1587
Arab Univ.
J. Agric. Sci.,
Ain Shams Univ., Cairo
Special Issue, 26(2B), 1587-1598, 2018

MOLECULAR CHARACTERIZATION OF SOME SEEDED TYPE DATE PALM TREES IN EGYPT

[118]

Abu-Afifeh[1] A.A., Neima K. Al-Sanosy[2], Heba M. Ibrahim[3] and Soliman[2] Kh.A.

1- The Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), Damascus, Syria
ali-abuafifeh@acsad.org
2- Genetics Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt
3- Genetics Dept., Fac. of Agric., Cairo Univ., Giza, Egypt

Keywords: *Phoenix dactylifera*, Fingerprinting, Genetic similarity, RAPD, ISSR

ABSTRACT

In the present study, molecular fingerprinting among selected ten seeded type date palm trees growing at Al Dakhlah Oasis, New Valley Governorate in Egypt was carried out, in this approach 10 RAPD and 10 ISSR primers were used. In RAPD markers, primers 1, 3, 4, and 6 showed the highest level of polymorphism with number of polymorphic amplicons of 100%. While primers 7 and 8 resulted in the lowest number of polymorphic bands with polymorphism level of 62% and 63%, respectively. The similarity between Acsad-Dakhla 6 and 7 (0.76%) was observed as maximum similarity. While, Acsad-Dakhla 5 exhibited a minimum degree of similarity with all the seeded type date palm trees ranging from 33% to 49%. According to ISSR, highest polymorphism was generated by primers 8 and 9 (100%). While primers 4 and 2 produced the lowest polymorphism 50% and 55.6%, respectively. The highest similarity was observed between Acsad-Dakhla 8 and Acsad-Dakhla 9. Acsad-Dakhla 5 and Acsad-Dakhla 7 showed close relationship but Acsad-Dakhla 10 was at distant from all the seeded type date palm trees and did not lie in any subcluster. In combined analysis, the similarity between Acsad-Dakhla 6 and 7 were similar to Acsad-Dakhla 9 and 10 showed maximum similarity (85%). While, Acsad-Dakhla 2 and 5 exhibited a minimum degree of similarity with all the seeded type date palm trees (47%). A high genetic diversity among selected ten seeded type date palm trees was present.

(Received 3 February, 2018)
(Revised 18 February, 2018)
(Accepted 20 February, 2018)

1- INTRODUCTION

Date palm (*Phoenix dactylifera*) is a perennial monocotyledon dioecious plant and belongs to *Arecaceae* family **(Ahmad and Al-Qaradawi, 2009)** with a very slow growth rate. The genome of date palm contains 18 pairs of diploid chromosomes (2n = 2x = 36). The date palm genome appears similar size about 658 Mb (**Al-Dous et al 2011**) It's one of the most important strategic fruit trees in the Middle East and North Africa regions, because its high tolerance to environmental stresses in arid and semi-arid regions (**Adawy et al 2004**). In Egypt, people are used date palm daily due to its high nutritional value and health benefits of the fruits (**Bekheet, 2013**). In addition, helps to prevent erosion and protection of cereals (**Aaouine, 1998**). Also, show low incidence rate of cancer and heart diseases (**FAO, 1982**).

In the Arab countries the average number of date palm is estimated to be about 62 million trees (**El-Khishin et al 2003**). In Egypt there are 16 millions palm trees, while three quarter of them are fruiting (**FAO STAT, 2009**). According to statistics of the (FAO) for the period 2013-2014; Egypt is the top of the producers of dates palm in the world (1,694,813 tons), and following Iran (1,065,704 tons), Algeria (1,029,596 tons), Saudi Arabia (964,536 tons), United Arab Emirates (671,891 tons), Iraq (615,211 tons), Pakistan (494,601 tons), Sudan (439,120 tons), Oman (348,642 tons) and Tunisia (241,000 tons) (**FAO STAT, 2016**).

During the past decades, molecular methods were recommended to evaluate genetic variation between cultivars of date palm. The DNA markers which are based on polymorphisms found in DNA has greatly facilitated research in a variety of many biological branches such as taxonomy, phylogenetic relationships and genetics (**Abdelsalam et al 1998**). There are a number of molecular techniques available for characterization of the variation at the DNA level used to identify genetic relationships between the date palm varieties; like Random Amplified Polymorphic DNA (RAPD) is based on the use of a single short, usually 10-mer primer of arbitrary sequence (**Williams et al 1990**). The RAPD technique used effectively and widely to characterize many date palm varieties (**Khanam et al 2012**). Moreover, successful tool for assessing the genetic relationships and the genetic variability among genotypes of date palm that closely related (**Seif El-Yazal et al 2017**). In addition, RAPD used for phylogenetic studies in plant species (**Xuemei et al 2012**).

Inter-Simple Sequence Repeats (ISSRs) are semi-arbitrary markers amplified by using a single primer composed of a microsatellite repeated sequences (**Marsafari and Mehrabi., 2013**). ISSR strategy was therefore performed to access the DNA diversity among crop genotypes (**Zehdi et al 2004**). ISSR markers was used to examine the phylogenic relationships among date palm cultivars Tunisian. Furthermore, ISSR was used for determining the phylogeny of date palm cultivars in Saudi Arabia (**Al-Issa et al 2008; Munshi and Osman 2010). Haider et al (2012)** reported that ISSR technique was efficient to determine the genetic relationships in date palm grown in Syria. **Khierallah et al (2014)** suggested that RAPD and ISSR were effective for defecting genetic relationship and assessing genetic diversity in date palm cultivars of Iraqi. **Soliman et al (2009)** found specific marker of abnormality in date palm Barhi cultivars, so, they suggested that RAPD and ISSR techniques can detect the abnormalities produced from tissue culture derived of Barhi cultivar.

This research aims to do molecular fingerprinting for selected distinctive ten seeded type date palm trees in Egypt using molecular techniques. To identify these date palm trees and preserve genetic resources, identifying unique markers characterizing each palm, and determine the genetic relationships among these seeded type date palm trees were carried out.

2. MATERIALS AND METHODS

2-1- Samples collection

All samples were collected from original home in Mut, Al Dakhlah Oasis, New Valley Governorate in southwestern of Egypt, in October 2015.

Ten seeded type date palm trees were selected for its high productivity and distinctive fruit qualities which are competitive in international markets and evaluated by morphological, physical and chemical measurements.

2-2- DNA extraction

Total genomic DNA was extracted from 150 mg of female fresh leaves, using ZR Plant/Seed DNA MiniPrep™ kit. DNA was quantified by nano drop (2000c, Thermo Scientific), and then concentrations were standardized.

2-3- RAPDs amplification

A list of primers analyzed in this study is provided in **Table (1)** (Operon Technologies Inc., USA). The primers included 10 sequences selected from previously published reports based on reproducibility **(Heba, 2017)** and **(Grzywacz et al 2012)**. The PCR reactions were performed in a final volume of 25 µl containing 50 ng total genomic DNA, 12.5ul 2X MyTaq™ Red Mix kit (BIOLINE catalog N. BIO-25043) and 50 p mole primer. The PCR cycling parameters consisted of an initial denaturation at 92°C for 5 min followed by 40 cycles of denaturation at 92ºC for 30 seconds, annealing at 35ºC for 1 min, ramp up to 72ºC for 5 min, extension at 72ºC for 2 min and a final extension at 72°C for 10 min. The amplified PCR fragments were separated on 1.5% agarose gel in 1x TAE buffer (40 mM Tris-acetate, 1.74 M of glacial acetic acid and 1 mM EDTA, pH 8.3 at 25°C), stained with ethidium bromide (0.5 µg/ml) and visualized by Gel documentation (G:BOX) (SYNGENE model 680XHR, UK).

Table 1. Sequences of RAPD primers

No.	Name	5´ seq. 3´
1.	B10	CAGGCACTAG
2.	S13	TTCCCCCGCT
3.	S55	CATCCGTGCT
4.	S61	TTCGAGCCAG
5.	S97	ACGACCGACA
6.	S102	TCGGACGTGA
7.	S361	CATTCGAGCC
8.	S379	CACAGGCGGA
9.	B12	CCTTGACGCA
10.	P8	GGAGCCCAG

2-4- ISSR amplification

A total of 10 primers selected in **Table (2)**. The PCR reactions were performed in a final volume of 25 µl containing 50 ng total genomic DNA, 12.5ul 2X MyTaq™ Red Mix kit (BIOLINE catalog N. BIO-25043) and 50 pmole primer. The PCR cycling parameters consisted of an initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95ºC for 30 seconds, annealing at 54ºC for 1 min, extension at 72ºC for 2 min and a final extension at 72°C for 10 min. The amplified PCR fragments were separated on 1.5% agarose gel.

Table 2. Sequences of ISSR primers

No.	Name	5´ seq. 3´
1.	UBC811	GAGAGAGAGAGAGAGAC
2.	UBC818	CACACACACACACACAG
3.	UBC849	GAGAGAGAGAGAGAGAT
4.	UBC-822	TCTCTCTCTCTCTCTCA
5.	UBC-835	AGAGAGAGAGAGAGAGYC
6.	HB 12	CACCACCACGC
7.	UBC-845	CTCTCTCTCTCTCTCTRG
8.	UBC-817	CACACACACACACACAA
9.	844A	CTCTCTCTCTCTCTCTAC
10.	17898A	CACACACACACAAC

3- RESULTS AND DISCUSSION

The aim of the present study was to identify and cluster unknown Egyptian seeded type date palm trees according to their genetic background distance among them using the barcoding and RAPD techniques.

3-1- Random amplified Polymorphic DNA (RAPD)

In this study ten arbitrary decamer primers were used for the 10- seeded type date palm trees (named Acsad-Dakhla Dakhla 1, 2, ……, 10) as shown in **Table (3)**, 121 DNA fragments (amplicons) were produced. The polymorphism level of each primer was different from each other. So that they can be used for the DNA fingerprinting of these seeded type date palm trees. For instant, primers 1, 3, 4, and 6 exhibited the highest level of polymorphism with number of polymorphic amplicons of 100%. While Primers 7 and 8 resulted in the lowest number of polymorphic bands with polymorphism level of 62% and 63%, respectively. **(Table 3 and Fig. 1)**.

Table 3. Total number of amplicons, monomorphic and polymorphic amplicons as revealed by RAPD primers among the ten seeded type date palm trees

Primer	Total No. of Ampicons	Polymorphic amplicons	Monomorphic amplicons	% of Polymorphism
1	14	14	0	100.0
2	11	8	3	72.7
3	18	18	0	100.0
4	13	13	0	100.0
5	12	11	1	91.7
6	10	10	0	100.0
7	8	5	3	62.5
8	11	7	4	63.6
9	12	9	3	75.0
10	12	11	1	91.7
Total	**121**	**106**	**15**	**87.6**

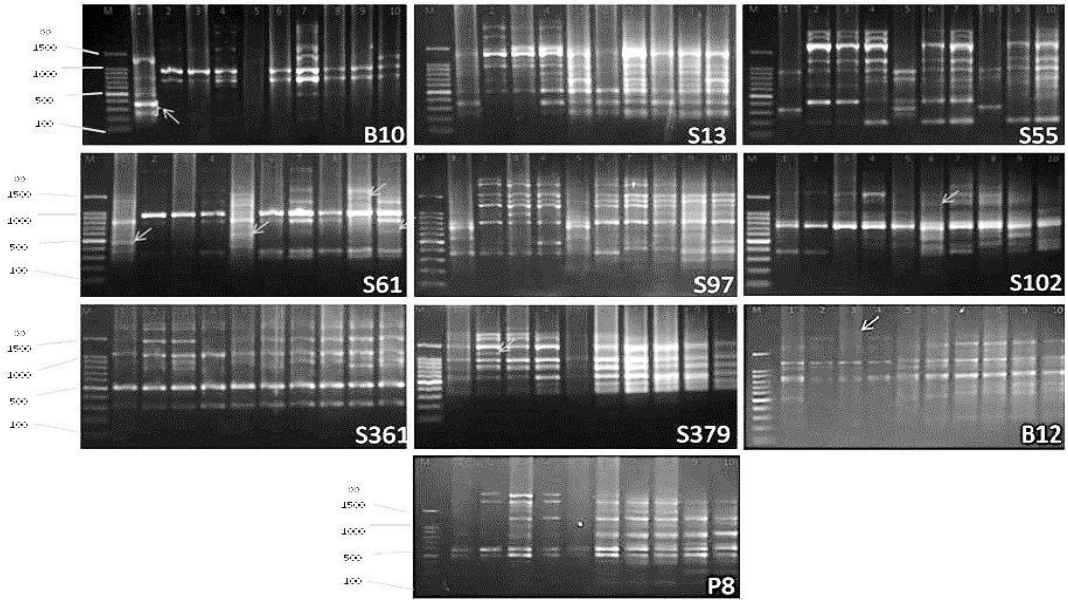

Fig. 1. Photographs showing RAPD products of the ten different seeded of Date palm using ten random primers. 1 (Acsad-Dakhla 1); 2 (Acsad-Dakhla 2); 3 (Acsad-Dakhla 3); 4 (Acsad-Dakhla 4); 5 (Acsad-Dakhla 5); 6 (Acsad-Dakhla 6); 7 (Acsad-Dakhla 7); 8 (Acsad-Dakhla 8); 9 (Acsad-Dakhla 9); 10 (Acsad-Dakhla 10) and M: DNA marker

The genetic distance based on Nei and Li's similarity coefficient are ranged from 0.33 to 0.76. Maximum similarity was observed between Acsad-Dakhla 6 and 7 (0.76). Acsad-Dakhla 5 in general showed a minimum degree of similarity with all the seeded type date palm trees ranging from 0.33 to 0.49 as shown in **Table (4)**.

Table 4. Similarity matrix based on Nei and Li's similarity coefficient of the 10 seeded type date palm trees obtained from RAPD markers

	Acsad-Dakhla 1	**Acsad-Dakhla 2**	**Acsad-Dakhla 3**	**Acsad-Dakhla 4**	**Acsad-Dakhla 5**	**Acsad-Dakhla 6**	**Acsad-Dakhla 7**	**Acsad-Dakhla 8**	**Acsad-Dakhla 9**	**Acsad-Dakhla 10**
Acsad-Dakhla 1	1.00									
Acsad-Dakhla 2	0.41	1.00								
Acsad-Dakhla 3	0.39	0.67	1.00							
Acsad-Dakhla 4	0.45	0.66	0.71	1.00						
Acsad-Dakhla 5	0.49	0.33	0.34	0.33	1.00					
Acsad-Dakhla 6	0.42	0.52	0.53	0.58	0.39	1.00				
Acsad-Dakhla 7	0.38	0.48	0.52	0.64	0.39	0.76	1.00			
Acsad-Dakhla 8	0.40	0.39	0.49	0.48	0.49	0.64	0.69	1.00		
Acsad-Dakhla 9	0.36	0.46	0.53	0.53	0.41	0.70	0.74	0.68	1.00	
Acsad-Dakhla 10	0.37	0.42	0.52	0.54	0.40	0.65	0.69	0.65	0.76	1.00

Cluster analysis by the unweighted paired group method of arithmetic mean (UPGMA) was generated and showed three clusters **(Fig. 1)**. Cluster A consisted of two seeded type date palm trees (Acsad-Dakhla 1, Acsad-Dakhla 5) with 0.49 Nei and Li's coefficient in the similarity matrix. Cluster B consisted of three seeded type date palm trees (Acsad-Dakhla 2, 3, and 4) with a 0.66-0.71 Nei and Li's similarity range. While, Cluster C consisted of five seeded type date palm trees (Acsad-Dakhla 6, 7, 8, 9 and 10) with a 0.64–0.76 Nei and Li's similarity range. This result was supported when a similar UPGMA dendrogram was generated using a statistically different multivariate software to analyze the data based on Nei's (1978) **(Fig. 2)** by generating bootstrap values.

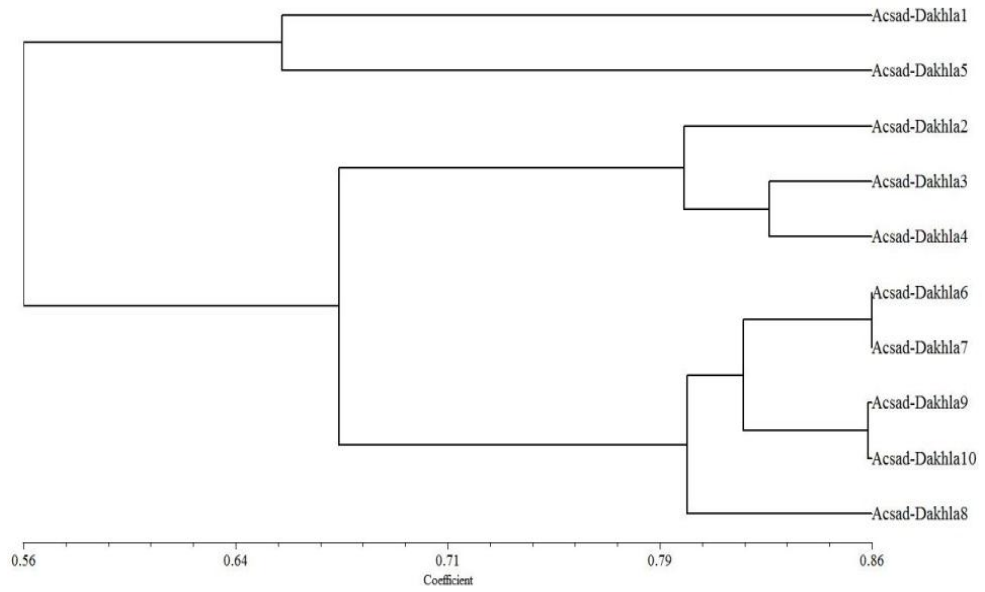

Fig. 2. A dendrogram of phylogenetic relationships among 10 seeded type date palm trees based on Nei and Li's similarity coefficient obtained from 10 RAPD primers

3-2- Inter Simple Sequence Repeats (ISSR)

Ten ISSR primers were used for this study as shown in **Table (2)**. The ten used primers successfully produced 94 total number of bands, of which 68 bands were polymorphic **(Table 5)**. The highest percentage of polymorphism (100%) was generated by primers 8 and 9. While primers 4 and 2 produced the lowest polymorphism with percentages 50 and 55.6, respectively.

Table 5. Total number of amplicons, monomorphic and polymorphic amplicons as revealed by ISSR primers among the 10 seeded type date palm trees

Primer	Total No. of Ampicons	Polymorphic amplicons	Monomorphic amplicons	% of Polymorphism
1	13	9	4	69.2
2	9	5	4	55.6
3	11	8	3	72.7
4	8	4	4	50.0
5	8	6	2	75.0
6	11	7	4	63.6
7	9	7	2	77.8
8	7	7	0	100.0
9	10	10	0	100.0
10	8	5	3	62.5
total	**94**	**68**	**26**	**72.3**

Similarity coefficient matrix was generated to cluster the data in order to compute precise relationships **(Table 6)**. The pair wise genetic similarity coefficient ranged from 0.58 to 0.82 indicating a genetic diversity of the tested seeded type date palm trees.

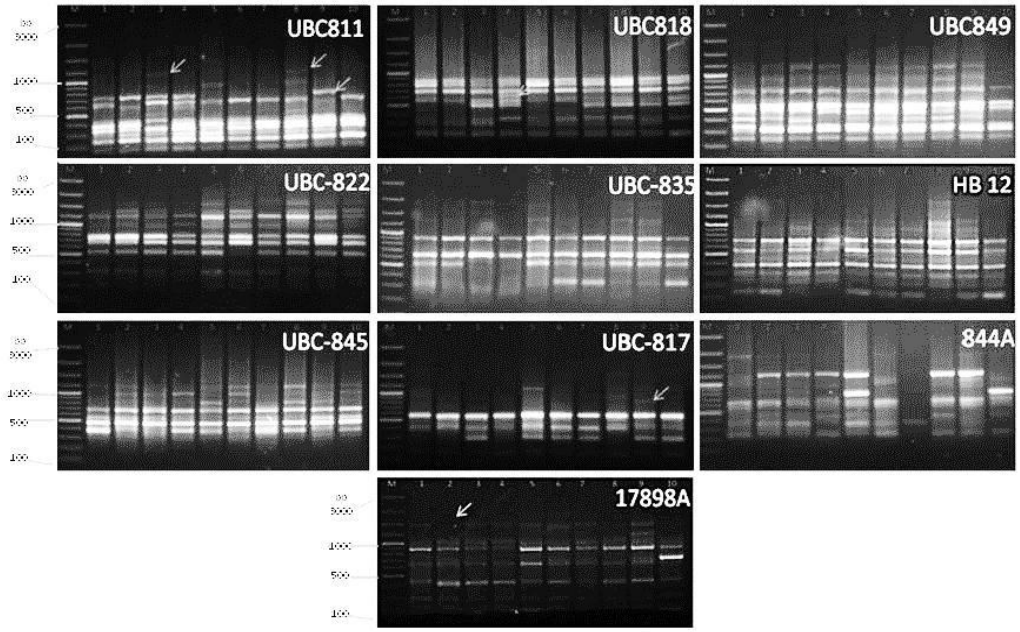

Fig. 3. Photographs showing ISSR products of the ten different seeded of Date palm using ten random primers. 1 (Acsad-Dakhla 1); 2 (Acsad-Dakhla 2); 3 (Acsad-Dakhla 3); 4 (Acsad-Dakhla 4); 5 (Acsad-Dakhla 5); 6 (Acsad-Dakhla 6); 7 (Acsad-Dakhla 7); 8 (Acsad-Dakhla 8); 9 (Acsad-Dakhla 9); 10 (Acsad-Dakhla 10) and M: DNA marker.

Table 6. Similarity matrix based on Nei and Li's similarity coefficient of the 10 seeded type date palm trees obtained from ISSR markers

	Acsad-Dakhla 1	**Acsad-Dakhla 2**	**Acsad-Dakhla 3**	**Acsad-Dakhla 4**	**Acsad-Dakhla 5**	**Acsad-Dakhla 6**	**Acsad-Dakhla 7**	**Acsad-Dakhla 8**	**Acsad-Dakhla 9**	**Acsad-Dakhla 10**
Acsad-Dakhla 1	1.00									
Acsad-Dakhla 2	0.77	1.00								
Acsad-Dakhla 3	0.69	0.79	1.00							
Acsad-Dakhla 4	0.66	0.72	0.69	1.00						
Acsad-Dakhla 5	0.68	0.65	0.65	0.66	1.00					
Acsad-Dakhla 6	0.76	0.72	0.67	0.69	0.69	1.00				
Acsad-Dakhla 7	0.71	0.68	0.67	0.66	0.76	0.76	1.00			
Acsad-Dakhla 8	0.62	0.62	0.67	0.68	0.77	0.69	0.71	1.00		
Acsad-Dakhla 9	0.65	0.65	0.68	0.62	0.72	0.74	0.66	0.82	1.00	
Acsad-Dakhla 10	0.65	0.60	0.58	0.63	0.63	0.68	0.65	0.63	0.61	1.00

The UPGMA (Unweighted Pair Group of Arithmetic Averages) analysis distributed the ten seeded type date palm trees into three main clusters **(Fig. 4)**. The data generated from ten ISSR primers were used to compute the genetic similarity index through Dice coefficient. The cluster 1 comprised 4 seeded type date palm trees Acsad-Dakhla 1, 6, 2, 3, and 4. The seeded type date palm trees Acsad-Dakhla 2 and 3 showed close relationship in comparison with Acsad-Dakhla 1 and 6 seeded type date palm trees. Acsad-Dakhla 4 was distant from other seeded type date palm trees. The cluster 2 was further divided into 2 subclusters, the highest similarity was observed between Acsad-Dakhla 8 and Acsad-Dakhla 9. Acsad-Dakhla 5 and Acsad-Dakhla 7 showed close relationship but Acsad-Dakhla 10 was at distant from all the seeded type date palm trees and did not lie in any subcluster.

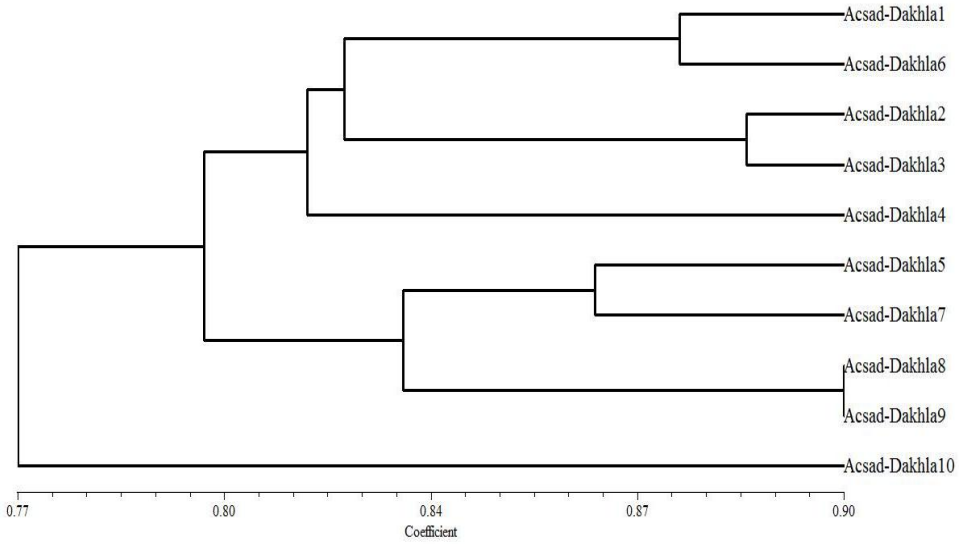

Fig. 4. A dendrogram of phylogenetic relationships among seeded type date palm trees based on Nei and Li's similarity coefficient obtained from 10 ISSR primers

3-3- Combined analysis

In the present study, dendrograms based on similarity values from RAPD and ISSR were constructed to reveal the genetic relationships among the ten seeded type date palm trees. The two applied techniques amplify different parts of the genomes (**Amel et al 2005**). This was partially reflected on the topology of the phylogenetic tree drawn from the data of the two assays **(Fig. 5)**. The dendrogram based on the data of two molecular DNA markers illustrates that the Acsad-Dakhla 6 and 7are most genetically similar (85%), and Acsad-Dakhla 9 and 10 also 85% similarity, among the ten studied seeded type date palm trees. This is followed by Acsad-Dakhla 7 and 9 with similarity of 83%. Acsad-Dakhla 3 is genetically related to both Acsad-Dakhla 2 and 4 with similarities of 82%. Both the Acsad-Dakhla 6 and 9 seeded type date palm trees are contained one group with similarity of 80%. Acsad-Dakhla 7 and 10 are related to each other with similarity of 80% too. On another hand, the lowest similarity is between Acsad-Dakhla 2 and 5 was observed 47%.

Table 7. Similarity matrix based on Nei and Li's similarity coefficient of 10 seeded type date palm trees obtained from from RAPD and ISSR markers

	Acsad-Dakhla 1	Acsad-Dakhla 2	Acsad-Dakhla 3	Acsad-Dakhla 4	Acsad-Dakhla 5	Acsad-Dakhla 6	Acsad-Dakhla 7	Acsad-Dakhla 8	Acsad-Dakhla 9	Acsad-Dakhla 10
Acsad-Dakhla 1	1.00									
Acsad-Dakhla 2	0.56	1.00								
Acsad-Dakhla 3	0.56	0.82	1.00							
Acsad-Dakhla 4	0.63	0.80	0.82	1.00						
Acsad-Dakhla 5	0.61	0.47	0.50	0.49	1.00					
Acsad-Dakhla 6	0.59	0.70	0.69	0.74	0.55	1.00				
Acsad-Dakhla 7	0.55	0.66	0.67	0.79	0.56	0.85	1.00			
Acsad-Dakhla 8	0.57	0.56	0.65	0.64	0.67	0.75	0.78	1.00		
Acsad-Dakhla 9	0.52	0.63	0.68	0.69	0.58	0.80	0.83	0.77	1.00	
Acsad-Dakhla 10	0.53	0.60	0.67	0.70	0.57	0.77	0.80	0.76	0.85	1.00

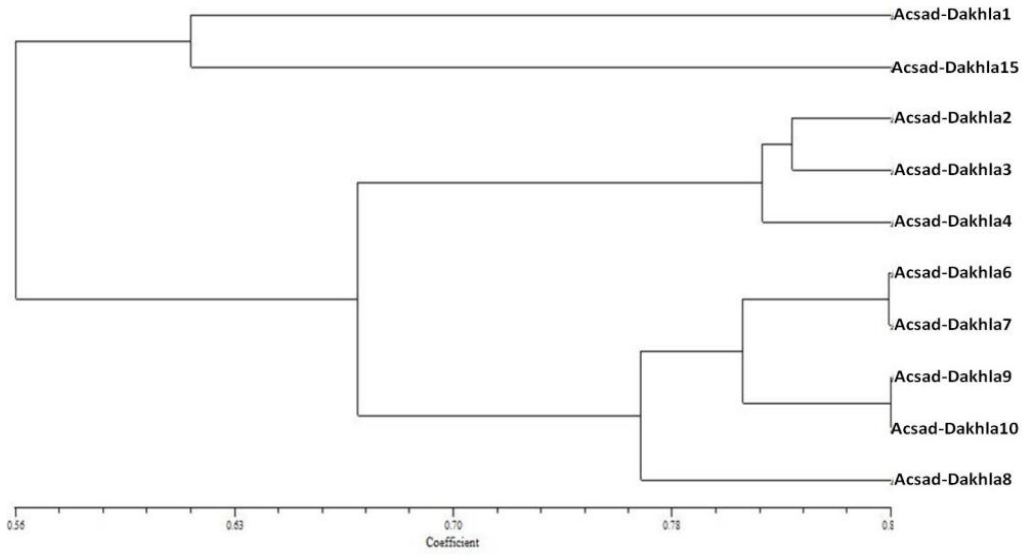

Fig. 5. A dendrogram of phylogenetic relationships among 10 seeded type date palm trees based on Nei and Li's similarity coefficient obtained from RAPD and ISSR markers

4- DISCUSSION

The present work was designed to study the genetic relationships among ten Egyptian seeded type date palm trees that growing at *Elwadi Elgadid*. To achieve this purpose, RAPD and ISSR were applied for each trees, different genomic markers and a wide range of polymorphism were identified. These results show that each seeded date palm tree is somewhat different and has its own specific genetic makeup at the level of coding sequences. Concerning the data of the DNA-markers, high genetic diversity for coding and non-coding sequences was indicated among the ten genomes. However, each technique exhibited different level of polymorphism and unique markers.

Overall comparison among seeded type date palm trees across the ten primers revealed the power of RAPD in distinguishing among date palm cultivars grown in the same location and these results were in line with (**Eissa et al 2009**) and are in harmony with (**Hemeid et al 2007**). RAPD technique is shown to be well appropriate to DNA fingerprinting (**Thormann et al 1994**). **Hemeid et al (2007)** constructed the dendogram based on similarity values from RAPD to reveal the genetic relationships between the nine cultivars of Date palm. The applied techniques amplify different parts of the genomes. Therefore, it found that the cultivars Q and K are genetically similar (71%), T and Q (69% similarity), both the O/T and G/HG cultivars were similar (68%), S and F are related with similarity of 67% and GA cultivar was genetically related to both Q and K cultivars with similarities of 78%.

Adawy et al (2004) detected the Polymorphism and genetic relationships among six Egyptian cultivars date palm *(Phoenix dactylifera L.)*; *Sakkoty, Bertmoda, Malkaby, Gandila, Fraihy and Siwi* using Combined RAPD, ISSR and AFLP data, and reported that different marker systems differ in the mechanism of detecting polymorphism, genome coverage and the ease of application. Therefore, they could complement each other to draw more accurate conclusions. Moreover, the RAPD and ISSR were efficient protocols for estimating the genetic variability and the genetic relationships among 14 accessions date palm in Egypt. Accordingly, the RAPD and ISSR are useful tools in date palm improving programs (**Ebtissam et al 2004**). **Younis et al (2008)** used RAPD and ISSR to identify sex-specific DNA markers for date palm cultivars, to select good male pollinators for increase the yield and improve the chemical and physical traits of fruits. Moreover, **Elsheikh et al (2014)** showed that ISSR marker able to estimate genetic relationships among the six different cultivars (*Saidi, Filfil, Degla, Megrew and Rutab Wadi, Saifi*) when were used ISSR for fingerprinting of date palm. Our results agree with **Xuemei et al (2012)** that the RAPD detect high levels of polymorphism among species indicating its efficiency to estimating the intra specific genetic diversity in the genus. Also, agree with **Sabir et al (2014)** used ISSR and AFLP protocols to differentiate among ten date palm cultivars growing at Saudi Arabia.

Seif El-Yazal et al (2017) used RAPD marker to analyzed five male seedlings of date palm and found that 75 bands were polymorphic and the two bands were monomorphic (96.4% Polymorphism percentage). Furthermore, the diagram exhibited that the five male seedlings different genotypes were classified into two major clusters. These results concluded that high genetic diversity among the date palm studied. Moreover, RAPD and ISSR have been used widely and effectively to characterize many date palm varieties.

REFERENCES

Aaouine, M. 1998. The application of biotechnology to date palm. In: Ives C, Bedford, B (eds.). Agric. Biotechnology in international development. **CABI, Wallingford, pp. 133-146.**

Abdelsalam, A.Z.E., El-Gewely, M.R., Ibrahim, S.A., Awad, A.A. and Abdelrazik, A.B. 1998. Biochemical and molecular genetic characterization of prokaryotic algae. **3rd Arab Conference Modern Biotech. and Areas of Application in the Arab World, 14-17 December, Cairo, Egypt, pp. 566-582.**

Adawy, S.S., Ebtissam Hussein, Dina El-Khishin, Moharam M. and Hanayia A. El-Itriby. 2004. Genetic variability studies and molecular fingerprinting of some Egyptian date palm (Phoenix dactylifera L.) cultivars: II. RAPD and ISSR profiling. **Arab J. Biotech., 5, 225-236.**

Ahmed, T. and Al-Qaradawi, A. 2009. Molecular phylogeny of Qatari date palm genotypes using simple sequence repeat marker. **Biotechnology, 8, 126–131.**

Al-Dous, E.K., George, B., Al-Mahmoud, M.E., Al-Jaber, M.Y., Wang, H., Salameh, Y.M., Al-Azwani, E.K., Chaluvadi, S., Pontaroli, A.C., DeBarry, J., Arondel, V., Ohlrogge, J., Saie, I.J., Suliman-Elmeer, K.M., Bennetzen, J.L., Kruegger, R.R. and Malek, J.A. 2011. De novo genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). **Nature Biotechnol, 29, 521-527.**

Al-Issa, A.M., Al-Helal, A.A. and Al-Saad, **F.A. 2008**. "DNA fingerprinting analysis for three date palm (Phoenix Dactylifera L.) cultivars grown at Al-Ahsa and Al-Qatif in Saudi Arabia using (ISSR-PCR) technique. **Magazine of Abd-Al-Aziz king," Astrology and Environment Sciences and Agriculture in Dry Lands, 19, 3-21.**

Amel, S.H., Khaled, Ch., Messaoud, M., Marrakchi, M. and Mokhtar, T. 2005. Comparative analysis of genetic diversity in two Tunisian collections of Fig cultivars based on Random Amplified Polymorphic DNA and Inter Simple Sequence Repeats Fingerprints. **Genetic Resources and Crop Evolution, 52, 563-573.**

Bekheet, S. 2013. Direct organogenesis of date palm (Phoenix dactylifera L.) for propagation of true - to - type plants. **Scientia Agric., 4(3), 85-92.**

Ebtissam Hussein, Adawy S. S., Ismail S. E. M. and El-Itriby, H.A. 2004. Molecular characterization of some Egyptian date palm germplasm using RAPD and ISSR markers. **Arab J. Biotech., 8, 83-98.**

Eissa, E.A., Abd EL-Razek, A., EL-Sharabasy, S. and Rizk, R. 2009. Morphological and molecular genetic characterization of softdate palm (Phoenix dactylifera L.) cultivars in Egypt. **Egypt. J. Genet. Cytol., 38, 269-284.**

El-Khisin Dina, Adawy, S.S., Ebtissam Hussein and El-Itriby, H.A. 2003. AFLP fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) **cultivars. Arab J. Biotech., 6, 223-234.**

Elsheikh, M.H., Elham, Z. Abd El-Motty, Elsabagh, A.S. and Yassin, R. 2014. Fruit properties and molecular characterization using ISSR markers of six Libyan date palm cultivars. **Research J. of Agric. and Biological Sci., 10, 47-52.**

FAO 1982. Plant production and protection. Paper No. 35. Date production and protection. FAO, Rome, Italy, **294 p**.

FAOSTAT 2009. Crop Production 2008. Statistics Division, Food and Agriculture Organization of the United Nations, Rome, Italy.

FAOSTAT 2016. Crop Production 2016. Statistics Division, Food and Agriculture Organization of the United Nations.

Grzywacz, B., Warchałowska-Sliwa, E., Banach, Z. and Pyza, E. 2012. Genetic variability and changes of elemental concentrations in cells of *Tetrix tenuicornis* (Orthoptera: Tetrigidae) from polluted and unpolluted areas. **Folia Biol (Krakow), 60, 17–25.**

Haider, N., Nabulsi1 I., and MirAli1 N. 2012. Phylogenetic relationships among date palm (*Phoenix dactylifera* L.) cultivars in Syria using RAPD and ISSR markers. **J. of Plant Biology Research, 1, 12-24.**

Heba, M. Ibrahim 2017. Assessment of Genetic Diversity and Relationships of Five *Mentha* Species Using RAPD Marker. **Current Science International. 6, 271-277.**

Hemeid A., Sanaa, A., Riad, A. and Abd El-Rahman, T. M. 2007. Molecular characterization of different date palm (*Phoenix datylifera* L.) cultivars grown in Siwa Oasis. **Egypt. J. Genet. Cytol., 36, 145-162.**

Khanam S., Sham, A., Bennetzen, J.L. and Aly, M.A.M. 2012. Analysis of molecular marker-based characterization and genetic variation in date palm (*Phoenix dactylifera* L.) - a review. **Aust.J. Crop Sci., 6, 1236–1244.**

Khierallah, H.S.M., Al-Sammarraie, S.K.I. and Mohammed, H.I. 2014. Molecular Characterization of some Iraqi date palm cultivars using RAPD and ISSR markers. **J. Asian Sci. Res., 4, 490-503.**

Marsafari, M. and Mehrabi, A.A. 2013. Molecular identification and genetic diversity of Iranian date palm (*Phoenix dactylifera* L.) cultivars using ISSR and RAPD markers. **AJCS, 7, 1160-1166.**

Munshi, A. and Osman, G. 2010. Investigation on molecular phylogeny of some date palm (*Phoenix dactylifra* L.) cultivars by protein, RAPD and ISSR markers in Saudi Arabia, **Aust. J. Crop Sci., 4, 23-28.**

Sabir, J.S., Abo-Aba, S., Bafeel Sameera, Zari T. A., Edris, S., Shokry, A.M., Atef, A., Nour, O. Gadalla, Ramadan, A.M., Al-Kordy, M.A., El-Domyati, F.M., Jansen, R.K. and Bahieldin, A. 2014. Characterization of ten Date Palm (Phoenix dactylifera L.) Cultivars from Saudi Arabia using AFLP and ISSR markers. **Comptes Rendus Biologies. 337, 6-18.**

Seif El-Yazal, S.A., Alharby, H.F., Seif El-Yazal, M.A., Hassan, G.M. and Rady, M.M. 2017. Molecular Identification of some Seedling of Date Palm (*phoenix dactylifera* l.) Males Trees. **The J. of Animal & Plant Sci., 27, 1287-1294.**

Soliman, A.Kh., Rizk, M.R. and El-Sharabasy, S.S.H. 2009. Genetic analysis of abnormalities in tissue culture-derived date palm (*Phoenix dacylifers L*.), Barhi cultivars. **Egypt. J. Biotechnol. 33, 122-134.**

Thormann, W., Sarah Molteni, Jitca Caslavska and Schmutz, A. 1994. Clinical and forensic applications of capillary electrophoresis. **Electrophoresis. 15, 3-12.**

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Res., 18, 6531-6535.**

Xuemei, P., Yan, C., Ziyi, Y., Weiming, Z., Qitang, L., and Changmei, L. 2012. DNA sequence and RAPD information re-affirms the taxonomic relationships between *Apocynumvenetum* L. and *Poacynumpictum* (Schrenk) Baill. **Pakistan J. Bot., 44, 1261–1266.**

Younis, R.A.A., Ismail, O.M. and Soliman, S.S. 2008. Identification of sex-specific DNA Markers for date palm (*Phoenix dactylifera* L.) using RAPD and ISSR techniques. **Res. J. Agric. Biol. Sci., 4, 278–284.**

Zehdi, S.L., Sakka, H.L., Rhouma, A., Salem, A.O.M., Marrakchi, M.L. and Trifi, M.L. 2004. Analysis of Tunisian date palm germplasm using Simple Sequence Repeat primers. **African J. of Biotech., 3, 215-219.**