

## Genetic Diversity of Egyptian Date Palms (*Phoenix dactylifera* L.) Using Morphological and Molecular Markers

Sara Aly<sup>1</sup>, Mohamed E. Saad<sup>2,3</sup>, E. A. Madboly<sup>4</sup>, Hoda S. Barakat<sup>1</sup> and H. El-Atroush<sup>1\*</sup>

1- Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

2- Genomics Facility, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.

3- Department of Biology, Faculty of Sciences, Taibah University, Al-Madinah Almonawarah, Kingdom of Saudi Arabia

4 -The Central Laboratory for Date Palm Research and Development (CLDPRD), Agriculture\ Research Center (ARC), Giza, Egypt

E-Mail : [hala\\_elatroush@hotmail.com](mailto:hala_elatroush@hotmail.com)

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### ABSTRACT

Morphological and molecular analyses were conducted to evaluate the genetic diversity among eleven Egyptian date palms (*Phoenix dactylifera* L.). Ten SSR primer pairs revealed a high percentage of polymorphism (97.4%) among 38 total number of alleles, while 11 SCoT primers produced 48.5% from 171 amplified fragments. SCoTs recorded effective multiplex ratio (EMR) and PIC values of 7.5 and 0.14, respectively, opposing to 3.7 and 0.26 for SSRs, respectively. A similar marker index has thus resulted in both markers. Nei's gene diversity (h) was generally low for both marker systems indicating homogeneous nature and narrow genetic background for the studied Egyptian date palms, which was also emphasized through their high genetic similarity estimates. The results of UPGMA cluster analysis and PCoA grouped the eleven date palms according to their geographical location and sex. In addition to classical morphological characters, the current study confirmed the importance of using SCoTs as functionally gene-based multi-loci markers besides nuclear SSR loci to assess the genetic variations and cultivar identification among Egyptian date palm genotypes.

### INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most ancient cultivated fruit crops belong to family Arecaceae. It is mainly grown in the arid regions of the Middle East and North Africa (Barrow, 1998; Zohary and Hopf, 2000; Chao and Krueger, 2007). Egypt is the world's largest date palm average yield producers, representing about 20% of global production (FAO, 2014). Biodiversity conservation of date palm is a crucial concern to maintain the diverse number of date palm cultivars in Egypt (Rizk *et al.*, 2004). Progress in any genetic conservation scheme relies on understanding the genetic variation existing in the gene pool (Jubrael *et al.*, 2005; Jaradat, 2014). Moreover, new cultivars have been introduced from different countries that may result in the establishment of novel recombinant

genotypes which introduce novel genetic variations among date palms in different geographical sites (Elshibli and Korpelainen, 2008). Also, the genetic diversity of date palm is endangered by some factors such as soil salinity, desertification, ecosystem and climate change. In addition to the fact that the genetic relationships between cultivars are not well documented (Jaradat, 2016), the precise number of Egyptian date palm cultivars is still argued because some cultivar names are synonyms as well as different cultivars are given the same name (Jain *et al.*, 2011; Bekheet and El-Sharabasy, 2015). The previous factors necessitate re-evaluating the genetic structure and diversity of date palm that helps in using these precious genetic resources for better identification, classification and conservation, which is crucial in developing breeding programs and also combating any potential genetic erosion (Arabnezhad *et al.*, 2011; Jaradat, 2016). Consequently, regional and international cooperation is needed to develop a complete DNA fingerprint database and phylogenetic relations for all the known date palm cultivars (Khanam *et al.*, 2012). This may help in building a date palm cultivar identification system through specific fingerprints for each cultivar. Also, will facilitate the verification of the identity of juvenile date palm offshoots to be easily implemented in any official certification scheme for new as well as existing cultivars.

Many efficient approaches have been developed to explore the genetic diversity of date palm. Morphological traits are one of the oldest methods that were used by many investigators to evaluate date palm genetic resources (Elhoumaizi *et al.*, 2002; Rizk *et al.*, 2004; El Sharabasy and Rizk, 2005; IPGRI *et al.*, 2005; Salem *et al.*, 2008; Hammadi *et al.*, 2009; Ahmed *et al.*, 2011). Despite the usefulness of using phenotypic characters in date palm biodiversity analyses, it is not completely dependable. This is owing to the plant's large size together with its long generation time. Further, the date palm cultivars have homogenous vegetative traits and vary mostly in their fruit parameters. In addition, some of the morphological features are prone to environmental influences (Wünsch and Hormaza, 2002; Jaradat, 2014).

Molecular techniques have been reported to be a shotgun approach that successfully provides better diversity analyses for date palm germplasm, and even can differentiate between closely related cultivars (Elhoumaizi *et al.*, 2002; Arabnezhad *et al.*, 2011). Numerous investigators estimated the genetic relationships and provided comprehensive information on the genetic diversity of date palm cultivars using several molecular marker techniques: RAPD (El-Yazal *et al.*, 2017), ISSR (Abd EL-Azeem *et al.*, 2011), AFLP (El-Assar *et al.*, 2005), 18S rRNA (Ahmed *et al.*, 2006), SSR (Ibrahim *et al.*, 2014; Al-Najm *et al.*, 2017; Naeem *et al.*, 2018; Mattat *et al.*, 2019), SCoT, CDDP and ITAP (Adawy and Atia, 2014; Al-Qurainy *et al.*, 2015). The present work was undertaken to detect the degree of genetic diversity and relationships among eleven Egyptian male and female date palm genotypes utilizing SSR and SCoT markers as well as morphological characters. The effectiveness of both used molecular marker systems was also addressed.

## MATERIALS AND METHODS

### Plant Materials:

Eleven Egyptian date palm (*Phoenix dactylifera* L.) genotypes were studied including six male and five female date palms. The female Sewi genotype was collected from two locations (Table 1). Sewi produces a semi-dry fruit while Amhat, Zagloul, and Hayani bear soft fruits. As the genotype names are usually given depending on fruit characteristics, that is why male cultivars are commonly possessing vague identification. Males 1, 2, 3, and 4 were collected from a private farm in Sakara, Giza Governorate, and were assigned as numbers rather than names for accuracy. The other two males collected

from Bahariya oasis, Giza were identified a long time ago by the Central Laboratory for Date Palm Research and Development (CLDPRD), Agriculture Research Center (ARC), Giza, Egypt.

**Table 1:** Identification of the eleven *Phoenix dactylifera* genotypes used in the study.

S.	Cultivar	Sex	Collection	
			Coordinates	Region
1	Male 1	Male	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
2	Male 2	Male	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
3	Male 3	Male	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
4	Male 4	Male	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
5	Sewi m1	Male	N 28° 22' 40'' E 29° 6' 54''	Bahariya Oasis , Giza
6	Sewi m2	Male	N 28° 22' 40'' E 29° 6' 54''	Bahariya Oasis, Giza
7	Sewi	Female	N 28° 22' 40'' E 29° 6' 54''	Bahariya Oasis, Giza
8	Amhat	Female	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
9	Sewi	Female	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
10	Zagloul	Female	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza
11	Hyani	Female	N 29°51'51.8'' E 31°13'49.7''	Sakara, Giza

### Morphological Assessment:

The morphological characterization of the eleven plants was performed in their natural fields. The palm trunk height and circumference were measured for each cultivar. Three mature leaves of each tested palm were detached and the length of leafleted part (LP), the spinted part length (SL), the number of leaflets (LN), and spines (SN) were determined. Also, the number and length of the new leaves, bunches, and the amount of the produced fruits were recorded.

### Molecular Assessment:

#### DNA Extraction:

Young leaves were thoroughly rinsed with water, washed in 70% ethyl alcohol and dried by clean tissues. The leaves were then cut into small pieces, directly immersed in liquid nitrogen and stored in -80°C or directly applied to nuclear DNA isolation protocol. Plant nuclei were first isolated and DNA was then extracted from nuclei using the DNeasy plant mini-kit (Qiagen Inc., USA) according to the manual's instructions (Al-Mssallem *et al.*, 2013). The resulting DNA concentration and quality were estimated using Thermo Scientific Nanodrop 2000 spectrophotometer and electrophoretic separation on agarose gel matrix (0.8%).

### Simple Sequence Repeat (SSR) Amplification:

SSR-PCR reactions were conducted using thirteen primers (Eurofins, Germany). Only ten of which gave amplification with clear patterns and used for further analyses. These primers were designed depending on the first published microsatellite-enriched library in *Phoenix dactylifera* that targets (GA)<sub>n</sub> repetitive nuclear loci (Billotte *et al.*, 2004). Sequences of primer pairs are indicated in Table 3. The reaction was performed in a final volume of 25 µL which contains 200 µM of dNTPs, 1.5 mM of MgCl<sub>2</sub>, 1X of green GoTaq Flexi buffer, 10 pM of each primer, 40 ng of template DNA, 1U of GoTaq Flexi DNA Polymerase (Promega, USA). Amplification was carried out in a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, USA) programmed as follows: 94°C/5 min (1 cycle); [94°C/45 sec., 50, 55 or 60°C/45 sec, 72°C/1 min] (35 cycles); 72°C/7 min (1 cycle) and 4°C (infinite). Annealing temperature 55°C was used for mPdCIR15, 60°C for SSRs mPdCIR50, mPdCIR48, and mPdCIR32, while 50°C was successful for the rest of primers. A volume of 10 µL of the SSR-PCR product was resolved using 3% agarose gel electrophoresis and further confirmed on 12% non-denaturing polyacrylamide gel for more precise scoring. Gels were stained with ethidium bromide and visualized under UV light using molecular imager gel doc XR<sup>+</sup> System with image lab software, (Bio-Rad, California, USA).

### Start Codon Targeted (SCoT) Amplification:

The SCoT technique is principally based on the preferential amplification of the ATG start codon using PCR. Eleven primers (18-mers) were employed (Eurofins, Germany) to estimate genetic variation among date palm genotypes (Table 4) according to Collard and Mackill (2009). Each 25 µL PCR reaction mixture consisted of 25 ng of nuclear DNA, 200 µM of dNTPs, 2 mM of MgCl<sub>2</sub>, 1X of green GoTaq Flexi buffer, 20 pM of primer, 1 U of GoTaq Flexi DNA Polymerase (Promega, USA). Samples were subjected to the following standard thermal profile for amplification in a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, USA) programmed as follows: 94°C/3 min (1 cycle); [94°C/1 min, 50°C/1 min, 72°C/2 min] (40 cycles); 72°C/5 min (1 cycle) and 4°C (infinite). PCR amplification products were resolved on 2% agarose gels in Tris-borate buffer, and then stained with ethidium bromide and visualized on a UV transilluminator.

### Data Analysis:

Only the clearest and strongest SSR and SCoT bands from the resulted patterns were scored manually as present (1) or absent (0) to be used for further analysis. The binary results were converted into similarity matrices according to Dice's coefficient (Sneath and Sokal, 1973). The numerical data resulted from the morphological examination were treated through the interval measure using Euclidean distance (Deza and Deza, 2009). Dendrograms based on the unweighted pair-group method with arithmetic averages (UPGMA) (Sokal and Michener, 1958) were constructed using SPSS software Version 16. Principal coordinates analysis (PCoA) was performed through PAST software (Hammer *et al.*, 2001). The program POPGENE Version 1.32 (Yeh *et al.*, 1997) was used to calculate variability parameters including the number of observed alleles (Na), the number of effective alleles (Ne), Nei's gene diversity (h), and Shannon index (I). The Polymorphic Information Content (PIC) was estimated by the Power Marker software Version 3.25 (Liu and Muse, 2005). The multiplex ratio (MR) was calculated as the total number of loci simultaneously detected per assay. The effective multiplex ratio (EMR) was estimated as the average number of polymorphic loci detected per assay (Powell *et al.*, 1996) and the marker index (MI) was used to calculate the overall utility of a marker system depending on the formula (Powell *et al.*, 1996; Azizi *et al.*, 2009):

$$MI = EMR \times PIC$$

## RESULTS AND DISCUSSION

**Morphological Characterization:**

The mean leaf length of the three Sewi cultivars (collected from Siwa oasis) was the highest one relative to others (Table 2). The leaf length varied from 370 cm for Zagloul and Male 4 to 510 cm for Sewi male 1 (Table 2). Sewi male 1 had the highest number of leaflets per leaf (250) where the lowest one was observed in Amhat and Sewi-Sakara (154). Sewi-oasis also recorded the lowest spine number (22). Although having long leaves and wide trunk, Sewi cultivars (collected from Siwa oasis) together with Sewi-Sakara showed the shortest trunks among the eleven date palms (Table 2). The number and length of the new leaves exhibited no great variation among the eleven palms in the four seasons. However, their number and length were generally greater in spring and summer relative to autumn and winter. The number of male bunches per palm ranged from 29 to 40 in the studied six males over two successive seasons, while it varied between 12 and 16 among the five female palms. Every female cultivar differed in yield: Zagloul cultivar had the lowest value and Amhat showed the highest one.

Morphological markers utilized in the current study to detect phenotypic variability among date palms have previously reported (Rizk *et al.*, 2004; El Sharabasy and Rizk, 2005). These authors examined 21 Egyptian dry and 21 soft date palms and illustrated that fruit and seed features were the most significant measures to distinguish cultivars while the leaf, crown, and spine characteristics were only useful in defining some soft cultivars. Our results agree with their findings. Further, surveying Moroccan, Mauritanian and Tunisian date palm cultivars has shown that vast differences were noticed using leaf and fruit characters (Elhoumaizi *et al.*, 2002; Salem *et al.*, 2008; Hammadi *et al.*, 2009). It was therefore concluded that morphological markers cannot be useful to identify the variability among date palm cultivars and other markers such as molecular ones might be promising in this issue (Hammadi *et al.*, 2009).

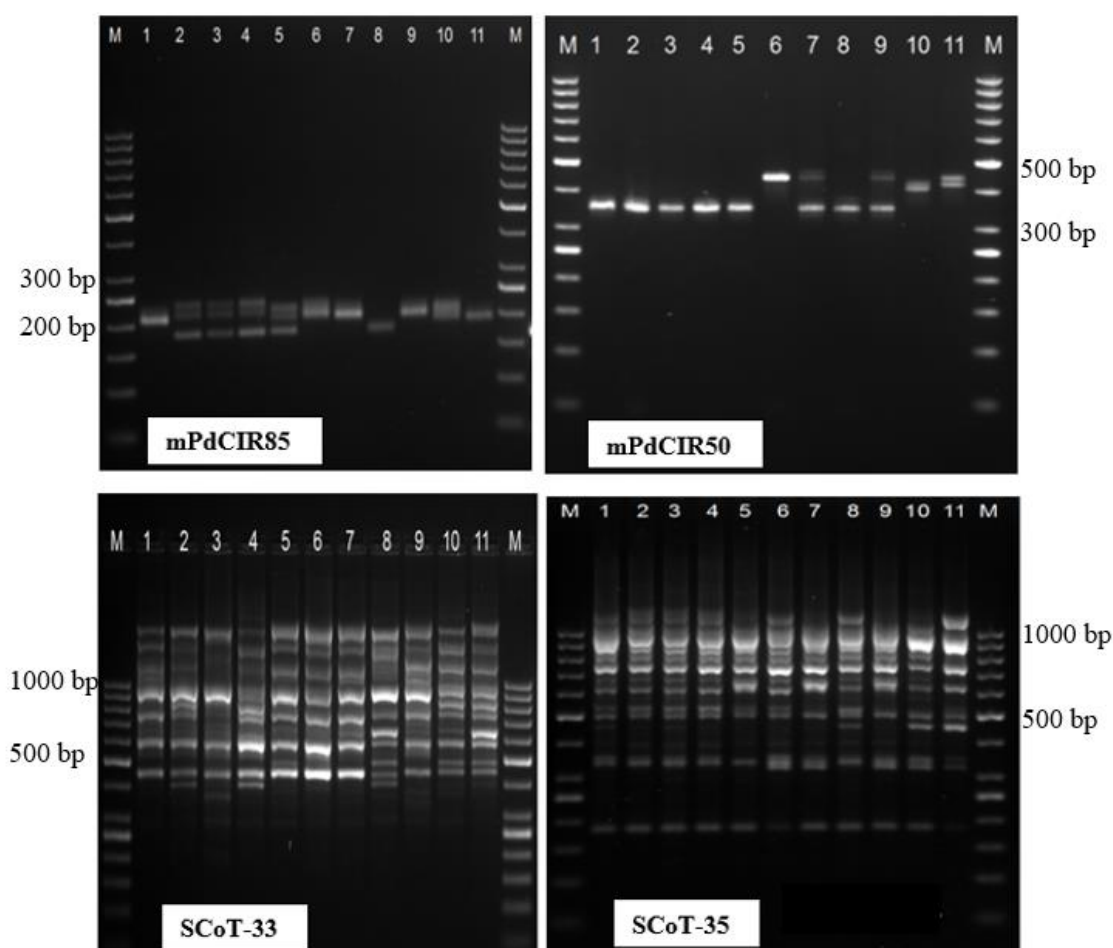
**Table 2:** Morphological characters of the eleven date palms. Each value is the mean of four seasons.

Genotype		Male 1	Male 2	Male 3	Male 4	Sewi m1	Sewi m2	Sewi-oasis	Amhat	Sewi-Sakara	Zagloul	Hyani
Character												
Leaf length (cm) LL		385	380	385	370	510	500	415	408	400	370	390
Length of leafleted part (cm) LP		315	305	305	290	380	375	320	330	290	275	290
Leaflets number LN		198	206	194	174	250	246	210	154	154	160	162
Spineted part length (cm) SL		70	75	80	80	130	125	95	78	110	95	100
Spine number SN		26	30	30	24	34	30	22	24	30	28	30
Palm dimensions	Circumference (m)	1.6	1.5	1.6	1.65	2.5	2.4	2.25	1.4	1.8	1.4	1.35
	Height (m)	6.5	6.25	6.5	6	4.5	4	5	5.5	5	7	6
Number of new leaves	Spring	7	7	6	7	7	6	6	5	6	5	6
	Summer	7	7	7	7	7	7	7	6	7	6	6
	Autumn	4	4	3	4	4	4	4	3	3	3	4
	Winter	3	3	3	3	3	3	3	3	4	3	3
Length of new leaves	Spring	1.5	1.6	1.7	1.8	1.9	1.7	1.7	1.4	1.6	1.5	1.6
	Summer	1.75	1.7	1.75	1.8	1.9	1.9	1.7	1.4	1.7	1.6	1.5
	Autumn	1.4	1.5	1.45	1.7	1.8	1.7	1.7	1.3	1.6	1.4	1.5
	Winter	1	1.1	1.2	1.2	1.3	1.2	1.2	1.1	1.3	1.2	1.2
No. of bunch / palm	Season1 (2014)	33	30	29	33	35	36	15	12	15	12	14
	Season2 (2015)	35	32	32	32	40	38	16	13	13	12	15
Yield / palm (kg)	Season1 (2014)	0	0	0	0	0	0	124	137	120	110	120
	Season2 (2015)	0	0	0	0	0	0	130	130	115	108	116



### Molecular Characterization:

SSR and SCoT primers used in this survey produced fingerprinting profiles that gave acceptable distinctions among date palm cultivars (Fig. 1) as they offer a wider genome coverage due to their diverse mechanisms in revealing polymorphism. A total number of 38 alleles (among which 37 were polymorphic) were produced with the 11 date palm genotypes using 10 SSR primer pairs in a size range of 90 to 463 bp (Table 3). However, 11 SCoT primers yielded a total number of 171 bands (of which 83 were polymorphic) from 210 to 2620 bp (Table 4). The SSR allele number per primer varied between 2 with mPdCIR32 locus and 6 for mPdCIR93 and mPdCIR50 loci, while the number of SCoT amplicons/primer varied between 10 with the primer SCoT-28 to 20 with the primer SCoT-13. The average numbers of microsatellite alleles per locus distinguished in this study (3.8) were similar to those produced by Ahmed and Al-Qaradawi (2009), Akkak *et al.* (2009), Arabnezhad *et al.*, (2011), Adawy and Atia (2014), and El Kadri *et al.* (2019) who recognized 4, 6.4, 4.8, 5.9 and 5.4 alleles per locus, respectively, when examining different date palm accessions. Other studies however reported higher allelic numbers: 21.4 (Elshibli and Korpelainen, 2008) and 31 allele per locus (Pintaud *et al.*, 2010) in studying 68 and 308 date palms, respectively. Adawy and Atia (2014) and Al-Qurainy *et al.* (2015) reported slightly lower averages for SCoT bands/primer than the present investigation (15.5) as they scored 10.1 and 7.1, respectively.



**Fig.1:** SSR and SCoT profiles for date palm cultivars separated on agarose gels. M refers to 50 bp DNA ladder (gene ruler). Date palm cultivars are numbered (1-11) as listed in table (1). Primer names are included in each image.

**Table 3:** List of SSR loci, primer sequences, number of alleles, polymorphic ones, percentage of polymorphism, allele size range and polymorphic information content (PIC).

S.	Locus		Primer sequence 5'-3'	Allele No.	Poly- morphic alleles (%)	Allele size range (bp)	PIC
1	mPdCIR93	F	ACCATTTATCATTCCTCTC	6	6 (100%)	164- 182	0.318
		R	GACCTTGGTAGCTGCGTTTCTT				
2	mPdCIR78	F	TTTTAGGAAGACGCACCACGAG	3	3 (100%)	289- 310	0.275
		R	CCTTCGCCATATACCCATCTTG				
3	mPdCIR85	F	TTAGCAGCAGAGGAAAAGGTTA	4	4 (100%)	182- 235	0.364
		R	CCCCCGAAGAGACGCTATT				
4	mPdCIR57	F	TCCGTAGCTCTGCGACCTCTGC	3	3 (100%)	270- 285	0.262
		R	AAGCTCGCCTACGCCTCCATTC				
5	mPdCIR50	F	CATCTGCCATTTCTTCTGACCA	6	6 (100%)	350- 463	0.247
		R	GACCGGCATTCTATTCTCTGTT				
6	mPdCIR48	F	GTAAAACGCACACCATAGAGGAT	5	4 (80%)	370- 413	0.206
		R	GAGCCCGTTGTTTAGAAGATGGAC				
7	mPdCIR32	F	TTGTCGTAATACCAGCAGGAA	2	2 (100%)	345- 350	0.318
		R	GGTGTGGAGTAATCATGTAGTAGA				
8	mPdCIR16	F	CCAGCGGGAAATGAAAAGGTAT	3	3 (100%)	170- 180	0.293
		R	GAAAGCAATCCCAATCGTCCAT				
9	mPdCIR15	F	GGTTGGCCGGTTGAGAG	3	3 (100%)	175- 200	0.151
		R	GGGCGCTTGTGTGGACTGAT				
10	mPdCIR10	F	CCGGACGTGAGGTGAAAAAG	3	3 (100%)	90-122	0.241
		R	TCGAGAAAGGAAGAGATGAAG				
	Total			38	37 (97.4%)		
	Average			3.8	3.7		

**Table 4:** List of SCoT primers, their sequences, total number of bands, polymorphic bands, percentage of polymorphism, allele size range and polymorphic information content (PIC). The ATG sequence is marked in bold in each primer.

S.	Primer name	Primer sequence	Total no. of bands	Poly- morphic bands (%)	Allele size range (bp)	PIC
1	SCoT-2	5'-CAACA <b>ATGG</b> CTACCACCC-3'	13	6 (46%)	375- 2300	0.156
2	SCoT-11	5'-AAGCA <b>ATGG</b> CTACCACCA-3'	14	4 (28.6%)	250- 2500	0.082
3	SCoT-13	5'-ACGAC <b>ATGG</b> CGACCATCG-3'	20	13 (65%)	285- 2620	0.183
4	SCoT-14	5'-ACGAC <b>ATGG</b> CGACCACGC-3'	17	4 (23.5%)	400- 1368	0.055
5	SCoT-16	5'-ACC <b>ATGG</b> CTACCACCGAC-3'	19	13 (68.4%)	210- 2015	0.186
6	SCoT-20	5'-ACC <b>ATGG</b> CTACCACCGCG-3'	17	6 (35.3%)	280- 2770	0.11
7	SCoT-22	5'-AACC <b>ATGG</b> CTACCACCAC-3'	11	6 (54.5%)	488- 1320	0.136
8	SCoT-28	5'-CC <b>ATGG</b> CTACCACCGCCA-3'	10	3 (30%)	280- 1230	0.072
9	SCoT-33	5'-CC <b>ATGG</b> CTACCACCGCAG-3'	18	13 (72.2%)	360- 1640	0.234
10	SCoT-35	5'-CA <b>ATGG</b> CTACCACCGGCC-3'	18	8 (44.4%)	185- 1180	0.149
11	SCoT-36	5'-GCAACA <b>ATGG</b> CTACCACC-3'	14	7 (50%)	545- 1950	0.118
Total			171	83 (48.5%)		
Average			15.5	7.5		

The present study showed a little number of SSR alleles and a high degree of polymorphism through the ten loci (97.4%) with an average of 3.7, while SCoT primers generated a lower percentage (48.5%) with a mean of 7.5. SSR polymorphic bands varied from 2 in mPdCIR32 to 6 in mPdCIR93 and mPdCIR50. The number of SCoT polymorphic bands varied from 3 in SCoT-28 to 13 in SCoT-13, SCoT-16, and SCoT-33. Locus mPdCIR85 recorded the highest PIC value (0.36) while mPdCIR15 had the lowest value (0.15). In addition, SCoT-33 recorded the highest PIC value (0.234) and SCoT-14 had the lowest one (0.05). Interestingly, both female Sewi cultivars showed an identical and specific pattern for mPdCIR50 locus with 3 alleles at 463, 445, and 350 bp. This could be utilized in cultivar identification strategies in date palm.

In agreement with the current results, the highly polymorphic nature of SSR was prominent as high levels of genetic polymorphism (81.6 and 93.3%) were observed across Libyan and Moroccan date palm samples (Bodian *et al.*, 2014; Racchi *et al.*, 2014). However, a lower percentage of polymorphisms (59 and 46%) among three and four Egyptian date palm cultivars, respectively, were indicated (Adawy and Atia, 2014; Ibrahim *et al.*, 2014). The percentage of polymorphism resulted from SCoT data (48.5%) in this study was similar to that reported by Adawy and Atia (2014) who showed 41.3%, and Al-Qurainy *et al.* (2015) who observed 42.6% in Saudi Arabian date palms. It is obvious that the more the number of individuals is examined, the higher the number of produced alleles and polymorphisms.

The comparison between the efficiency of both molecular marker systems (Table 5) showed that SCoTs reported a higher multiplex ratio (MR) and effective multiplex ratio (EMR) of 15.5 and 7.5, respectively, compared with 3.8 and 3.7 for SSR. However, the number of observed alleles (Na), the number of effective alleles (Ne), Nei's gene diversity (h), Shannon index (I) and Polymorphic information content (PIC) indices gave elevated values for SSR ( $1.97 \pm 0.16$ ,  $1.45 \pm 0.3$ ,  $0.28 \pm 0.14$ ,  $0.44 \pm 0.18$  and 0.268) when compared with SCoT ( $1.48 \pm 0.5$ ,  $1.3 \pm 0.39$ ,  $0.18 \pm 0.2$ ,  $0.26 \pm 0.29$  and 0.14), respectively. The marker index (MI) showed no difference between SCoT and SSR markers.

**Table 5:** Levels of polymorphism and comparison of informativeness with SCoT and SSR markers in the 11 date palm cultivars.

Parameter	Value	
	SCoT	SSR
Number of assay units	11 primers	10 primer pairs
Total number of amplicons	171	38
Multiplex ratio (MR)	15.5	3.8
Number of polymorphic amplicons	83	37
Polymorphism % per assay	48.5	97.4
Number of observed alleles (Na)	$1.48 \pm 0.5$	$1.97 \pm 0.16$
Number of effective alleles (Ne)	$1.3 \pm 0.39$	$1.45 \pm 0.3$
Nei's gene diversity (h)	$0.18 \pm 0.2$	$0.28 \pm 0.14$
Shannon index (I)	$0.26 \pm 0.29$	$0.44 \pm 0.18$
Effective multiplex ratio (EMR)	7.5	3.7
Polymorphic information content (PIC)	0.14	0.268
Marker index (MI)	1.05	1.02

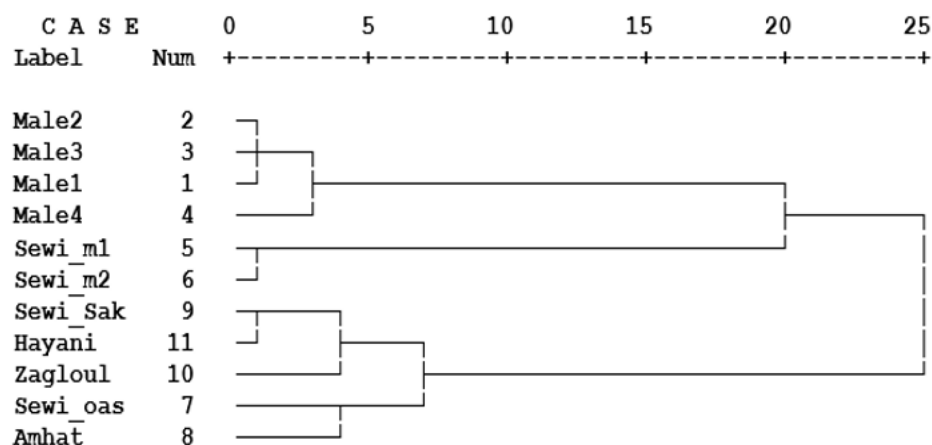
It is worth mentioning that SSRs offered an estimate for diversity parameters among various date palm genotypes. Higher PIC values, compared with the current study, were reported by Akkak *et al.* (2009) ranging from 0.15 to 0.79 in Algerian and Californian date palm cultivars. Congruently, an average PIC value of 0.60 among date palm genotypes from Iran, Iraq, and Africa was reported (Arabnezhad *et al.*, 2011). Furthermore, an average PIC value of 0.67, Shannon's information index of 2.067, and the number of effective alleles (Ne) of 7.76 were observed through 60 Australian, 12 Iraqi, and 10 Jordanian cultivars (Al-



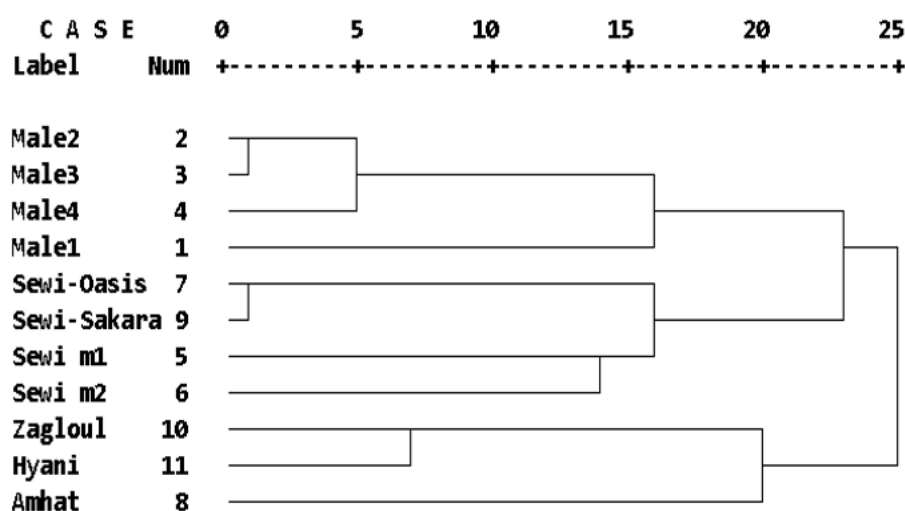
Najm *et al.*, 2017). In addition, the average genetic diversities of 0.8 and 0.66 were noticed across different Qatari date palm samples (Elmeer *et al.*, 2011; Elmeer and Mattat, 2015). However, Jaskani *et al.* (2016) reported a PIC mean value of 0.39, which agrees with PIC (0.26) obtained in this research. We believe that this was because the study used correlated date palm samples that represent parents and their progenies. It can be therefore concluded that lower diversity parameters detected in this study may reflect a more homogeneous nature and narrow genetic background of the studied Egyptian date palms, comparing with higher divergence found in cultivars investigated earlier or those studied samples from different counties. The narrow divergence among the Egyptian date palms, despite the recent introduction of some foreign cultivars, most likely attributed to the contention that the off-shooting process is the principal method for replantation in Egypt rather than cultivation from seeds that produce great variations. Depending upon only a few selected males that are frequently exchanged between farmers during hand pollination is also presumably one more factor that contributes to the narrow genetic background of the Egyptian date palms. On the other hand, Al-Qurainy *et al.* (2015) reported a much lower Nei's gene diversity (0.090) and Shannon's Information index (0.155) across 24 Saudi date palm cultivars using 11 SCoT primers relative with what was observed in this study. This may be explained by the notion that an actual lower genetic diversity among date palms cultivated in Saudi Arabia over the Egyptian ones or because some of the mutational gene states does not essentially exist in the studied populations.

#### **Genetic Relationships and Cluster Analysis:**

The dendrogram depending on phenotypic data (Fig. 2) obviously separated the six-date palm males in a distinct cluster from the five females. The male cluster is further subdivided where the 4 males collected from Sakara occupied one group and the two Sewi males in another group. The highest similarity was recorded between Males 2 and 3 and the lowest one was between Amhat and 'Sewi m1. The dendrogram resulted from the combined molecular data clearly separated the 11 date palm cultivars into two major groups (Fig. 3), with a genetic similarity estimate that ranged from 0.78 to 0.944. The first cluster is divided into two sub-clusters: the first comprises Males 1, 2, 3 and 4 with a shorter genetic distance between Males 2 and 3, similar to their morphological analyses, with 94.4% genetic similarity, and the lowest resemblance between Male 3 and 'Hyani (78%). The second sub-cluster includes the four Sewi cultivars shown in two branches. The remaining female cultivars Amhat, Zaghloul, and Hyani occupied the second group, with a closer distance between Zaghloul and Hyani than Amhat. It is obvious that morphological and molecular markers gave slightly different relations among genotypes, which can be attributed to fact that one morphological character can be produced from a combination of genes (Elhoumaizi *et al.*, 2002). Similarly, Adawy and Atia (2014) reported high genetic similarity among three Egyptian cultivars (i. e. Zaghloul, Hayani, and Samany) using SCoT primers that ranged from 0.85 to 0.95 while SSR primer pairs produced a genetic similarity range of 0.7 to 0.99. This high genetic similarity among Egyptian date palms was also confirmed by other investigations using different marker systems (RAPD, Soliman *et al.*, 2003; AFLP, El-Khishin *et al.*, 2003; Adawy *et al.*, 2005; and ISSR, Hussein *et al.*, 2005). In agreement with the cluster results, Adawy *et al.* (2005) grouped Sewi cultivars together in one sub-cluster, and Eissa *et al.* (2009) grouped Zaghloul and Hayani in the same cluster. In contrast to, other studies using SSR loci showed diverse genetic distances among Sudanese, Moroccan, Qatari, Iraqi, Mauritanian, Nigerian and Saudi date palm genotypes (Elshibli and Korpelainen, 2008; Ahmed and Al-Qaradawi, 2009; Khierallah *et al.*, 2011; Bodian *et al.*, 2012; Bodian *et al.*, 2014; Yusuf *et al.*, 2015).



**Fig. 2:** Dendrogram of eleven date palm cultivars constructed from the morphological data using Un-weighted pair-group Arithmetic Average (UPGMA) and similarity matrix computed according to Euclidian's coefficient.

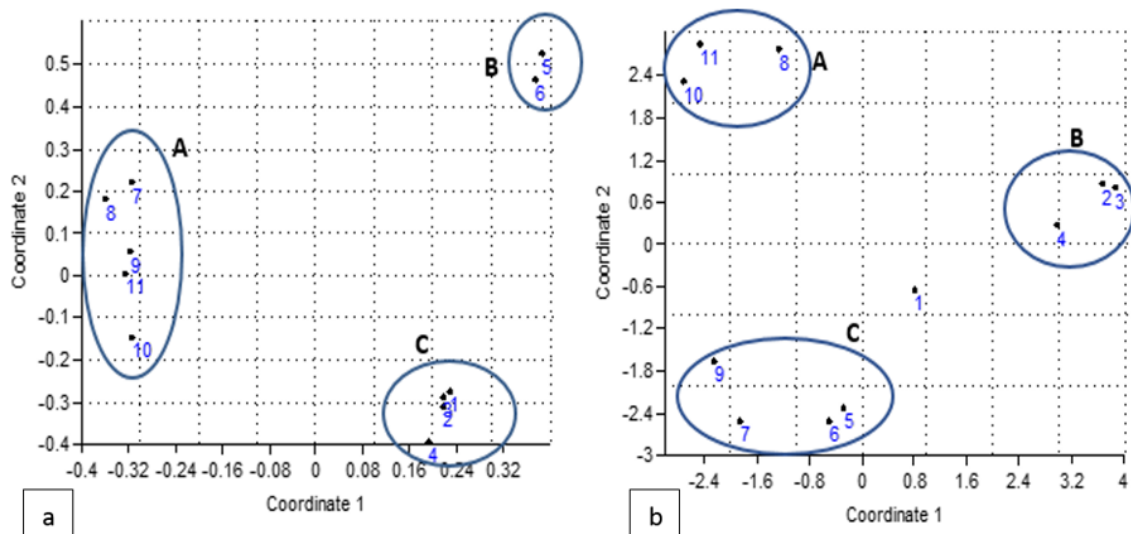


**Fig. 3:** Dendrogram of eleven date palm cultivars constructed from the combined SSR and SCoT data using Un-weighted pair-group Arithmetic Average (UPGMA) and similarity matrix computed according to Dice's coefficient.

### Principal Coordinates Analysis (PCoA):

PCoA analysis better-revealed distances amongst major groups while cluster analysis better resolved the relations between closely related individuals. It could be proposed that the relationships remarked in the principal coordinate analysis (PCoA) were congruent with the UPGMA analyses that grouped the eleven date palms according to their geographical location and sex. Morphological characters resulted in grouping date palm genotypes in 3 groups: one comprised the female cultivars as indicated by cluster analysis, and one had the 4 males from Sakara, while placed apart from the two male Sewi cultivars (Fig. 4a). The highest eigen values were reported for the first two axes representing about 97% of the total variation. PCo1 recorded 71% and PCo2 with 26.6%, with all other axes having small eigen values. PCoA scatter plot resulted from combined SSR and SCoT analyses showed three groups (Fig. 4b): one included the four Sewi cultivars, the other with 3 male cultivars from Sakara and the last with the remaining female cultivars, while Male 1 failed to be categorized in either one of these groups. It was noticed that the first three principal axes described about

70% of the total variation present in date palm samples with relatively large eigen values, where PCo1 accounted for 35.18%, while 23% and 13.8% were observed for PCo2 and PCo3, respectively.



**Fig. 4:** Scatter plot of principal coordinate analysis (PCoA) of eleven date palm cultivars based on morphological data, a; and combined molecular data, b. Date palm cultivars are numbered 1-11 as listed in Table 1.

## Conclusion

In this study, morphological and molecular approaches showed narrow divergence among Egyptian date palm cultivars. However, molecular markers had higher potential over morphological ones in discriminating between date palms by generating a unique molecular profile for each genotype. SCoT marker system proved its efficiency in producing a higher effective multiplex ratio compared with SSR. SSRs were superior in recording the variations among the studied population which was clear from their higher PIC value. Nevertheless, both molecular markers can complement each other. In addition, it was confirmed that SSR can accurately participate in cultivar identification strategies in date palm. Applying more markers and samples from different locations, however, can be more useful in the proper identification of economically important cultivars and more efficiently reassess their genome variations.

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