DNA Fingerprinting and Characterization of some Egyptian Date Palm Cultivars Using Simple Sequence Repeats (SSRs)

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Abstract: The Date Palm (*Phoenix dactylifera* L), germplasm commonly cultivated in Egypt, shows a wide range of repining periods and fruit quality and is an unexploited resource for breeding programs. The main purpose of this study was to fingerprint 45 date palm genotypes and accessions and to construct a molecular database including the cultivars commonly grown in Egypt. An analysis of thirty three microsatellite simple sequence repeat (SSR) loci out of thirty five markers was performed to define distinct specific alleles across all loci, genetic similarity within cultivars in each region of five different locations and possibility of linking morphological traits with molecular data. In general, the results indicated the possible use of SSR analysis to detect cultivar-specific markers for forty five Date palm cultivars and accessions under investigation that can be used to discriminate among the cultivars and genotypes. The genetic distance among populations compared to each other (Hayani, Samany and Zagholol) which presented at different locations in this study, may or may not correlate with the geographical distance between them in some species, depending on natural and artificial factors involved in shaping the population genetic structure of the species. Based on our findings, it is clear that there is some discordance between the data corresponding to molecular variability and those related to the variability available for breeding purposes (phenotypic variability). However, the use of 33 polymorphic microsatellite markers to study Egyptian date palm germplasm suggested that this is reliable, efficient and effective marker system that can be used for diversity analysis, and subsequently in crop improvement programs.

***Keywords**:* Microsatellite loci, Specific alleles, genetic distance, morphological traits.

INTRODUCTION

Date palm (*Phoenix dactylifera* L., 2n = 36) is a perennial monocotyledonous fruit plant, belonging to the family of Arecaceae (Coryphoideae). The genome size is estimated to be approximately 658-Mbp long (Al-Dous *et al*., 2011). Palm tree is an excellent candidate for cultivation in arid and semi-arid regions of the world due to its high tolerance to environmental stresses. In Egypt, date palm is one of the most important fruits and widely distributed in different districts. There are 3 main types of dates based on fruit moisture content, i.e., soft, semi - dry and dry cultivars (Adway *et al.,* 2005). Date palm is an important economic crop in Egypt where the world's largest producer over the last two years is Egypt with 1,470,000 Mt followed by Islamic Republic of Iran (1,066,000 Mt) and Saudi Arabia (1,050,000 Mt) (FAO stat, 2012).As with many other plants, genetic diversity of date palm is threatened by habitat loss due to population pressure and clearance for agriculture development. Moreover, developing elite cultivars using a few genetic materials from gene pool and using off-shooting propagation intensively in date palm breeding could cause loss of genetic diversity (Zhao *et al.*, 2013).

A variety of morphological characters of date fruits (viz., shape, size, weight, color, aspects of fruit skin, consistency, texture, etc.) and biochemical markers like isozymes and proteins (Abdulla and Gamal, 2010) have earlier been employed for the identification of date fruits. However, these traits are greatly influenced by environmental factors as well as the developmental stages of the plant.

Nowadays, molecular markers, based on polymorphisms at DNA level, are increasingly used and proved effective to assess genetic diversity. Data based on molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLPs), have been used to characterize date palm genotypes. Among molecular markers, microsatellites, also known as Simple-Sequence Repeats (SSRs), because of their particular features such as their codominant nature and their typically high levels of allelic diversity at different loci, represent a suitable tool for genotyping. The usefulness of microsatellite markers for measuring the genetic variability in a wide range of plants has been recently reviewed (Elsheikh *et al.,* 2014). Because of their high mutation rates and the ease of the analysis, Microsatellite markers were proved useful and effective for phylogenetic studies genetic fingerprinting and cultivar identification among different date palm accessions in Egypt (Adway *et al.*, 2005).

However, genetic variation is a basic requirement for plant breeding, whereas a high genetic variation is needed for genetic improvement of date palm. In recent years, genetic markers are increasingly for the study of genetic diversity. Therefore, the polymorphism determined by these markers is one of the valuable parameters for studying cultivars and understanding their genetic difference. The high reproducibility of Microsatellite markers may be because of their large number, distribution throughout the genome, co-dominant inheritance, neutrality with respect to selection and easy automation of analytical procedures of SSR technique. Microsatellite markers were used for the analysis of genetic differentiation among date palm cultivars, in which 33 loci out of 35 microsatellite loci for 45 date palm genotypes and accessions allowing the estimation of genetic diversity within.

MATERIALS AND METHODS

Plant materials:

The date palm materials were collected from different locations in Egypt. Forty-five genotypes and accessions were chosen for their good fruit quality

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Table (1). Three trees per each genotype were selected; most of them were vigorous vegetative propagated using off shooting.

DNA Isolation:

For DNA extraction, three young leaves were collected from each adult tree and three plants per cultivar were subjected to molecular analysis. Total genomic DNA was extracted according to the basic DNA extraction protocol of (Dellaporta *et al.,* 1983) with slight modifications by (Porebski *et al.,* 1997). A weight (0.2 g) from young leaves were ground in liquid nitrogen to fine powder and extracted using 10 ml preheated (65º C) cetyl hexadecyl-trimethyl ammonium bromide **(**CTAB**)** extraction buffer [3% CTAB (w/v), 100 mMTris- HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) PVP (Polyvinyl pyrrolidone)], then 1% (v/v) of β-mercaptoethanol (15 mM) with further grinding.. The mixture was incubated at 65º C for 60 min, followed by two extractions with chloroform/isoamyl alcohol (24:1). The nucleic acids were precipitated with cold isopropanol, and the pellet was dissolved in 1 mL TE 0.1X (Tris-EDTA) buffer (10 mMTris-HCl, pH = 8 and 1 mM EDTA, pH = 8). Co-precipitated RNA was removed by digestion with RNAase A. 4 µl (10 mg/mL).

Table (1): List of Date Palm Genotypes and Accessions, Geographic Origin and Consistency in the Present Study.

No.	Name	Location	Consistency	Origin
1	**DegletNoor**	Aswan	Semi-Dry	Algeria
2	**Malkabi**	Aswan	Dry	Sudan
3	**Bartamoudawardy**	Aswan	Dry	Sudan
4	**BartamoudaAdia**	Aswan	Dry	Sudan
5	**Balady**	Aswan	Dry	Egypt
6	**Shamiya**	Aswan	Dry	Egypt
7	**Sakkoty**	Aswan	Dry	Egypt
8	**Gondailawardy**	Aswan	Dry	Sudan
9	**GondailaAdia**	Aswan	Dry	Sudan
10	**Maghal 1**	Ismailia (elkasasen)	Soft	Egypt
11	**Samany 1**	Ismailia (elkasasen)	Soft	Egypt
12	**Zaghlool 1**	Ismailia (elkasasen)	Soft	Egypt
13	**Kabooshy**	Ismailia (elkasasen)	Soft	Egypt
14	**Amry 1**	Ismailia (elkasasen)	Semi-Soft	Egypt
15	**Hayani 1**	Ismailia (elkasasen)	Soft	Egypt
16	**Amry 2**	Sharqiya (elkoreen)	Semi-Soft	Egypt
17	**Bent-ashaa**	Sharqiya(elkoreen)	Soft	Egypt
18	**Aglany**	Sharqiy(elkoreen	Semi-Soft	Egypt
19	**Hayani 2**	Sharqiya (elkoreen)	Soft	Egypt
20	**Khadrawi**	KanaterKhairia	Semi-dry	Iraq
21	**OmalDehn**	KanaterKhairia	Semi-dry	Iraq
22	**Nabotseaif**	KanaterKhairia	Semi-dry	Iraq
23	**Halawi**	KanaterKhairia	Soft	Iraq
24	**Galbi**	KanaterKhairia	Soft	Iraq
25	**Avanda**	KanaterKhairia	Semi-dry	Iraq
26	**Hayani 3**	KanaterKhairia	Soft	Egypt
27	**Zaghlool 3**	KanaterKhairia	Soft	Egypt
28	**Samany 3**	KanaterKhairia	Soft	Egypt
29	**Amhat 3**	KanaterKhairia	Soft	Egypt
30	**Hayani 4**	Arish	Soft	Egypt
31	**Maghal 4**	Arish	Semi-dry	Egypt
32	**Maghaltamr**	Arish	Semi-dry	Egypt
33	**Khalas**	Ismailia (Al-RAJHI)	Soft	Saudi Arabia
34	**Barhiisoidy**	Ismailia (Al-RAJHI)	Soft	Saudi Arabia
35	**NabtetSoltan**	Ismailia (Al-RAJHI)	Semi-dry	Saudi Arabia
36	**Sakey**	Ismailia (Al-RAJHI)	Semi-dry	Saudi Arabia
37	**Samany 5**	Ismailia (Al-RAJHI)	Soft	Egypt
38	**Zaghlool 5**	Ismailia (Al-RAJHI)	Soft	Egypt
39	**Medjool**	Ismailia (Al-RAJHI)	Semi-Soft	Saudi Arabia
40	**Hayani 5**	Ismailia (Al-RAJHI)	Soft	Egypt
41	**Agwet –Almadina**	Ismailia (Al-RAJHI)	Soft	Saudi Arabia
42	**Zaghlool 6**	Rasheed	Soft	Egypt
43	**Samany 6**	Rasheed	Soft	Egypt
44	**Hayani 6**	Rasheed	Soft	Egypt
45	**Araibee**	Rasheed	Soft	Egypt

The DNA was further purified by 300 µl phenol: chloroform: isoamyl alcohol (25:24:1), then left overnight at (-20º C) using 1/10 vol. from 2 M sodium acetate (pH = 8.0) and one volume of cold isopropanol alcohol. The precipitate was washed twice with 10 mM ammonium acetate in 76 % ethanol, and the pellet was dissolved in 0.1 XTE buffer. The purified total DNA was quantified by gel electrophoresis, and its quality verified by Nano drop spectrophotometer model ND1000. DNA samples were then stored at 4º C. DNA samples of each cultivar were analyzed individually to detect intra-cultivar variations and bulked to detect inter-cultivar variations.

DNA amplification and PCR Conditions:

An initial screening of 35 SSR primer pairs (Successfully utilized in other date palm genotypes, was performed in order to test their readability and amplification profiles for polymorphism. After this screening procedure, 33 SSR primers were selected (Table 2) and these primers were synthesized by Oligo Macrogen, Seoul, Korea.

Table (2): A list of 35 SSR loci used in the present study

No.	Locus	SSR primers sequence 5---»3	No.	Locus	SSR primers sequence 5---»3
1	DPALM_100	F: GCCACTATCACCATTGCTGT R: CAATGGAGGTCGTAGTGGTG	19	DP 175	F: ACACACACACACACACACC R: GTGGCTTCTTTTTGGCTGTC
2	DPALM_103	F: TTCCATCCCTGGAGAAAGG R: AACCAAGACATCGTCCCAAG	20	mpdCIR010	F: ACCCCGGACGTGAGGTG R: CGTCGATCTCCTCCTTTGTCTC
3	DPALM_104	F: GGAAAGTTTCGGAACATTTTGT R: AACCCAACCTAAGCCCTACC	21	mpdCIR015	F: AGCTGGCTCCTCCCTTCTTA R: GCTCGGTTGGACTTGTTCT
4	DPALM_107	F: GGAAGGCGTCAAGGTATCTC R:ACAACACGGGGAAAGAACAT	22	mpdCIR016	F: AGCGGGAAATGAAAAGGTAT R: ATGAAAACGTGCCAAATGTC
5	DPALM_110	F: TGTCACATTTGAGCATAATCCA R: ACCCTTTGTTGATGCACCTC	23	mpdCIR032	F: CAAATCTTTGCCGTGAG R: GGTGTGGAGTAATCATGTAGTAG
6	DPALM_112	F: AGCAGGTTCATGGTTTGCTT R: AGAACCAGGGAGGATGAGGT	24	mpdCIR035	F: ACAAACGGCGATGGGATTAC R: CCGCAGCTCACCTCTTCTAT
7	DPALM_113	F: GGTCCCGACGCCTATTTTAT R: AGCAAAGTCCACCCCTTTTT	25	mpdCIR044	F: ATGCGGACTACACTATTCTAC R: GGTGATTGACTTTCTTTGAG
8	DPALM_119	F: TGCGCTAAATAGTTCCCTTCA R: CACATTCACAAGGCCTGCTA	26	mpdCIR048	F: CGAGACCTACCTTCAACAAA R: CCACCAACCAAATCAAACAC
9	DPALM_120	F: TTCAATTCATCCCACTGCAA R: CACCAACATGAGCAAATGGA	27	mpdCIR050	F: CTGCCATTTCTTCTGAC R: CACCATGCACAAAAATG
10	DP 151	F: TTGCTGGTTGAAATGGTGTT R: GCAACAGATGCTCTTGCTCA	28	mpdCIR057	F: AAGCAGCAGCCCTTCCGTAG R: GTTCTCACTCGCCCAAAAATAC
11	DP 157	F: TGGACAATGACACCCCTTTT R: GCCCACACAACAACCTCTCT	29	mpdCIR063	F: CTTTTATGTGGTCTGAGA R: TCTCTGATCTTGGGTTCTGT
12	DP 159	F: AGCTCCAATTTGCTGCAGAG R: GCTGACCTGGAGTCCAAAAC	30	mpdCIR070	F: CAAGACCCAAGGCTAAC R: GGAGGTGGCTTTGTAGTAA
13	DP 160	F: AAGAGCGACAATCATGACCA R: GGAAATTGAAGGGCATCTTG	31	mpdCIR078	F: TGGATTTCCATTGTGAG R: CCCGAAGAGACGCTATT
14	DP 168	F: GCAGCAAAAGCCCTTAGGC R: GGTGTTATGTGCAGCCAATG	32	mpdCIR085	F: GAGAGAGGGTGGTGTTATT R: TTCATCCAGAACCACAGTA
15	DP 169	F: GCATGGACTTAATGCTGGGTA R: GGTTTTCCTGCCAACAACAT	33	mpdCIR090	F: GCACGAGAAGGCTTATAGT R: CCCCTCATTAGGATTCTAC
16	DP 170	F:TCTTTGGGCTTACGACAACC R: GTATGGCCCAAGATGCAGAT	34	mpdCIR044	F: ATGCGGACTACACTATTCTAC R: GGTGATTGACTTTCTTTGAG
17	DP 171	F: GTGGGAGTAGCGAGGTAT R: GTCCGGCACTTTAGGAAGTT	35	mpdCIR015	F: AGCTGGCTCCTCCCTTCTTA R: GCTCGGTTGGACTTGTTCT
18	DP 172	F: ACCCCGGACGTGAGGTG R: CGTCGATCTCCTCCTTTGTCTC			

PCR reaction was performed in 25 µl volume contained 2µl (20 ng) of template DNA, 1µl (20 pmol) forward primer, 1µl (20 pmol) reverse primer, 12.5 µl Master Mix, and 8.5 µl PCR water. The amplification was carried out in a thermos cycler (Eppendorf Master Cycler Gradient Eppendorf, Hamburg, and Germany). After a first denaturation step at 95º C for 5 min, the reaction went through 35 cycles at 95º C for 15 sec., 51º C for 15 sec., 72 º C for 30 sec. followed by a final extension step of 5 min at 72º C.

Primers used in the SSR analysis:

Thirty-three specific primers were used in this study; fourteen of these microsatellite markers selected from (Billotte *et al.,* 2004), and the others microsatellite markers issued by WCMC-Q was download from this web site address: http://qatar-weill.cornell.edu/research/datepalmGenome/download.html

The sequence file is named as (pdactyKAssembly1.0.fasta – 329328KB) and contained 271804 fast sequence clones.

Data Analysis

The similarity matrix was used in the cluster analysis. The cluster analysis was used to organize the observed data into meaningful structures, that is, to develop taxonomies. At the first step, when each genotype represents its own cluster, the distances between these genotypes defined by the chosen distance measure (Jaccard coefficient). However, once several genotypes have been linked together, the distance between two clusters is calculated as the All those analyses were computed by the program SPSS version 16.0.

Allelic composition of each genotype and the number of total alleles was determined for each SSR locus. Putative alleles were indicated by the estimated size in bp. The genetic information was assessed only for single locus SSRs using the following parameters: Observed number of alleles per locus (na), counts the number of alleles with nonzero frequency. The observed heterozygosity (Ho, direct count), expected heterozygosity (He) and polymorphic information content values for each locus (PIC) were calculated as follows:

He or PIC = 1- $\sum$ pi2 where pi is the frequency of the ith allele, and summation extends over n alleles (Nei, 1973), effective number of alleles (Ne) = (1/1-He), and heterozygosity level of Date palm genotypes assayed.

The computations were performed with the programs, GENEPOP version 1.31 Raymond and Rouset (1995), Quantity one, Irfanview and Microsoft Excel.

RESULTS AND DISCUSSION

I-Genotype-specific alleles:

The difference between the number of alleles in each locus and number of effective loci showed the existences of private/specific alleles. Private alleles occurred on one or more genotypes (Kohpayegani and Behbahani, 2008). However, Genetic analysis of the 45 date palm genotypes based on 33 polymorphic SSR markers detected 428 distinct specific alleles across all loci. Thirty three of the 45 genotype samples had alleles were unique that genotype alone (Table not shown). 'Hayani' had the most unique alleles (59), following by 'Samany' (38). While the lowest number of genotypes-specific alleles (3) was scored for 'GondialaAdia' and 'Maghal' genotypes. The palm family seems to have a unique ratio of common retrotransposons so that it may relate to genome dynamics, such as the generation of new genes (Al-Mssallem *et al.,* 2013).

However, the specific alleles could be used to identify the genotypes by means of combination of some genetic loci. For example, using Dpalm-113 can be used as primer to identify genotypes which the gene loci were different from each other. In addition, Khadrawi and Khals genotypes can be differentiated from the other date palm genotypes using a set of (2and 7) primers which those generated (6 and 8) privet alleles, respectively. Al-Qurainy *et al.* (2011) reveled that eight cultivars among them: Khadrawi and Khals were morphologically different to each other, and also differed in sequence up to some extent at both loci (psbA-trnH&rpoB) and thus each sequence would act as a molecular signature for each cultivar.

These SSR markers that generate unique alleles will play a key role in creating a cost effective Egyptian date palm cultivars fingerprinting technique. However, the presence of many unique alleles can be taken as an indication of high and adaptation based genetic diversity in date palm. The presence of high number of unique SSR alleles can be accompanied with a high number of novel functional alleles, which can be utilized in crop improvement. In addition, the availability of a molecular marker for any interested trait will allow breeders to rapidly identify potent genitors that carry a specific allele, and to introgress the trait into main elite genotypes during the next breeding cycle for seed production (Morcillo *et al.*, 2013).

II. Geographical distribution for date palm genotypes

It is important to understand the genetic makeup of date palm at the regional level for efficient use of germplasm, classification, maintenance and conservation of date palm populations and their utilization in the improvement strategies. The dendrogram, for genotypes geographical origins demonstrated that all genotypes tested in this study were obtained from a reliable source with definite origin which is important for true estimates of genetic diversity in date palm molecular analysis. In addition several factors might affect the estimation of genetic diversity: at least the number of used markers, the distribution of markers throughout the genome and the nature of evolutionary mechanisms underlying the measured variation (Soumaya *et al.,* 2011).

1-**Aswan area:**

The genetic similarities between cultivars are quite variable (ranging from 0.076 to 0.252). These values suggest that there are cultivars that are genetically very close and others that are very far; they reflect their high variability in DNA. "Balady" and "Shamiya" cultivars

are genetically the closest because they have the highest value of similarity (0.252). Since all date palm genotypes are originated by hybridization, it may be assumed that they have a common genetic basis, and cultivars diverged from others by mutational events that arise during selection (Zehdi *et al.,* 2004). While "Malkabi" and "GondelaWardy" are the most genetically distance with the lowest similarity value (0.076). The present results are not in good accordance with those of Adawy *et al*. (2004b) as they showed that the dendrogram based on the AFLP analysis, the cultivars "Gondela" and "Malkabi" were the most genetically similar and "Shamiya" and "Sakkoty" come next. It is likely that different marker systems differ in the mechanism of detecting polymorphism, genome coverage of date palm cultivars .Hussein *et al*. (2005) found difference between different palm cultivars in Egypt: among these cultivars; "Sakkoty", "Malkaby", "Bartamouda" and "Gondela". However,in the south of Egypt it is usually multiplied using basal offshoots (Riyadh, 1996).

The traditional practice of vegetative multiplication of plants by offshoots, which was generally performed with good skill by farmers, ensures the identity and uniformity of the cultivars. Nevertheless, cases of misclassification can occur during propagation because of the difficulty faced sometimes in the identification of the cultivars on the base of morphology (Racchi *et al*., 2014).

The dendrogram constructed (Figure 1) for 9 date palm cultivars analyzed was in close agreement with Proximity matrix. Two main clusters were observed. The first cluster contained two cultivars ("Deglet Noor' and Malkabi''). The second cluster included the remaining 7 cultivars. This cluster was further divided into sub clusters; the first sub cluster contained two cultivars ("GondelaAdeia" and "GondelaWardy"). The latter sub cluster was further divided into two groups; one containing only two cultivars, namely "Bartamouda Wardy" and "Sakkoty" and the second containing the remaining three cultivars which included the most closely related cultivars studied namely "Balady" and "Shamyia" (similarity value =0.252) while "Bartamouda Adia" was an individual cultivar. In a few instances, related cultivars originated from the same country ("Malkabi", "BartamoudaWarday" and "Shamyia") however clustering was independent from their geographic (Saudi) origin.

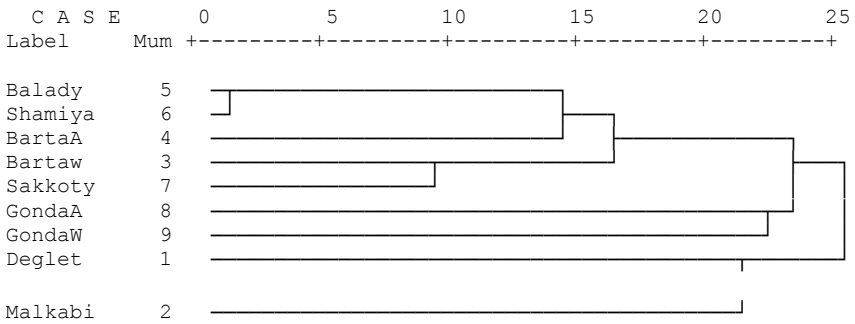

Figure (1): Dendrogram for nine date palm cultivars and accession constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

2. Ismailia area:

The genetic similarity values ranged from 0.041 to 0.248. The highest similarity value was observed between "Maghal" and "Samani"/Elkasasen which seem to be the nearest two cultivars and can be closely regrouped. Therefore, it is likely that they are from the same progenitor material or alternatively are the same cultivar but growing in a different location and so have been given a different name (Nartvaranant and Kantiporn, 2011). While "Hayani"/Elkasasen and "Khalas" had the smallest similarity value. Zhaghlol/Elkasasen and Amery/Elkasasen had also a high genetic similarity value (0.240).

The dendrogram constructed (Figure 2) for 15 date palm genotypes analyzed was in very good agreement with Proximity matrix. Two main clusters were observed. The first cluster contained five genotypes (Khalas, NabtSaltan, Bahri, Samany/Al-raghay and Sakey).

The second cluster included the remaining 10 genotypes. This cluster was further divided into two sub clusters, the first sub cluster contained Hayani and AgwatAlmadina. The latter sub cluster was further divided into two groups; one containing only Medjool and Hayani/El-kasasen and the second containing the remaining six genotypes which included the most closely related genotypes studied namely "Amry/El-kasasen" and "Zaghlol" and Samany El-Kasasen and Magahal while Zaghlol and Kabososh were formed a distance group.

3. Sharquia area:

The genetic similarity values ranged from 0.129 to 0.250 (data not shown). "Amry'' and ''Hayani'' had the smallest similarity value. While, the highest similarity value was observed between "Bentashaa'' and "Aglany" which seem to be the nearest two cultivars. ''Bentashaa'' and ''Amry'' had also a high genetic similarity value (0.242). However, it is possible that the

specimens of 'Aglany' and 'Amry' we used originated from the same Bentashaa cultivar.

UPGMA tree based on Jaccard's coefficient divided date palm in Shraquia location into three groups (Figure 3). ''Bentashia'' and ''Aglany'' cultivars showed more genetic affinity and were placed close to each other, while ''Amry'' cultivar was more distance. ''Hayani'' cultivar was the most distance. Since, continuous selection for date palm was carried following asexual reproduction implemented by farmers, may resulted in new cultivars emergence. Exchange of propagules, which are a mixture of vegetative and seed-propagated materials, has been conducted among farmers. All these processes together may result in a mixed genome within the same country (Khierallah *et al*., 2014).

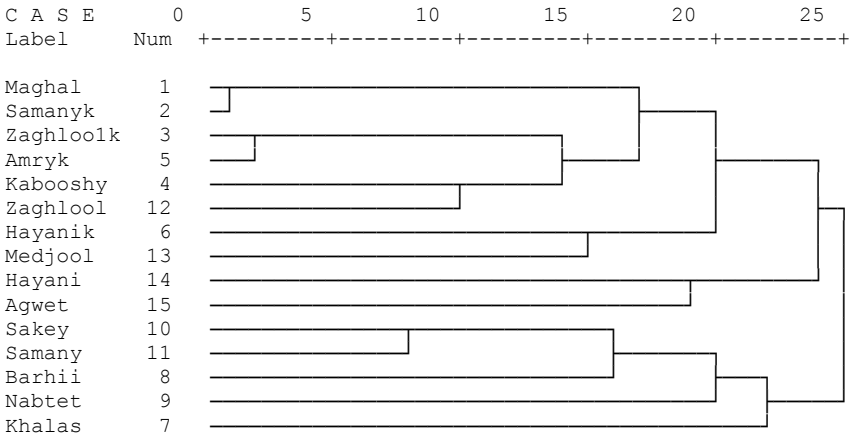

Figure (2): Dendrogram for fifteen date palm cultivars and accession constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

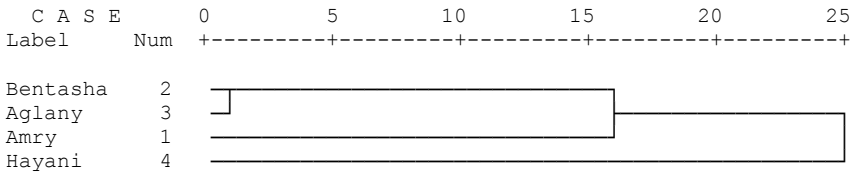

Figure (3): Dendrogram for three date palm cultivars and accession constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

4-Kanater area:

The genetic similarity values ranged from 0.078 to 0.201. The highest similarity value was observed between "Halawi'' and "Avanda" which seem to be the nearest two cultivars. While "Halwai'' and ''Samany'' had the smallest similarity value.

The dendrogrom based Jaccard's coefficient revealed 3 groups (Fig. 4). The largest cluster, cluster 1 consists of date palm germplasm which originated from Iraq. In this cluster, there are two sub clusters as follows: first sub cluster contained "Omal Dehn" and "Khadrwai" cultivars. The later sub cluster contained "Avanda" and "Halawi" cultivars were placed to each other while "Galbi" cultivar was more distance. ''Zaghlool'', ''Amhat'', ''Hayani'' and ''Samany ''cultivars were grouped in Cluster 2 as cultivars of Egyptian origination. Whereas, ''Nabotseaif'' formed a separated cluster (cluster3).

Some cultivars of date palm with different folk names like' Zaghlool'', ''Amhat' which are morphologicaly different, were not found to be distinct using SSRs. This could be attributed to different reasons. First, they may not be genetically distinct from each other in which case the morphological differences between these cultivars may have little genetic basis, instead it could be due to farmers' directional selection for different morphological traits for different purposes. Another reason could be that the observed morphological differences might not be detected using neutral genetic markers (Adugna, 2014).

5. Arish area:

In the north of Egypt, approximately 2/3 of the palms are propagated from seed (Riyadh, 1996). Since, seed dispersal takes place by different means such as travelers and traders across different locations (Zahdi *et al.,* 2004).

The genetic similarity values ranged from 0.195 to 0.233. ''Hayani''and Maghol had the highest similarity value. While, the smallest similarity value was observed between "Maghal and Maghaltamr. Although the two genotypes had the same name but they had the smallest similarly value which could be genetically different and could be homonyms. Dendrogrom (Figure 5) showed three genotypes were grouped in one cluster. (Hayni and Maghale) were seemed to be close to each other while Maghal was most distance to the other two genotypes.

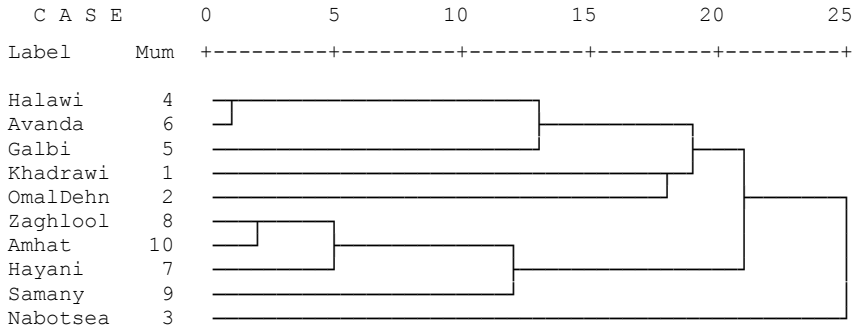

Figure (4): Dendrogram for ten date palm cultivars and accession constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

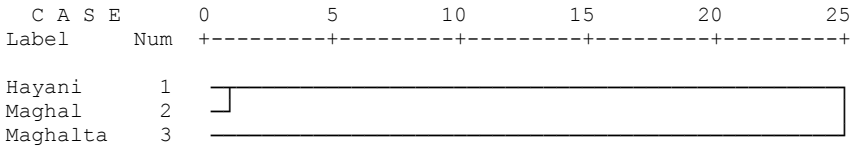

Figure (5): Dendrogram for three date palm cultivars and accession constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

6. Rashid area:

The genetic similarity values ranged from 0.104 to 0.169. "Samany' and ''Arabi'' had the smallest similarity value. While, the highest similarity value was observed between "Hayani'' and "Arabi" which seem to be the nearest two genotypes. ''Samany'' and ''Zaghlol'' had also a high genetic similarity value (0.163).

Dendrogram tree constructed using Jaccid revealed two main groups. Samany and Zaghlol were formed one group. Whereas, Hyani and Arabi were placed to close to each other. The SSR markers also revealed the narrow genetic diversity among the four genotypes. This narrow genetic diversity probably might be due to several different reasons mainly exchanging of varieties between different plantation areas, clonal propagation of ecotypes, development of new recombinant by seedling selection and limited sexual reproduction. Arbitrary or random selection by farmers may represent only a small fraction of the date palm diversity (Zahdi *et al.,* 2009).

The genetic distance among populations compared to each other (Hayani, Samany and Zagholol) which they presented at different locations in this study, may or may not correlate with the geographical distance between them in some species depending on natural and artificial factors involved in shaping the population genetic structure of the species. Most of the date palm trees originated by sexual reproduction from seeds, high level of genetic variation within populations (Gurevich *et al., 2005*).The large genetic variation represent the heterogeneity of date palms.

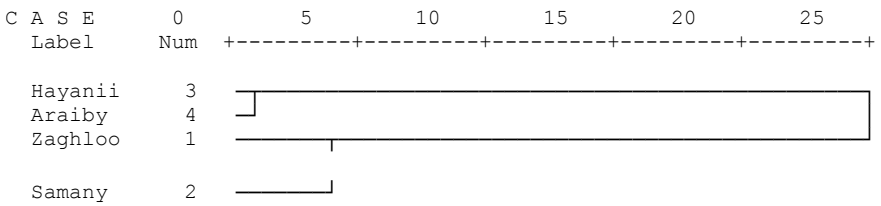

Figure (6): Dendrogram for four date palm cultivars constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

III. Phenotypic Diversity:

Genetic diversity can be estimated using both morphological and molecular markers. Morphological trait measurements can provide a simple technique of quantifying genetic variation. However, assessment Similarity values matrix among the date palm cultivars, Table (3): based on characters data calculated using Jaccard's similarity coefficient of morphological traits are generally influenced by environments and plant developmental stages (HU *et al*., 2012).

Cluster analysis was conducted to generate a dendrogram (Figure 7) illustrating possible relationships among 25 genotypes out of the studied 45 date palm genotypes based on 6 morphological traits (Table 3). This phenogram clustered into main phenotypical related groups. The first cluster was composed of (Shamiya, Bartamoda, Bartam_w, Malkabi, Gondilia, Gondaial, Medjool,and Amry) together while 'Degletnoor' was delimited in separate group from the others. However, (Bartamoda, Bartam_w) showed more phenotypical affinity and were placed to each other and

the same attitude for Gondial and condial wardi in this sub cluster. Genotypes (Kabooshy, Samany, Amhat, Aglany, Sakkoty, Agwet Almedina and Nabotese) were arranged in the second cluster, which exhibited two sub clusters. In addition to Khadraw and Barhi which were placed to each other and Khalas and Sakey were similar in the first sub cluster while all the remaining genotypes (Bentasha, Zaghlool, Hayani, Aloraiby and Halwai) composed the second sub cluster. Aloraiby showed more phenotypical affinity with Halwai and place to each other, Based on these findings, it is clear that there is some discordance between the data corresponding to molecular variability and those related to the variability available for breeding purposes (phenotypic variability). This disparity could arise from the fact that neutral molecular markers, such as SSR, commonly used in molecular diversity studies, may be located in non-coding regions of the genome (Collard *et al.,* 2005) and therefore be of limited use in predicting the phenotypic diversity of individuals, especially in complex traits such as yield.

Table (3): Performance of 25 genotypes out of the studied 45 date palm genotypes against 6 morphological traits

Characters	Cultivars	Deglet Noor	Malkabi	Bartamoudawardy	BartamoudaAdia	Shamiya	Sakkoty	GondailaAdia	Gondailawardy	Samany	Zaghlool	Amry	Hayani	Bent-ashaa	Aglany	Khadrawi	Halawi	Amhat	Khalas	Barhiisoidy	Nabotseaif	Kabooshy	Agwet-Almadina	Sakey	Medjool	Aloraiby
Fruit color (khelal)	Red	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1
	Yellow	1	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	1	1	1	1	1	0	1	0	0
	Brown	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Fruit size	Big	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0
	Medium	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	1	1	1	0	1	1	0	1
	Small	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Average production by tree	Large	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Medium	0	0	0	0	1	0	0	0	0	1	1	1	0	0	1	1	1	0	1	0	0	0	0	1	1
	Small	0	1	1	1	0	1	1	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	0	0
Marketing	Early	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	middle	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	0	1	1	1	0
	Late	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1
Majority of sugar contents	Inverted	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	Mainly sucrose	1	1	1	1	1	1	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Moisture content	More than 30%	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	1	1	1	1	0	1	0	1	0	1
	20-30%	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	1	0
	Less than 20%	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

However, high morphological variability is not always reflected at the molecular level (Wang *et al.,* 2006). That might interpret why the clusters formed using morphological data were different from the one formed using SSR.

Similarly, the genotypes that were detected as outliers in our molecular analysis were also not unique based on phenotypic data. Hamza *et al*. (2011) also did not detect correlation between the genetic distances estimated based on morphological data and genetic distances estimated based on molecular data in SSR markers. In both cases, the natural and human selective forces acting on molecular variation apparently differed from those acting on morphological traits. A further consideration is that morphological traits are heavily affected by the environment when they are expressed, whereas molecular markers are not subject to such variation and their variation is based directly on DNA sequence variation (Collard *et al*., 2005). Consequently, date palm plantations are a mixture of plants both clonally or seed propagated with a high genetic variability within cultivars. Nevertheless cases of misclassification can occur during propagation because of the difficulty in identifying some cultivars on the base of morphology (Elshibli and Korpelainen, 2008).

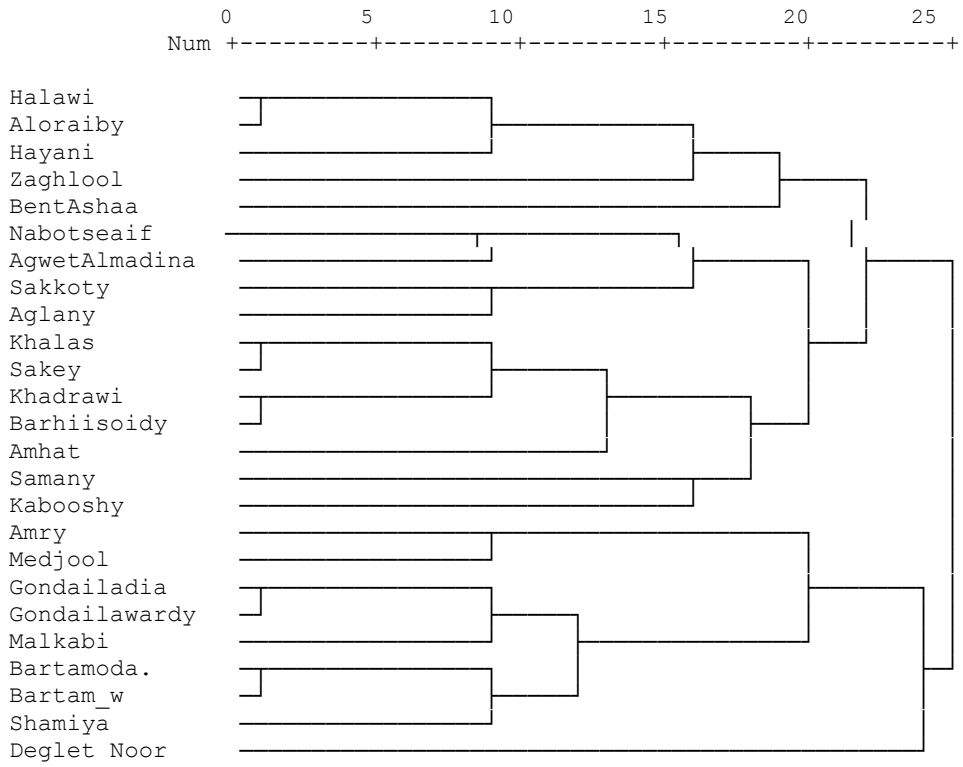

Figure (7): Dendrogram for twenty five date palm cultivars from characters data based on Average Linkage (Between Groups), using Similarity computed according to Jaccard's coefficient

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البصمة الوراثية و توصيف بعض أصناف نخيل البلح المصرية باستخدام معلمات التتابعات المكررة البسيطة

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تهدف الدراسة الحالية بشكل أساسي لتحديد البصمة الوراثية لعدد ٤٥ صنف وتركيب وراثي منزرع في مناطق مختلفة من مصر بالإضافة إلى المساعدة في عمل قاعدة بيانات على أساس جزيئي من خلال التوصيف الجزيئي لمجموعة الأصناف والتراكيب الوراثية تحت الدراسة. وتم استخدام معلمات الميكروستاللیت أو التتابعات المكررة البسيطة لتحقيق تلك الأهداف. أظهرت نتائج التضاعف لل ٣٣ موقعا وراثيا من التتابعات المكررة البسيطة للدنا، أظهرت التمييز الكامل لكل أو معظم أصناف نخيل البلح تحت الدراسة، بواسطة حزمة منفردة أو أكثر حيث تم تحديد عدد إجمالي ٤٢٨ كشاف متخصص (حزمة منفردة)، جميعهم كشافات متخصصة موجبة كانت مفيده لتحديد وتمييز الأصناف تحت الدراسة. وكان أكبر عدد من الكشافات المتخصصة تم تسجيله مع صنف حيانى حيث تم تحديد ٥٩ كشاف متخصص يليه صنف سمانى (تم تحديد ٣٨ كشاف). بينما كان أقل عدد من الكشافات المتخصصة (ثلاثة كواشف) تم تسجيلها في صنف جنديلة وردى بواسطة البوادئ - DP160 _ 422 mpdCIR063_110 -pdCIR063_4009.. تم تحديد قيم معامل التشابه (أو درجة التشابه) بين الأصناف والتراكيب الوراثية في المناطق المختلفة وكانت النتائج كالتالي:

منطقة أسوان: ظهرت أعلى قيمة لمعامل التشابه أو درجة التشابه (٠.٢٥٢) بين بلدي و شامية بينما كانت أقل قيمة لمعامل التشابه هي (٠.٦٧) بين ملكابى و جنديلة وردى. وفي منطقة الإسماعيلية: كانت أقل قيمة لمعامل التشابه (أو درجة التشابه) هي (٠.٤١) بين خلاص و حيانى (القصاصين) بينما كانت أعلى قيمة لمعامل التشابه هي (٠.٢٨٤) بين مجهل و سمانى (القصاصين).

منطقة الشرقية: كانت أعلى قيمة لمعامل التشابه (أو درجة التشابه) هي (٠.٢٥٥) بين بنت عيشة وعجلانى بينما كانت أقل قيمة لمعامل التشابه هي (٠.١٢٩) بين عمري وحيانى. بينما منطقة القناطر الخيرية: كانت أعلى قيمة لمعامل التشابه (أو درجة التشابه) هي (٠.٢٠١) بين بنت حلاوى و أفندا بينما كانت أقل قيمة لمعامل التشابه هي (٠,٠٧٨) بين حلاوى و سمانى.

في حين منطقة العريش: كانت أعلى قيمة لمعامل التشابه (أو درجة التشابه) هي (٠,٢٣٣) بين مجهل و حيانى بينما كانت أقل قيمة لمعامل التشابه هي (١٩٥و) بين مجهل و مجهل تمر. بينما منطقة رشيد: كانت أعلى قيمة لمعامل التشابه (أو درجة التشابه) هي (٠,١٦٩) بين عريبى و حيانى بينما كانت أقل قيمة لمعامل التشابه هي (٠,١٠٤) بين عريبى و سمانى.

كما أظهرت نتائج الدراسة الحالية أن بعض الكشافات المتخصصة لمعلمات الميكروستاللیت ربما تكون قد ارتبطت ببعض الصفات البستانية الاقتصادية الهامة في بعض أصناف نخيل البلح تحت الدراسة (لون الثمرة، حجم الثمرة، متوسط إنتاجية النخلة، موعد التسويق، نوعية السكريات ومحتوى الرطوبة). حيث يمكن الاستفادة منها في برامج التربية، من خلال الانتخاب بمساعدة معلمات الحامض النووي دنا (DNA MAS) والذي يعتمد على وجود ارتباط وراثي بين معلمات الحامض النووي دنا و المواقع الوراثية المسئولة عن توريث مختلف الصفات البستانية الهامة. مثل هذه الارتباطات تسمح لأي مربي بالانتخاب للمعلم الجزيئي البسيط بدلا من الانتخاب للصفة نفسها والتي تتأثر بعديد من العوامل، كما يتطلب ذلك عدة أجيال من الهجن الرجعية.

مما يساعد على توفير الوقت والمجهود وتقليل تكلفة برنامج التربية نظرا لإمكانية الانتخاب على مستوى البادرة، هذا بالإضافة لفائدتها في الاستيراد حيث أنها تقدم اختباراً سريعاً ودقيقاً لتوصيف وتحديد الأصناف كذلك الصفات الهامة لأصناف نخيل البلح.