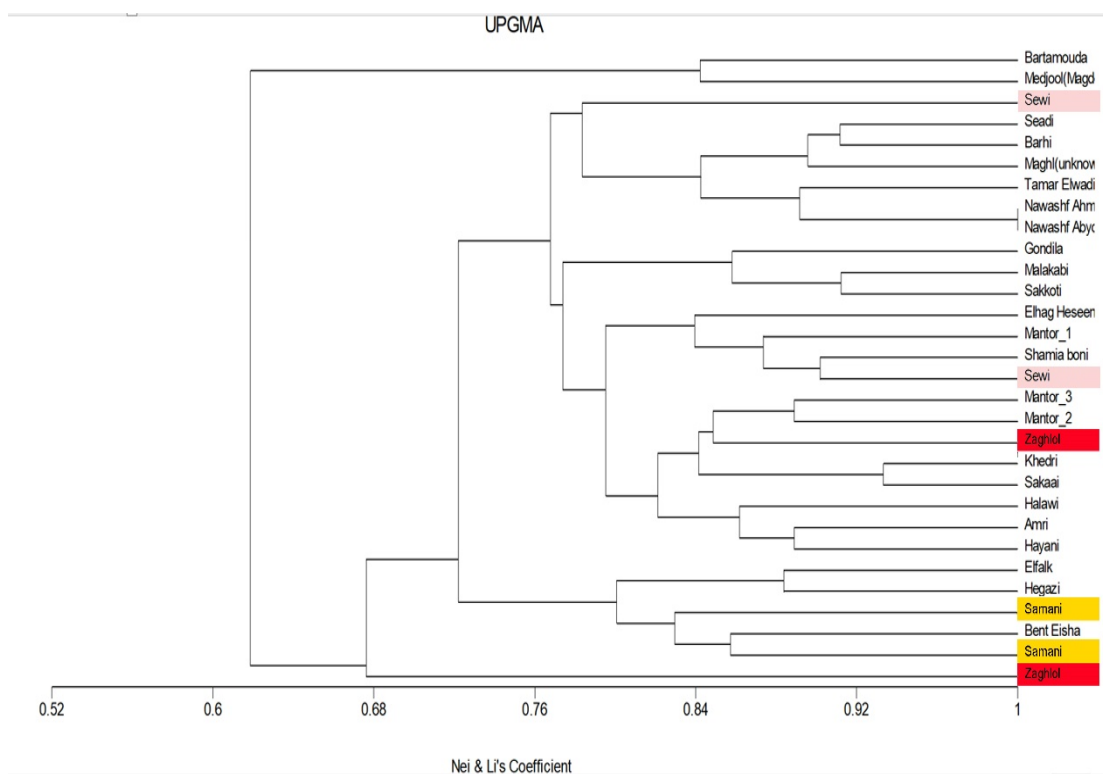
Figure 10. Dendrogram demonstrating the relationship among thirty Egyptian date palm cultivars based on RAPD data (Cultivars with same highlighted color have the same name but cultured in different locations).

Results in Figure (10) showed that RAPD primers couldn't group the cultivars into clusters associated with the studied fruit characteristics or with geographical location of collection.

RAPD-PCR was used to genetically differentiate between the cultivars that have the same name but collected from different locations. Results in Table (5) showed the genetic similarity between Sewi from Assiut1 and Sewi* from Assiut2 were 0.77., figure 10 shows that they were located in different clusters in the dendrogram. Moreover, they showed significant differences in the tested morphological traits (Table 6). From these results, we could conclude that these 2 cultivars are genetically and morphologically different, but they were wrongly named with the same name. Farmers are using morphological characteristics to identify and name a certain cultivar (Jarvis *et al.*, 2007).

Similar results (Table 6) were obtained with Zaghlol and Samani from different locations. Thus, it could be confirmed that there are genetic variations between the cultivars with same name cultivated in different locations. These results were also obtained and compatible with Elmeer *et al.*, (2019) who assessed the genetic diversity of the same cultivar (shishi) in different locations (saudi arabia and qatar) using microsatellite markers.

On the other hand, a high genetic similarity (0.81) was observed between both the Sewi cultivar collected from Assiut and Seadi collected from New Valley. Results in Table (6) also showed that there were no significant differences in the tested traits between them. So, we assume that they are the same cultivar, but the name was changed wrongly after years of transferring and adaptation.

Table 6. Shows the genetic similarity between cultivars from different locations and their fruit physical characteristics

cultivars	Location	Genetic similarity	Fruit physical characteristics					
			Length	Diameter	Shape index	Weight	seed weight	Flesh weight
Sewi	Assiut 1	0.77	0.0016399**	0.0010053**	0.6872981[NS]	0.0010053**	0.0010053**	0.0010053**
Sewi*	Assiut 2							
Sewi	Assiut 1	0.81	0.8999947[NS]	0.8999947[NS]	0.8999947[NS]	0.8999947[NS]	0.44966[NS]	0.8999947[NS]
Seadi	New Valley							
Sewi*	Assiut 2	0.85	0.0013127**	0.0010053**	0.6470641[NS]	0.0010053**	0.0010053**	0.0010053**
Seadi	New Valley							

**highly Significant at 0.01 level., NS non-Significant

The domestication of date palm and the characteristics of date palm geographical culture may have had a significant influence on the genetic makeup of date palm. Mixing the ways of propagation by farmers sometimes with offshoots which have the same characteristics of the established clonal mother tree, and sometimes with seeds followed by selection of the most productive varieties, would have obtained new date palm varieties. The combined consequences of all these actions could result in a mixed genome within the same geographical location (Elshibli and Korpelainen, 2008).

Based on these findings two problems could be addressed: 1. There are cultivars with same name over different locations that have significant differences

between them.2. There are cultivars that have no morphological or genetical significant differences but have different names. So, naming the different cultivars based on morphological characterization is not useful or accurate. As a result, a new way of naming is strongly recommended based on genetic background using molecular markers or sequencing. These results led the researchers to use molecular genetic differences to differentiate between the date palm varieties (Jarvis *et al*., 2007).

Conclusion

Fruit physical characteristics and RAPD-PCR were used to differentiate between 30 date palm cultivars collected from different locations. RAPD-PCR was also used to obtain molecular markers for date palm identification of some economic cultivars. Cultivars naming problem was observed, so a new way of naming is strongly recommended based on the genetic background using molecular markers or sequencing.

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التوصيف الجزيئي لبعض أصناف نخيل البلح المصرية باستخدام تقنية الـ RAPD-PCR

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الملخص

تم اختبار ثلاثين صنفًا من أصناف النخيل المصرية المختلفة الأنواع المجمعة من ثلاث محافظات هي أسيوط والوادي الجديد وأسوان. تم دراسة الصفات الظاهرية للثمرة كأبعاد الثمرة ووزن الثمرة والنواة ووزن اللحم للأصناف المختلفة وأظهرت النتائج اختلافات معنوية بين الأصناف وبعضها البعض في كل الصفات المدروسة وأظهر صنفي السماني والمجدول أعلى النتائج بالنسبة لصفات أبعاد الثمرة ووزن الثمرة ووزن اللحم. تم دراسة الاختلافات الوراثية بين الأصناف باستخدام الواسم الجزيئي RAPD. أظهرت جميع البادئات الخمسة معدلات عالية لتعدد الأشـــــــــكال بين الأصناف تراوحت بين 75- 100 %. وتم إيجاد ثلاثة عشر واسم جزيئي سلبي وإيجابي مرتبط ببعض الأصـــناف. تم حسـاب درجة التشـــابه الوراثي بين الأصـــناف المختبرة وتراوحت النسـبة بين 40 – 100 %. وكان صـنفي النواشـف الأحمر والأبيض هما الأقرب وراثيًا بنسـبة 100%. وأظهرت النتائج أن الأصناف التي لديها نفس الاسـم، ولكن تم زراعتها في مواقع جغرافية مختلفة تختلف وراثيًا. كما أنها تختلف عن بعضها البعض اختلافًا معنويًا على مسـتوى صفات الثمرة.