Journal of Agriculture and Environment for International Development - JAEID 2018, 112 (1): 161-184
DOI: 10.12895/jaeid.20181.776

The date palms of Al Jufrah - Libya: a survey on genetic diversity of local varieties

Milvia Luisa Racchi and Alessandro Camussi

Department of Agri-Food Production and Environmental Sciences - Genetics Section, University of Florence, Italy

**Corresponding author: milvia.racchi@unifi.it*

Submitted on 2018, 23 May; accepted on 2018, 30 May. Section: Reviews

Abstract: Date palm (*Phoenix dactylifera*) a long-lived dioecious monocotyledon is one of the oldest plants on the earth cultivated by humans and the most important fruit crop of arid climate region in North African and Middle East countries. This review refers to the genetic characterization of a set of the more relevant date palm cultivars in Al Jufrah Oasis. This oasis, due to its position represents one of the most interesting Libyan regions for date palm cultivation. Molecular typing of 18 date cultivars, which are common genotypes in the five oases of Al Jufrah, was performed using 16 highly polymorphic SSR loci. The high level of genetic diversity observed among the cultivars is sound with data obtained with similar analysis on accessions from other countries of North Africa and Middle East.
To understand the genetic history of date palms growing in Al Jufrah oases, we investigated the polymorphism of a plastidial minisatellite that discriminate between African and Middle Eastern varieties. Al Jufrah date palm revealed a very high frequency of western chorotype similar to that reported for Tunisian varieties and greater than those observed in Algeria, Morocco and Mauritania. Most of the time the cultivar of origin of male plant is unknown because of seed propagation. The assignment of male plants already growing in the farms to a cultivar of origin could give the possibility to choice the pollinators. The use of a genetic fingerprinting can be used to trace back the origin of the male plants. Three different methodologies for the analysis of the SSR data were compared to assign male plants to cultivars of origin. SSR markers, especially when different methods of analysis of polymorphisms are compared, can offer important indications, albeit not exhaustive, to assign unknown plants to a specific cultivar.

Keywords: Libyan date palms, genetic diversity, SSR

Introduction

The date palm, *Phoenix dactylifera*, is a perennial monocotyledonous dioecious fruit plant belonging to Aracaceae family. It is one of the oldest plants on the earth cultivated by humans and the most important fruit crop of arid climate region in North African and Middle East countries. Date palm represents an emblematic species of the oasis agriculture. In fact, this plant and its fruits, wood and leaves make the life in the desert possible and play a significant role in fighting desertification keeping fertile areas of desert and contributing to the appearance of rural communities.

Evidences of date palms cultivation were found in many archaeological sites of the Fertile Crescent and Arabia and date back to the 7100 BP (Hazzouri *et al.*, 2015). These trees have been present continuously in the early human societies and they are rich of symbolic meanings for the religions born in the South regions of Mediterranean Sea. Jewish, Christians and Muslims, but much earlier Phoenixes, Carthaginians Greeks and Romans used date palm as image of abundance, fertility and victory on coins and monuments.

Date palm is one of the first plant pollinated by humans. Sexual propagation has been carried out at the dawn of civilizations and afterwards clonal propagated by offshoots of female date palms have been mainly developed to preserve the characteristic of tree and the organoleptic features of fruits. Male trees represent an exception and originate mainly from seeds of undefined derivation.

The origins of the date palm are still unknown and controversial (Chao and Krueger, 2007; Hazzouri *et al.*, 2015). This species was probably domesticated around 6000 BP and its progenitor is believed to be Phoenix reclinata Jacq. from tropical Africa, or *Phoenix sylvestris* (L.) Roxb. from India or a hybrid of these two. However, ancestral palm populations have not been characterized yet. Many researchers agree that date palms originated in the Mesopotamia-Arabic Gulf area (Zohary and Hopf, 2000; Tengberg, 2012; Al-Abdoulhadi *et al.*, 2012) and subsequent introgression with a wild or semi-cultivated population in North Africa. Nonetheless, the research on genetic diversity of accessible date palm resources provides new insights on geographic origins of the cultivated part of this species. These studies suggest the existence of two domestication events: one in the Middle East and a second late domestication in North Africa, possibly from separate gene pools that had diverged before the onset of domestication in either sites (Zehdi-Azouzi *et al.*, 2015; Hazzouri *et al.*, 2015). Further detailed evolutionary demographic analysis may help discriminate between these alternative scenarios. More recently, whole genome sequencing of wild and cultivated individuals points out a complex domestication history of at least two wild sources to African cultivated palms (Gros Balthazar *et al.*, 2017).

The spreading of the date palm cultivation occurred during the past centuries following two distinct directions: one starting from Mesopotamia to Iran, to reach the Valley of the Indus and Pakistan, the other starting probably from Egypt towards Libya, the Maghreb and Sahel countries. Nowadays date palms grow in more than 50 countries in the world particularly in the Middle East and North Africa regions, which account for approximately more than 67 per cent of world production (El-Juhany, 2010). On the base of FAOSTAT data (Table 1, Figure 1), among the different date-producing countries, Egypt dominates date production with 22.6 per cent of the total production in the world, followed by Iran, Algeria, Saudi Arabia and United Arab Emirates. The next five most important countries include Pakistan, Sudan, Oman and Tunisia. Libya is ranked eleventh.

The Libyan date fruit production is estimated to represent only 2.5 per cent of total production. The past and recent political events deeply affected trade and consequently export is quite limited in comparison with the neighboring countries. Such a circumstance, which beyond question represents a limit from the economic point of view, resulted in a benefit for the conservation of date palm germplasm.

Unlike other North African countries, in which the predominance of elite cultivars determined severe genetic erosion and the overall impoverishment of date palm agrobiodiversity, Libya, free from market incentives, preserves a huge richness of date palm germplasm.

More than 400 different date varieties still grow in the country of which about one hundred are of commercial interest. This incredible genetic richness has served as a highly effective natural defence for the plantations, which have remained safe from pathogens as *Fusarium oxysporum f. sp. albedinis* (Bayoud disease). Consequently, date palm germplasm of Libya deserves to be preserved and evaluated.

The first studies on date palm germplasm trace back to the work done by Italian agronomists and botanists in the twenties of the last century commissioned by Italian Ministry of Colonies. In fact, the census of the date palm varieties of Tripolitania started in 1912 and resulted in a report published in 1923 by Emanuele De Cillis containing data about the date palm varieties and the distribution of trees in the different oases of that area. Similarly, in 1933 Giulio Vivoli reported data on the Fezzan date fruits. Giovanni Zucco in 1922 in a publication edited by the Italian Ministry of Colonies presented the first survey of Libyan economy dealing with date palm cultivation and its products.

Morphology and features of fruit at harvest represent the bases of date classification; according these criteria Libya's date varieties fall into three major groups. The fleshy-fruited coastal varieties, the semi-soft varieties from the central zone, mostly consumed fresh (cvs. Kathari, Abel, Tagiat) and the less succulent varieties from the southern oases (cvs. Amjog, Emeli, Awarig, Tascube, Intalia, Tamjog). These latter cultivars are suited for drying and can be stored for up to ten years, making them highly appreciated by the caravans that formerly crossed the desert (for more details see Battaglia *et al.*, 2015).

Table 1 - Date fruit yield and production in North Africa and Middle East Countries (FAOSTAT data, 2018).

Country	Element	Year	Unit	Value	Description
Algeria	Yield	2016	hg/ha	61,550	Calculated data
	Production	2016	tons	1,029,596	Official data
Egypt	Yield	2016	hg/ha	351,965	Calculated data
	Production	2016	tons	1,694,813	FAO data based on imputation meth.
Iran (Islamic Republic of)	Yield	2016	hg/ha	55,113	Calculated data
	Production	2016	tons	1,065,704	FAO data based on imputation meth.
Iraq	Yield	2016	hg/ha	19,830	Calculated data
	Production	2016	tons	615,211	Official data
Israel	Yield	2016	hg/ha	103,746	Calculated data
	Production	2016	tons	43,200	Official data
Libya	Yield	2016	hg/ha	53,635	Calculated data
	Production	2016	tons	173,546	FAO data based on imputation meth.
Morocco	Yield	2016	hg/ha	21,563	Calculated data
	Production	2016	tons	125,329	Official data
Oman	Yield	2016	hg/ha	144,545	Calculated data
	Production	2016	tons	348,642	Official data
Pakistan	Yield	2016	hg/ha	51,628	Calculated data
	Production	2016	tons	494,601	FAO data based on imputation meth.
Qatar	Yield	2016	hg/ha	119,998	Calculated data
	Production	2016	tons	28,877	Official data
Saudi Arabia	Yield	2016	hg/ha	66,284	Calculated data
	Production	2016	tons	964,536	FAO data based on imputation meth.
Sudan	Yield	2016	hg/ha	118,005	Calculated data
	Production	2016	tons	439,120	Official data
Tunisia	Yield	2016	hg/ha	39,353	Calculated data
	Production	2016	tons	241,000	Official data
United Arab Emirates	Yield	2016	hg/ha	71,813	Calculated data
	Production	2016	tons	671,891	FAO data based on imputation meth.

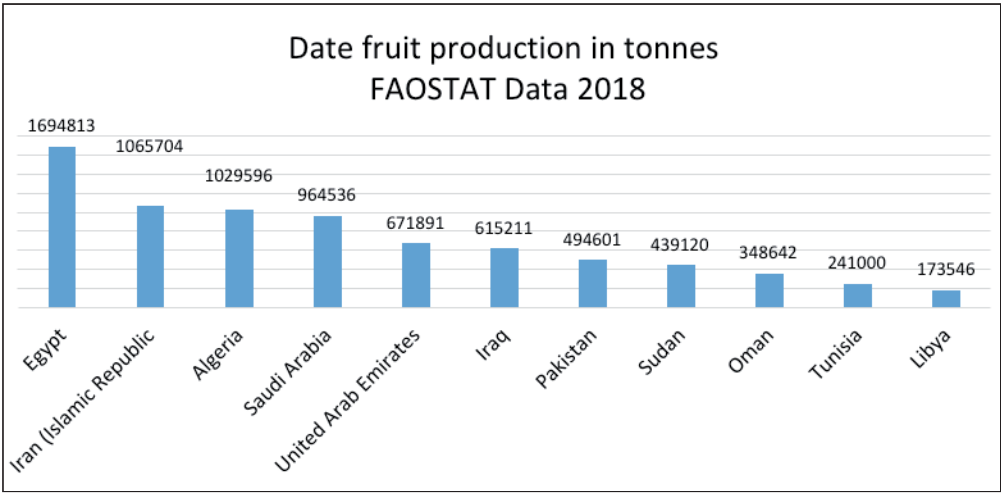

Figure 1 - Date fruit production in tonnes.

Dates are very high in nutritional value. This fruit consists of 70 percent carbohydrates (mainly sugars including glucose and fructose). It is an important nutritional source of minerals, protein, free amino acids, fats, vitamins and dietary fibres, making the date fruit one of the most nourishing natural foods to people.

The Al Jufrah oasis and its date palms

The Al Jufrah oasis, due to its position represents one of the most interesting Libyan regions for date palm cultivation aimed to soft date production and consequently this region was chosen for the integrated study of the palm dates cropping system. These studies were performed in 2009-2010 in the framework of the Italy - Libya bilateral cooperation project "Improvement and Valorisation of Date Palm in Al Jufrah Oasis", carried out by Istituto Agronomico per l'Oltremare (Italy) and the Board of Improving and Developing Olive and Palm Trees (Tripoli, Libya).

The detailed description of morphometric and nutritional characteristics of the date palm local varieties were reported in pomological cards (Bashir *et al.*, 2010; Battaglia *et al* 2015).

The Shabia of Al Jufrah is a broad region extending for about 117.000 Km^2 between the two mountains of Djebel Waddan and Djebel es-Soda. In this area, several smaller depressions cause the raise of water table and the consequent formation of a number of oasis, the three major ones being Waddan, Hun and Sokna plus the ancient oasis

Figure 2 - The Al Jufrah region and the oases considered in the study

of Zellah, located in the eastern part of the Shabia, and the oasis of Al Fugha located at south of Djebel es-Soda (Figure 2).

The region belongs climatically to the pre-desert belt. Mediterranean Sea exerts a certain influence on rainfall, considering the relatively abundant winter rains on the surrounding mountains, but the lower part of the depression has a typically desert climate and rain rarely exceeding 25 mm/year. Temperatures regime shows a great daily excursion, while variations on annual basis are modest. During summer months, maximum temperatures often reached very high levels. Sandy or very sandy soils are predominant in the Waddan and Hun areas, with a transition towards finer textured soils (sandy loam or loamy soils) in the depression of Sokna. A detailed description of the study area was reported by Delli in "The Geographic Information System of date plantations of Waddan, Hun, Sokna, Al Fugha and Zellah Oasis. 2010".

Date palm plantations can be classified in three typologies. Modern plantations, where more commercially valuable varieties as Deglet, Kathari and Abel are grown. Integrated farm, where palm date is the main crop but a range of other crops, annual or perennial, are cultivated. These palms are usually more than 20 years old, but in Waddan and Hun is not rare to find trees more than 100 years old. The varietal composition of such plantations shows a greater variability: the most widespread varieties in these farms are Tagiat, Kathari, Hamria and Deglet, but Abel, Bestian and Saiedi are also very common. Furthermore, a high number of plants obtained from seed and therefore not identifiable is present. Finally, there are traditional plantations that include the abandoned ones. The most widespread varieties in such plantations are Kathari and Tagiat (Delli, 2010).

In general, each palm plantation show a distinct cultivar composition due to local selection within the oases. Clonal propagation by shoots is the main method for date palm multiplication. Seed propagation using the pollen available from male trees of undefined origin occurs in just a few cases. In general, each cultivar derives from an individual seed, cloned thereafter by vegetative multiplication to ensure the identity and uniformity of the cultivar. However, intra-cultivar variation could potentially cause problems in cultivar identification.

The demonstration of the true-to-type character of the plants is an important part of quality assurance and it requires the use of markers effective in distinguishing the cultivars. Morphological markers, being influenced by environmental conditions and dependent on developmental stage, are unreliable as indicators of plant genotype. Nowadays, a numbers of molecular markers have been developed for varietal identification and for analysis of genetic diversity in *P. dactylifera*.

Identification and conservation of date palm germplasm

There is an increasing demand for date fruits and total world date production has risen by 2.9 per cent in the last decade. The need to fulfil market request with high quality products resulted in a severe genetic erosion with loss of cultivars. Consequently, conservation of date palm germplasm is a fundamental topic for date production and food security in desert and semi-desert areas.

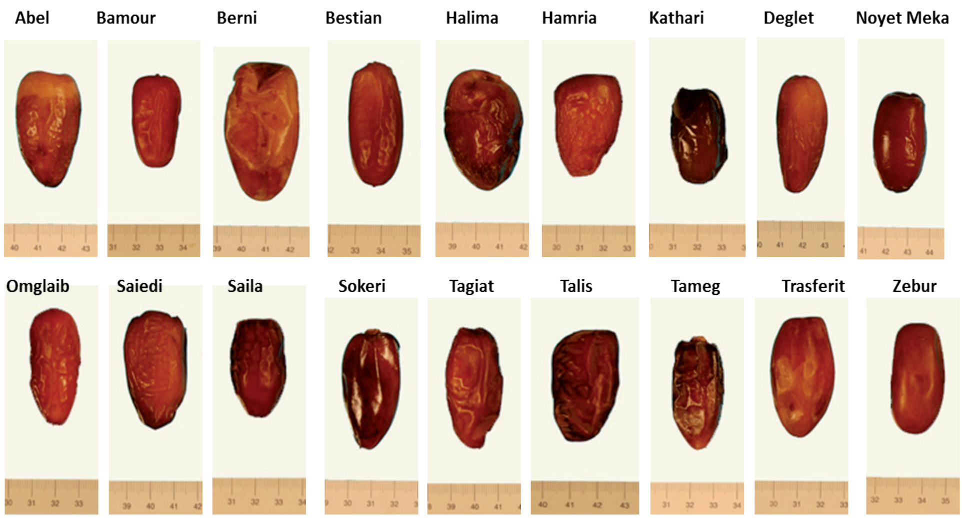

Figure 3 - Fruits of date palm cultivars grown in Libya. Source: (IAO photograph archive)

Table 2 - Cultivars analysed and the number of genotyped plants in the different localities of Al-Jufrah oasis.

Cultivars	Sokna	Hun	Waddan	Zellah	Al Fugha	Al Jufrah
Abel	6	7	10	0	0	23
Bamour	1	0	6	0	0	7
Berni	5	7	9	0	0	21
Bestian	7	10	16	3	4	33
Deglet	10	7	7	1	0	25
Halima	6	3	6	0	0	15
Hamria	8	9	7	5	5	34
Kathari	12	10	6	6	0	34
Noyat Meka	0	1	8	0	0	9
Omglaib	1	4	7	0	0	12
Saiedi	4	7	6	4	0	21
Saila	3	8	0	0	0	11
Sokeri	8	2	9	0	0	19
Tagiat	7	10	11	6	5	39
Talis	8	6	6	5	0	25
Tameg	6	9	7	0	0	22
Tasferit	4	4	4	0	0	12
Zebur	6	7	2	0	0	15
Total	102	111	120	30	14	327

Studies on date palm genome have been intensely carried out in the last decade thanks the availability of molecular tools for genetic analysis. Molecular markers, based on polymorphisms at the DNA level, have proved particularly effective to assess genetic diversity and currently used. Among molecular markers the microsatellite, or Simple-Sequence Repeats (SSR), represent a tool particularly suitable for genotyping because of their particular features. In fact, because of their co-dominant nature and typically high levels of allelic diversity at different loci (Kalia *et al.*, 2011), SSR have greatly contribute to study the genetic diversity of *Phoenix dactilyfera* in the main areas of cultivation. The microsatellites analysis performed on date palm accessions from different Nord Africa and Middle East evidenced the great genetic diversity existing among them. In general, date palms from North Africa and Middle East showed a considerable genetic differentiation with observed heterozygosity levels that vary in different studies due to the heterogeneous or homogeneous nature of the different sets of cultivars examined. SSR analysis were performed on accessions of Tunisia (Zehedi *et al*., 2004a, b; 2006), Sudan (Elshibli *et al*.,) 2008), Oman (Al Rugaishi *et al*., 2008), Qatar (Ahmed Talaat and Al-Qaradawi., 2009; Elmer *et al*., 2011, 2015), Iraq and Iran (Arabnezhad *et al*., 2012), Morocco (Bodia *et al*. 2012) and Saudi Arabia (Al-Khalifah S. and Askari, 2003; Al Faifi et al., 2016).

Identification of al Jufrah cultivars using molecular markers

SSRs were similarly used to identify the date fruit cultivars and to investigate the genetic diversity of date palms in the oases of Al Jufrah, in the framework of the Italy - Libya bilateral cooperation project, as a first step in ascertaining the genetic richness of Libyan date palm germplasm.

Eighteen cultivars, which are probably landraces common in Al Jufrah oasis and selected for their good fruit quality, were analysed using 16 highly polymorphic microsatellite loci.

Plant materials for DNA extraction consisted of young leaves of adult trees randomly sampled in the localities of Sokna, Hun, Waddan, Zellah and Al Fugha. The number of plants sampled is presented in Table 2. DNA amplifications were performed and PCR products sequenced (details in Racchi *et al*., 2013).

A large number of SSR alleles were revealed with a mean of 6.88 alleles per locus and the genetic variability detected was relatively high (Table 3). In total 110 alleles with an average of 6.88 alleles per locus were scored.

The number of alleles per locus ranged from four for locus PDCAT1 to eleven for locus mPDCIR78; expected heterozygosity values ranged from 0.46 (mPdCIR10) to 0.85 (mPdCIR78 and mPdCIR85) indicating that the Libyan date palm germplasm is characterized by a high degree of genetic diversity. Similar result was previously obtained using both isoenzymes and SSR markers in date palm accessions of other Countries. In fact, high level of polymorphism was observed for date palms cultivars of Algeria, Morocco, Tunisian Sudan and Saudi Arabia (Bennaceur et al., 1991; Elhoumaizi et al., 2006; Elshibli and Korpelainen, 2008, 2009; Zehdi *et al*., 2004 a, b; Al Faifi, 2016). More recently, the presence of higher polymorphism within the date palm genome was shown by the results obtained from parallel sequencing (Al-Dous *et al*., 2011).

Each cultivar results from an empirical selection carried out by the farmers in the oases based on morphological characters and fruit quality. This fact justifies the presence, at the same time, of fixed alleles, 28 out of 110, due to random drift and the high level of heterozygosity due to a clonal breeding procedure with direct selection

Table 3 - Descriptive genetic parameters for 16 SSR loci analysed on individual Libyan date palms of 18 cultivars (modified from Racchi et al. *2013).*

Source	Locus	Allele size	Alleles	Genotypes	H_{obs}	H_{exp}
Billotte *et al.* (2004)	mPdCIR10	138 - 176	6	13	0.41	0.46
	mPdCIR15	142 - 157	5	15	0.87	0.77
	mPdCIR25	219 - 257	6	17	0.90	0.76
	mPdCIR32	306 - 321	5	13	0.71	0.66
	mPdCIR70	205 - 227	9	32	0.91	0.83
	mPdCIR78	126 - 173	11	36	0.85	0.85
	mPdCIR85	175 - 199	8	39	0.83	0.85
	mPdCIR93	181 - 197	7	17	0.77	0.77
Akkak *et al.* (2009)	PDCAT1	103 - 123	4	10	0.23	0.63
	PDCAT2	186 - 209	7	20	0.85	0.79
	PDCAT6	142 - 172	7	17	0.82	0.71
	PDCAT8	222 - 258	6	14	0.78	0.68
	PDCAT11	154 - 177	6	20	0.75	0.79
	PDCAT14	141 - 163	9	20	0.42	0.63
	PDCAT17	131 - 157	6	14	0.45	0.63
	PDCAT18	123 - 149	8	29	0.88	0.77
	Total		110			

for the best performing individuals, i.e. those with heterotic vigour. Interestingly, all the alleles at PDCAT1 locus were fixed even in different cultivars. The 120 pairwise comparisons among the 16 SSR loci did not show significant linkage disequilibrium (Racchi *et al.* 2013). Both number and frequencies of alleles vary among the localities due to a different presence of the cultivars in the localities. A good example was represented by locus mPdCIR10, which exhibits six alleles: the allele 154 is fixed in 9 out of 18 cultivars, while alleles at locus CAT11 are greatly polymorphic (Figure 4). These loci well exemplify the different distribution among the oases; in fact, while CAT11 alleles are present in all the oases, some CIR10 alleles are not equally distributed.

Analysis of the genetic structure of the cultivars reveals that all were characterized by negative values of the fixation index (F) due to an excess of heterozygotes respect to

Hardy-Weinberg (HW) equilibrium, though at different level (Table 4). In particular cvs Talis, Halima, Omglaib, Saiedi, Tagiat, Saila and Zebur present F= -1. This aspect is not very common in plants and can be explained by a strong heterotic selection at the base of the clonal breeding of these cultivars, as reported also in Cassava (Pujol *et al.*, 2005). On the other hand, an F value close to zero is expected under random mating, as observed in Sokeri that is traditionally seed propagated.

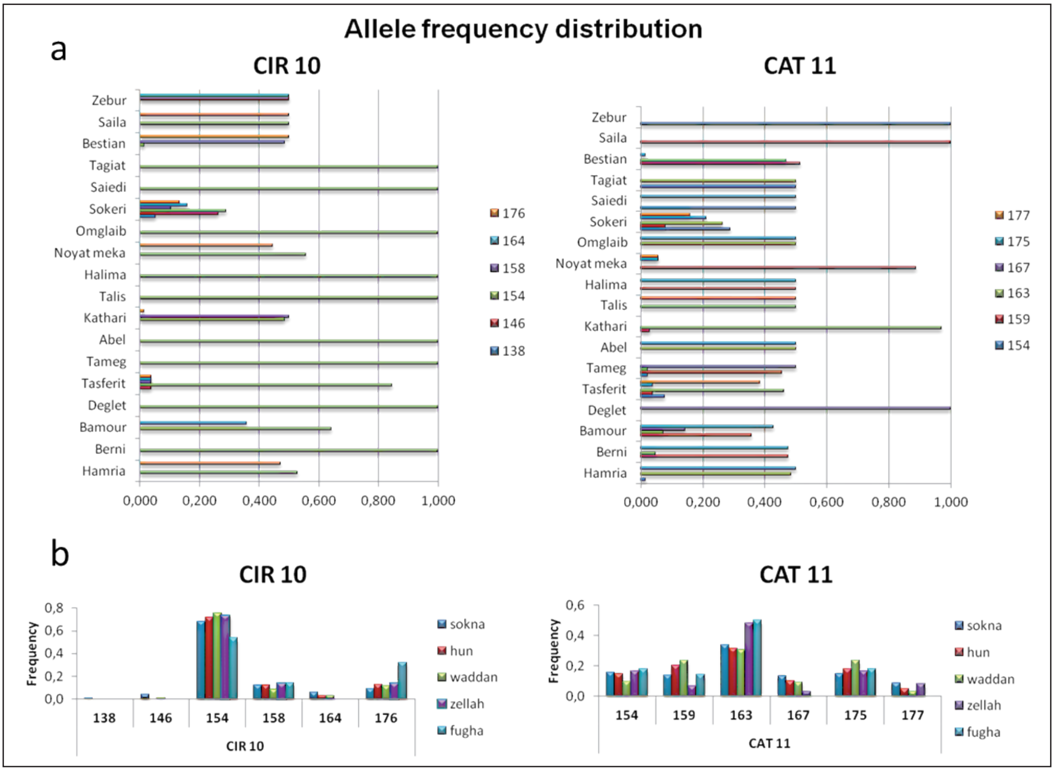

Figure 4 - Frequency distribution of alleles of SSR lociCIR10 and CAT11 that varies both among cultivars (a) and oases (b) .

Genetic diversity among cultivars was estimated as Codominant Genotypic Distances (Smouse and Peakall, 1999) obtained from SSR profiles.

The genetic distance matrix was then submitted to analysis of Principal Coordinates, a multivariate technique that allows to find and to plot the major patterns within the matrix in two or more dimensions. The plot is presented in Figure 5. The first twocoordinates account for 45 % of total variability and the third explains a further 18 % (not shown in Figure).

Table 4 - Genetic diversity indices for 18 Libyan date palm cultivars [N: number of sample; Na: Number of alleles; $H_{obs:}$ Observed Heterozygosity; H_{exp}: Expected Heterozygosity at the HW equilibrium; UH_{exp} Unbiased Expected Heterozygosity; F: Fixation Index] (Racchi et al. *2013).*

Cultivar	N	Na	Ne	H_{obs}	H_{exp}	UH_{exp}	F	P%
Abel	23	1.938	1.767	0.747	0.379	0.387	-0,973	75.00
Bamour	7	2.813	2.121	0.653	0.491	0.530	-0,329	100.00
Berni	21	2.625	1.733	0.622	0.348	0.356	-0,790	87.50
Bestian	33	2.750	1.684	0.629	0.339	0.344	-0,855	93.75
Deglet	25	1.688	1.630	0.625	0.314	0.320	-0,992	62.50
Halima	15	1.750	1.750	0.750	0.375	0.388	-1,000	75.00
Hamria	34	3.313	2.049	0.915	0.497	0.504	-0,842	100.00
Kathari	34	2.500	1.861	0.807	0.425	0.432	-0,897	93.75
Noyat Meka	9	2.563	1.755	0.576	0.365	0.387	-0,578	93.75
Omglaib	12	1.563	1.563	0.563	0.281	0.293	-1,000	56.25
Saiedi	21	1.813	1.813	0.813	0.406	0.416	-1,000	81.25
Saila	11	1.625	1.625	0.625	0.313	0.327	-1,000	62.50
Sokeri	19	5.125	3.630	0.708	0.656	0.674	-0,079	100.00
Tagiat	39	1.813	1.813	0.813	0.406	0.412	-1,000	81.25
Talis	25	1.625	1.625	0.625	0.313	0.319	-1,000	62.50
Tameg	22	2.750	1.833	0.685	0.380	0.389	-0,802	81.25
Tasferit	13	3.938	2.124	0.630	0.478	0.497	-0,319	100.00
Zebur	14	1.625	1.625	0.625	0.313	0.324	-1,000	62.50
Mean	20.9	2.434	1.889	0.689	0.393	0.406	-0.803	81.60
S.E.	0 .5	0.071	0.043	0.025	0.014	0.014	0.028	3.63

The first two coordinates reveal the separation of cultivars in two main groups. The use of codominant genotypic distances allows estimating the average similarity internal to each cultivar. Consequently, genetic heterogeneity of each cultivar is indicated by the coefficient of genetic dissimilarity (number in bracket). The coefficient of genetic dissimilarity values range from zero to 20.98. Cvs Talis, Halima, Omglaib, Saiedi, Tagiat, Saila and Zebur showed value 0, indicating absence of genetic difference within cultivar in agreement with their fixation index.

This result evidences that farmers have good skills in propagation by offshoot. In contrast, the high value (20.98) shown by cv. Sokeri is explained by the practice of seed propagation of this cultivar. Clonal propagation, beyond to guarantee genetic uniformity of the cultivars, also limits the negative effect of inbreeding.

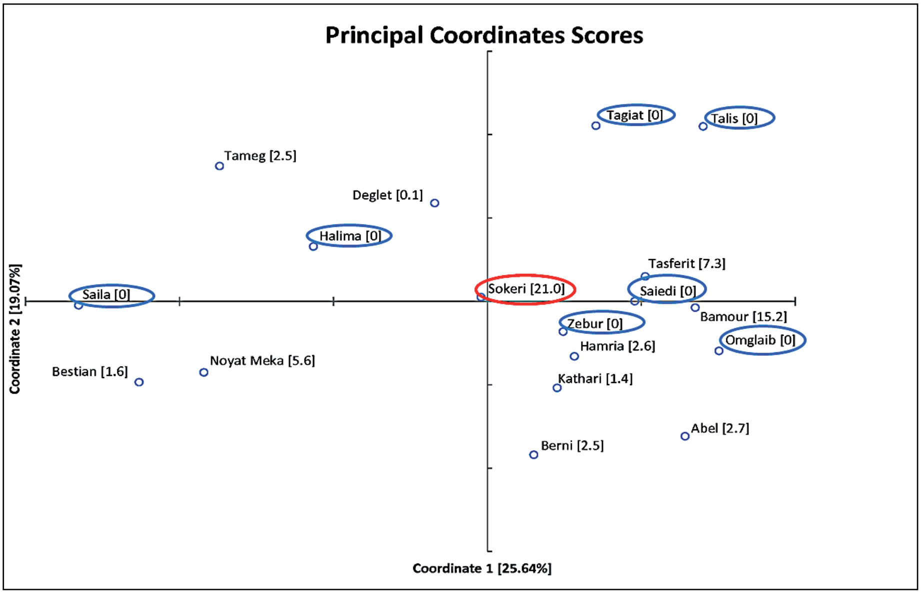

Figure 5 - Analysis of principal coordinates of SSR loci.

In fact, it provides the maintenance within cultivars of high level of heterozygosity achieved by assorting heterotic positive characteristics resulting from the empirical selection of plants with good pomology features and fruit quality. However in Libya as in Sudan (Elshibli and Korpelainen, 2008), seed propagation still occurs because of the ease and rapidity of seed reproduction coupled with their large availability. Accordingly, 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. The effectiveness of SSR in discriminating among all the accessions and cultivars evidences the usefulness of these markers for clonal fingerprinting and cultivar identification. Thus, because of the strong cultivar genetic identity observed, it was possible to design an identification key that allowed the labelling of all the cultivars studied. For each cultivar, the detected genotypes for mPdCIR78, mPdCIR93, mPdCIR25 microsatellite loci were scored. The allele frequencies of these SSR loci vary among the localities but with few exceptions, they are similarly distributed (Figure 6). 23 alleles were identified in these loci: ten alleles for mPdCIR78 locus, seven alleles for mPdCIR93 locus and six alleles for mPdCIR25 locus.

Since each cultivar was identified by a unique profile, it is possible to generate an individual barcode using the multi-locus. Zehdi and coworkers (2006) in the analysis of 49 Tunisian accessions with three SSR loci previously obtained similar results. DNA barcoding so far proposed as an ideal supplementary tool for palm systematic (Jeanson *et al.*, 2011, Ballardini, 2013) could become useful also in the certification and the control of the origin of date palm fruits and products.

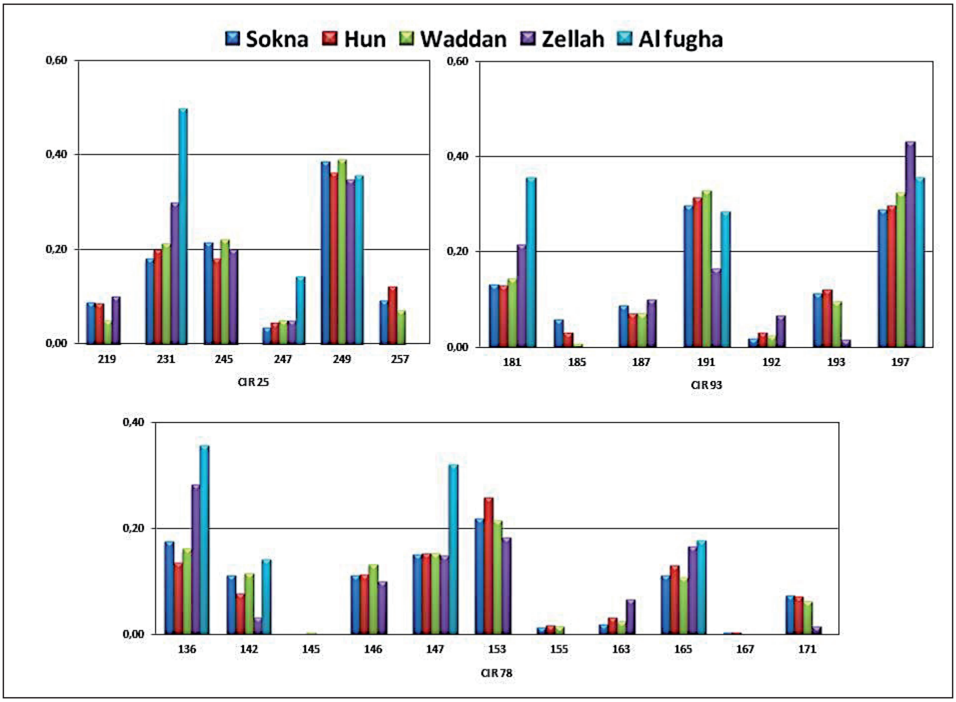

Figure 6 - Distribution of allele frequencies of SSR loci CIR25, CIR93, CIR78, in the oases.

The Structure software, based on a Bayesian procedure (Pritchard et *al.*, 2000), represents a different approach for genetic characterisation of cultivars. The program implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers, as in our case. Applications of the method include identifying distinct genetic populations and assigning individuals to populations. We assumed a model in which the number of populations (the parameter K) is 18 (the number of cultivars), each of which is characterized by a set of allele frequencies at each locus. The Figure 7 shows how the single plants were assigned (probabilistically) to the cultivars represented by different colours. The assignment was very efficient, evidencing, as in Hamria, Berni, Abel, Tasferit, Katari and Bestian, some sampling mistakes. The cultivar Sokeri confirmed its genetic heterogeneity. The Bayesian approach was effective even if the program assumes that, within populations, the loci are at Hardy-Weinberg and linkage equilibrium.

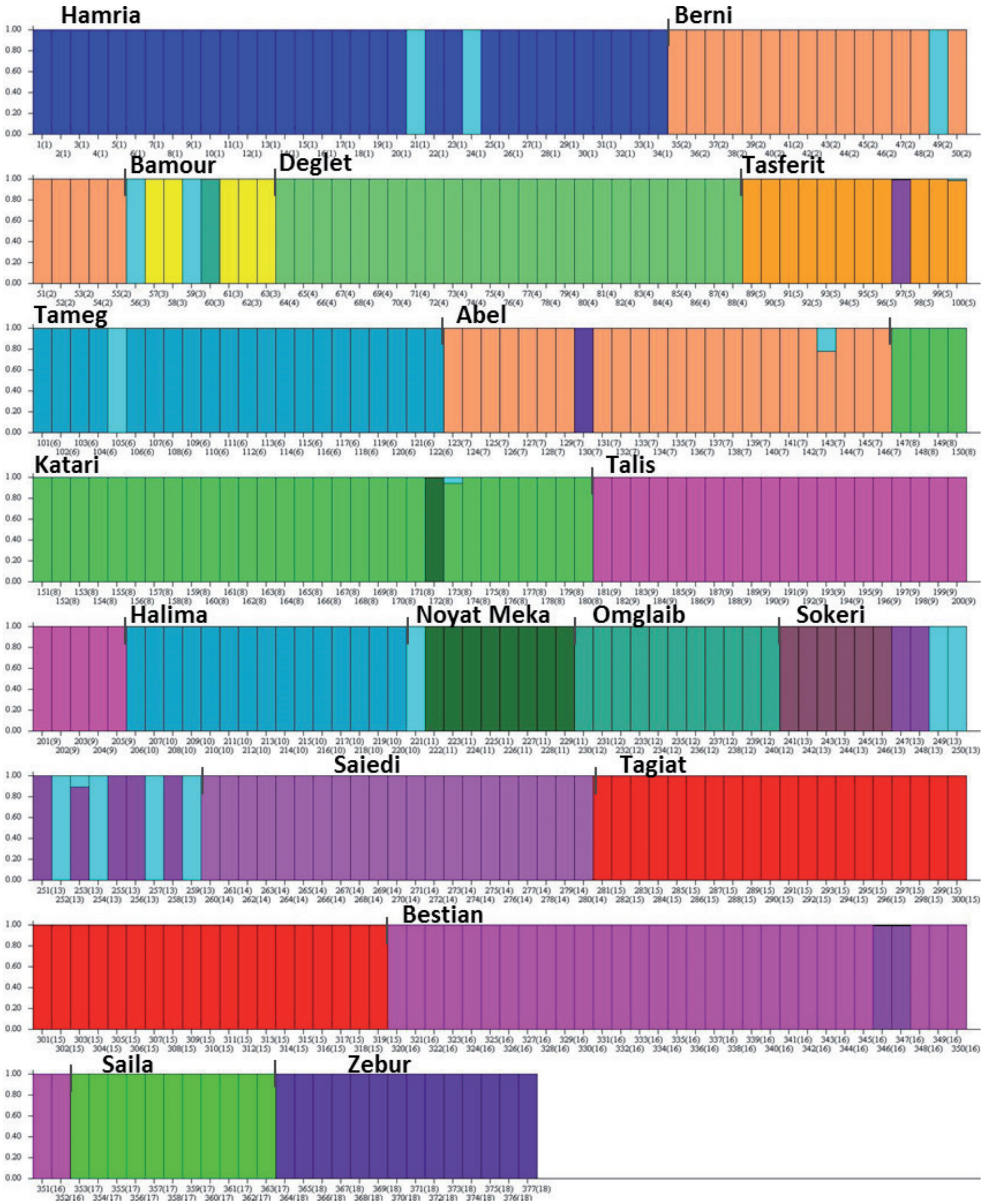

Figure 7 - Plant assignment to 18 cultivars according to STRUCTURE. Parameters: no admission model; K = 18; 5000 Burn-in period; 50000 Reps

About the genetic history of date palm cultivars in Al Jufrah

To understand the genetic history of date palms growing in Al Jufrah oases, we investigated the polymorphism of a plastidial minisatellite located in the *trnG*(GCC)-*trnfM*(CAU) spacer (Henderson *et al.*, 2006). Two chlorotypes were reported: the western chlorotype is chracterised by three repetitions of the 12 bp motif (haplotype 242 or type 3 chlorotype) while the eastern chlorotype has four repetition (haplotype 254 or type 4 chlorotype) (Zehdi-Azouzi et al., 2015). The analysis of Al Jufrah varieties revealed that the cultivars, with the only exceptions of Deglet and Saiedi, presented the western variant with 3-repetition. Interestingly, we observed a very high frequency of western chorotype similar to that reported for Tunisian varieties and greater than those observed in Algeria, Morocco and Mauritania (Figure 8).

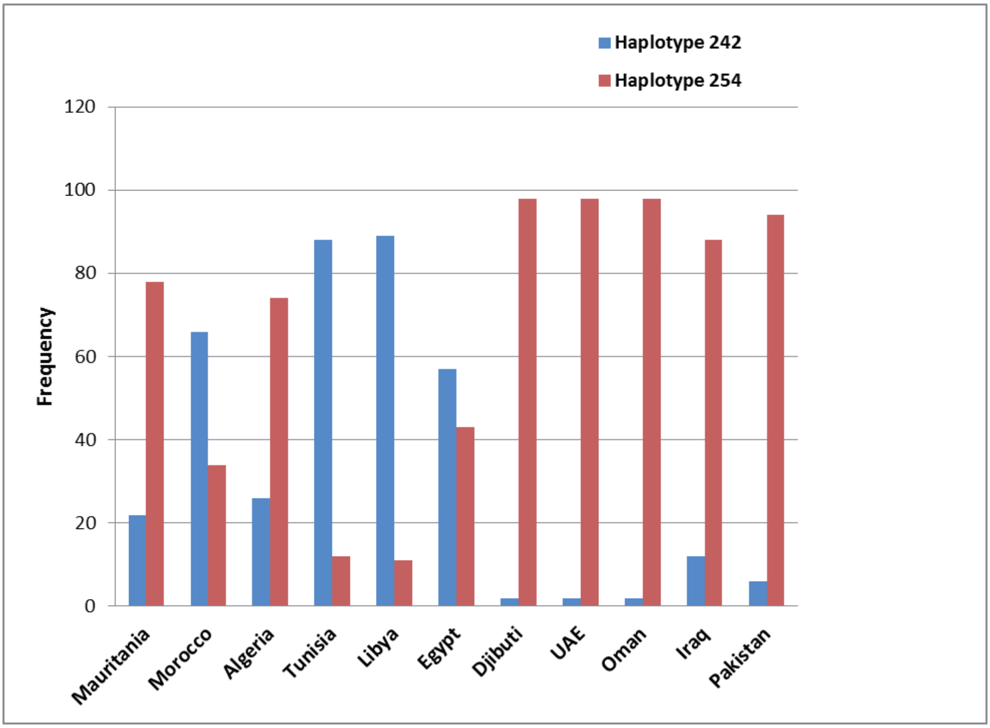

Figure 8 - Frequencies of western and eastern chlorotypes in date palms of different Middle East and North Africa Countries.

These data suggest different diffusion of date palms in North Africa countries. In contrast to the other countries, in Libya and Tunisia the importation of seeds or offshoots from the Middle East has been only occasional. In fact, the local farmers asserted that the varieties Deglet and Saiedi, which presented the eastern type 4 chlorotype, have been introduced the former from Algeria and the latter from Egypt. The absence of the cultivar Deglet in the ancient and presently abandoned plantations as reported by Delli (2010) seems sound with this assertion.

Assign cultivar of origin to male date palm from SSR data

The improvement of yield and quality of fruit is based on the possibility to know the genotype of male plant to use as pollinator. The identification of male plants before flowering, because of the long juvenile phase of the date palm, represents a major constraint to breeding programs. Nowadays the current extensive knowledge of the date palm genome provide different molecular techniques to identify male plants in early stages of development (Solliman *et al.*, 2017) and consequently to design convenient breeding program.

Traditionally the choice of the pollinator is not based on the knowledge of genotype. Most of the time the cultivar of origin of male plant is unknown because of seed propagation and exchanges that often occur among farmers. In fact, pollination of female plants is generally carried out mixing pollen from the few male plants available in the farms at the time of female flowering.

The assignment of male plants already growing in the farms to a cultivar of origin could give the possibility to choice the pollinators. Consequently, we performed a parentage analysis by comparing genotypes of male plants and cultivars. For this purpose, three different methodologies for the analysis of the SSR data obtained on pollinating plants sampled in the Al Jufrah Oasis were applied.

a) The use of the "Cultivar Identification Key" based on three microsatellite loci, turned out to be effective in identifying the 18 palm cultivars (Racchi *et al.*, 2013).

b) The Maximum Likelihood Paternity Assignment, by means of Cervus Package (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007), based on all the markers and on the complete collection of available genetic profiles of the 18 cultivars (Battaglia *et al.*, 2014).

c) The Structure software, based on a Bayesian procedure (Pritchard *et al.*, 2000), used as a different approach for genetic characterisation of male plants, comparing them to the profiles of the eight most predominant cultivars (Racchi and Camussi, 2014).

Given that the male plants considered derive from seed and a high variability due to segregation and recombination is expected, the three methods can hardly give unequivocal assignment responses. Tables 5a, 5b, 5c, show the most probable hypothesis of assignment of the 61 pollinating plants fully characterized by SSR markers.

The three methods applied were found to be consistent in the attribution only in two cases (names in bold in the table). In other cases, the most probable identification resulted from the comparison of results of the three approaches. The attribution to a cultivar was impossible only in 10 cases out of 63. Such result was expected because the small number of the reference cultivars. In fact, the 18 cultivars considered are a small sample compared to the about 400 ones existing in the Libyan germplasm.

It is evident that a simple and inexpensive system, such as that of the Identification Key, poorly fits in the case of segregating populations such as that of male plants; in fact, one allelic substitution is sufficient to attribute a plant to a different cultivar. On the other hand, the efficiency of SSR markers, especially when different methods of analysis of polymorphisms are compared, can offer important indications, albeit not exhaustive, to assign unknown plants to a specific cultivar. However, the whole genome analysis and re-sequencing data will provides essential information to develop markers for varietal identification and attribution of a male plant to its cultivar of origin.

Table 5a Proposed male plants attribution in the Oasis of Sokna

Farm	Male plant	Meth.	Proposed cultivar	Cultivars in the farm
AK	1		*unknown*	Abel, Bestian, Berni, Deglet, Kathari, Sokeri, Tagiat, Tameg, Talis, Zebur
	2	I,C,S	Bestian	
	3	S	Tasferit	
HB	1		*unknown*	Deglet, Hamria, Kathari, Saila, Saiedi, Tajat
	2	I	Abel	
MA	1	I	Berni	Alima, Berni, Bestian
	2	S	Tagiat	
ME	1		*unknown*	Abel, Deglet, Halima, Katari, Sokeri, Tagiat, Zebur
	2	S	Tagiat	
	3	C,S	Tasferit	
MM	1	I	Tagiat	Halima
	2	S	Tasferit	
	3	I	Tagiat	
	4	C	Kathari	
MT	1	C	Tasferit	Hamria, Kathari, Zebur
	2	S	Hamria	
SA	1	C,S	Deglet	Deglet, Abel, Berni, Deglet, Halima, Katari
	2	C,S	Deglet	
	3		*unknown*	

Meth.= method of identification: I identification key, C Cervus, S Structure

Table 5b - Proposed male plants attribution in the Oasis of Hun.

Farm	Male plant	Meth.	Proposed cultivar	Cultivars in the farm
3F	2	S	Tagiat	Abel, Berni, Bestian, Kathari, Tajat,Talis, Zebur
	3	C,S	Tameg	
3H	1	C	Tameg	Abel, Berni, Bestian, Kathari, Omblaib, Saila, Tagiat, Tameg
	2	C,S	Bestian	
5H	1	S	Tasferit	Bestian, Deglet, Hamria, Noyat Meka, Omglaib, Sokari, Talis, Tameg, Zebur
	2	C	Abel	
	3	S	Bestian	
	4	S	Tasferit	
6E	1		*unknown*	Abel, Bestian, Berni, Deglet, Kathari Saiedi, Tagiat, Tameg
	2	S	Tagiat	
	2	C	Bamour	
6I	1	I	Abel	Abel, Deglet, Hamria, Saiedi, Tagiat, Zebur
	2	C,S	Deglet	
	3	C	Tagiat	
EO	2	S	Tagiat	Hameg, Saila, Tameg
RG	1	C,S	Tagiat	Berni, Halima, Hamria, Saiedi, Talis, Tameg, Tasferit
	2	S	Tagiat	
	3		*unknown*	
SM	1		*unknown*	Deglet, Saiedi, Saila, Tasferit
	2	I,C,S	Bestian	
	3	S	Tagiat	

Meth.= method of identification: I identification key, C Cervus, S Structure

Table 5c - Proposed male plants attribution in the Oasis of Waddan.

Farm	Male plant	Meth.	Proposed cultivar	Cultivars in the farm
4B	1	S	Tagiat	Abel, Bamour, Berni, Halima, Nojat Meka, Omglaib, Tagiat
	2	I	Bamour	
	3	C,S	Hamria	
AD	2	S	Tagiat	Abel, Bamour, Berni, Bestian, Deglet, Kathari, Nojat Meka, Sokeri, Tameg, Tagiat, Talis,
	3	S	Tagiat	
AM	1	S	Tagiat	Abel, Bestian, Kathari, Nojat Meka, Sokeri, Tagiat, Tameg, Zebur
	2	S	Kathari	
BB	A	I	Talis	Bestian, Deglet, Hamria, Noyat Meka, Sokari, Saiedi, Tameg
	B	C,S	Tasferit	
	C		*unknown*	
BH	1	S	Bestian	Abel, Bestian, Deglet, Kathari, Nojat Meka, Sokeri, Tagiat, Tameg
	2	S	Hamria	
FZ	1	I	Nojat Meka	Bamour, Harria, Omglaib
	2		*unknown*	
	3	C,S	Tagiat	
HS	1	C,S	Tagiat	Abel, Bestian, Deglet, Kathari, Saiedi, Sokeri, Tagiat, Tameg
OE	2	C,S	Abel	Abel, Berni, Hamria, Kathari, Sokeri, Tagiat, Talis, Tansferit
	3	S	Tasferit	
SK	1		*unknown*	Bamour, Omglaib, Saiedi, Talis, Tasferit
	2	I	Hamria	
	3	I	Berni	

Meth.= method of identification: I identification key, C Cervus, S Structure

References

Ahmed T. and Al-Qaradawi Y., 2009. Molecular Phylogeny Of Qatari Date Palm Genotypes Using Simple Sequence Repeats Markers Biotechnology 8 (1): 126-131, 2009ISSN 1682-296X.

Akkak A. *et al.*, 2009. Development and evaluation of microsatellite markers in *Phoenix dactylifera* L. and their transferability to other Phoenix species. Boil Plant 53(1):164-166.

Al-Dous E. e*et al.*, 2011. De novo genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). Nat Biotech 29-6:521-527.

Al-Faifi S. A. *et al.*, 2016. Development, characterization and use of genomic SSR markers for assessment of genetic diversity in some Saudi date palm (*Phoenix dactylifera* L.) Cultivars. Electronic Journal of Biotechnology 21 (2016) 18–25.

Al-Khalifah N.S. and Askari E., 2003. Molecular phylogeny of date palm (*Phoenix dactylifera* L.) Cultivars from Saudi Arabia by DNA fingerprinting. Theor Appl Genet 107:1266–1270.

Al-Ruqaishi I.A. *et al.*, 2008. Genetic relationships and genotype tracing in date palm (*Phoenix dactylifera* L.) In Oman based on microsatellite markers. Plant Genet Resour 61:70–72.

Arabnezhad H. *et al.*, 2012. Development, characterization and use of microsatellite markers for germplasm analysis in date palm (*Phoenix dactylifera* L.). Scientia Horticulturae 134 150–156.

Ballardini M. *et al.*, 2013. The chloroplast DNA locus psbz-trnfm as a potential barcode marker in *Phoenix* L. (Arecaceae) Zookeys. (365): 71–82.

Bashir G. *et al.*, 2010 Date palm germplasm in Al Jufra oasis. Pomological cards. in Improvement and valorisation of date palm in the Al Jufrah oasis" Edited by IAO-MAE.

Battaglia M. *et al.*, 2015. Springer Science+Business Media Dordrecht J.M. Al-Khayri et al. (eds.), Date Palm Genetic Resources and Utilization: Volume 1: Africa and the Americas, 1_7 Chapter 14 Date Palm Status and Perspective in Libya DOI 10.1007/978-94-017-9694-.

Benaceur M. *et al.*, 1991. Genetic diversity of the date palm (*Phoenix dactylifera* L.) From Algeria revealed by enzyme markers. Plant Breed 107:56-57.

Billotte N. *et al.*, 2004. Nuclear microsatellite markers for the date palm (*Phoenix dactylifera* L.): characterization and utility across the genus Phoenix and in other palm genera. Mol Ecol Notes 4:256-258.

Bodia A. *et al.*, 2012. Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) Cultivars from Figuig oasis (Morocco) using SSR markers. IJSAT 2012; 2:96–104.

Chao C. and Krueger R. 2007. The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. Hortscience 42, 1077–1082.

De Cillis E., 1923. Saggio di "Fenicigrafia libica" Studi sopra alcune razze di palma da datteri coltivate in Tripolitania. Estratto dal Bollettino di Informazioni economiche n 6. Ministero delle Colonie, Roma.

Delli G., 2010. "The Geographic Information System of date plantations of Waddan, Hun, Sokna, Al Fugha and Zellah Oasis" Final report " Improvement and valorisation of date palm in Al Jufrah oasis" Edited by IAO-MAE.

Elhoumaizi M. *et al.*, 2000. Phenotypic diversity of date-palm cultivars (*Phoenix dactylifera* L.) From Morocco. Genet Resour Crop Evol 49:483-490.

Elhoumaizi M. *et al.*, 2006 Confirmation of 'Medjool' date as a landrace variety through genetic analysis of 'Medjool' accessions in Morocco. J Amer Soc Hort Sci 131:403-407.

El-Juhany L., 2010. Degradation of date palm trees and date production in Arab countries: causes and potential rehabilitation Aust. J. Basic Appl. Sci., 4 (8) (2010), pp. 3998-4010.

Elmeer K. *et al.*, 2009. New microsatellite markers for assessment of genetic diversity in date palm (*Phoenix dactylifera* L.) 3 Biotech, 1(2): 91–97. Published online 2011 May 27. doi: 10.1007/s13205-011-0010-z.

Elshibli S. and Korpelainen H., 2008. Microsatellite markers reveals high genetic diversity in date palm (*Phoenix dactylifera* L.) Germplasm from Sudan. Genet 134:251-260.

Elshibli S. and Korpelainen H., 2009. Biodiversity of date palms (*Phoenix dactylifera* L.) In Sudan: chemical, morphological and DNA polymorphism of selected cultivars. Plant Genet Resour. Doi: 10.1017/s147926210819748.9.

Gros Balthazar M. et al., 2017. The discovery of wild date palms in Oman reveals a complex domestication history involving centers in the Middle East and Africa. Curr Biol. 27(14):2211-2218.e8. doi: 10.1016/j.cub.2017.06.045.

Hazzouri K. *et al.*, 2015. Whole genome re-sequencing of date palms yields insights into diversification of a fruit tree crop. Nature Communications | 6:8824 | DOI: 10.1038/ncomms9824.

Henderson S. *et al.*, 2006. Genetic isolation of Cape Verde Island Phoenix atlantica (Arecaceae) revealed by microsatellite markers. Conservation Genetics 7: 213–223. Doi: 10.1007/s10592-006-9128-7

Jeanson M. *et al.*, 2011. DNA barcoding: a new tool for palm taxonomists? Annals of botany, Volume 108, Issue 8, Pages 1445–1451.

Kalia R. *et al.*, 2011. Microsatellite markers: an overview of the recent progress in plants. Euphytica 177:309-334.

Kalinowski S. *et al.*, 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16: 1099-1106.

Marshall T. *et al.*, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7: 639-655.

Moussouni S. *et al.*, 2017. Diversity of Algerian oases date palm (*Phoenix dactylifera* L., Arecaceae). Heterozygote excess and cryptic structure suggest farmer management had a major impact on diversity. PLOSONE/https//doi.org/10.1371/journal.pone.0175232.

Peakall R. and Smouse P., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295.

Pritchard J. K., 2000. Inference of Population Structure Using Multilocus Genotype Data Genetics 2000 vol. 155 no. 2 945-959.

Pujol B. *et al.*, 2005. Microevolution in agricultural environment: how a traditional Amerindian farming practice favours heterozygosity in cassava (Manihot esculenta Crantz, Euphorbiaceae). Ecology Letters 8: 138-147.

Racchi M.L. and Camussi A., 2014. Date palms of Al Jufrah oasis: genetic fingerprinting of local cultivars and impollinators. In: Dies Palmarum, Sanremo, 5-7/12/2013, Centro Palme Sanremo, pp. 1-9.

Racchi M. L. *et al.*, 2014. Genetic characterization of Libyan date palm resources by microsatellite markers 3Biotech 4: 21-32.

Smouse P. E. and Peakall R., 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure Heredity; 82 (Pt 5):561-73.

Solliman M. *et al.*, 2017. Identification and sequencing of Date-SRY Gene: A novel tool for sex determination of date palm (*Phoenix dactylifera* L.) Saudi Journal of Biological Sciences. Https://doi.org/10.1016/j.sjbs.2017.08.002.

Tengberg M., 2012. Beginnings and early history of date palm garden cultivation in the Middle East. J. Arid Environ. 86, 139–147 (2012).

Vivoli G., 1933. I datteri del Fezzan. Relazioni e Monografie Agrario-coloniali N. 24. Istituto Agricolo Coloniale Italiano Firenze.

Zehdi S. *et al.*, 2004a. Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) Revealed by nuclear microsatellite polymorphism. Hereditas 141:278-287.

Zehdi S. *et al.*, 2004b. Analysis of Tunisian date palm germplasm using simple sequence repeat primers. Afri J Biotech 3:215-219.

Zehdi S. *et al.*, 2006. Etablissement d'une clé d'identification variétale chez le palmier dattier (*Phoenix dactylifera* L.) Par les marqueurs microsatellites. Plant Genet Resour Newsl 145:11-18.

Zehdi-Azouzi S. *et al*., 2015. Genetic structure of the date palm (*Phoenix dactylifera*) in the Old World reveals a strong differentiation between eastern and western populations. Annales of Botany; 116(1):10112.doi:10.1093/aob/mcv068.

Zohary D. and Hopf M., 2000. Domestication of Plants in the Old World. The Origins and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley (third ed.) Oxford.

Zucco G., 1922. La palma da datteri e i suoi prodotti. Estratto dal Bollettino di Informazioni nn 5-6 Ministero delle Colonie, Roma.