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Genetic Characterization of Algerian Minor Date Palms (*Phoenix dactylifera* L.) Cultivated in the Oases of Biskra using Nuclear Microsatellite Markers

Ahmed Simozrag and Ziane Laiadi*

Laboratory of Genetic, Biotechnology and Valorization of Bioresources (LGBVB), University of Biskra, BP 145RP, Biskra 07000, Algeria.

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Abstract

In this study, minor and neglected cultivars of *Phoenix dactylifera* L. previously not reported were discriminated by molecular analysis using thirteen SSR markers in order to evaluate their genetic diversity and the relationships among them. The used set of markers could distinguish all eighty genotypes analyzed here according to the uniqueness of genotypes, and no similarity was found among the cultivars. A total of 101 polymorphic alleles were identified with an average of 7.77 alleles per locus, indicating the high level of polymorphism existing among the cultivars. The most informative loci was mPdCIR085, with the highest number of effective alleles (Ne=5.88) and had the lowest probability of identical genotypes (PI=0.052). The cumulative probability for genotype sharing among unrelated cultivars combining the 13 loci was $5.95*10^{-13}$; this value is low was enough to allow the check for synonymies in the samples. The study of genetic relationships among cultivars from different areas in Biskra oases showed the existence of close relatedness within some groups of cultivars. These facts suggest a common origin of them due to potential paternity relationships or easy exchange of plant materials by virtue of neighbor lines and mutual social relations between farmers.

Keywords: Biskra oases,date palm,genetic diversity, relationship.

1. Introduction

Date palm (*Phoenix dactylifera* L., $2n = 36$) is a dioecious perennial monocotyledon belonging to the family Arecaceae or Palmaceae (Munier,1973). Palm trees are the most visible and undisputed trees of the populations in oases and arid regions; through germplasm exchange, date palm agriculture has expanded to Australia, Southern Africa, South America, Mexico and the United States of America (Jain *et al.*,2011), and where considered of great socio-economic importance in the Arabian region (Khierallah *et al.*,2011). It has a halo of holiness for all Muslims because it is mentioned in the Quran and Sunnah, as dates constitute the favorite meal during Ramadan and Muslims generally break their fast by eating them. Algeria is characterized by a rich, complex and diversified date palm heritage, for according to recent statistics in 2015. Algerian date palm groves contained 18 million trees occupying 169,380 ha (Al-Khayri *et al.*,2015; Moussouni *et al.*,2017). Existing cultivars in all oases result from an empirical selection carried out traditionally by the farmers whereby the process of selection is done independently in every oasis (Elhoumaizi *et al.*,2002). However, date palm production has shifted from traditional cultivation in rich and diverse agrosystems to intensive monocultures (Jain *et al.*,2011). As a consequence, cultivars of minor economic interest have been abandoned favouring international varieties such as Deglet Nour, Ghers, Mech degla. The identification of date palm cultivars has been traditionally carried out by morphological markers, where the most common characters used are the morphology of leaves, spines and fruit characters, features which require a large set of phenotypic data that are sensitive to environmental factors and can be observed only in mature trees (Nixon,1950; Sedra *et al.*,1998; Elshibli and Korpelainen, 2008) as well as the developmental stages of the plant (Elhoumaizi *et al.*,2002).

Data based on molecular markers such as RFLPs, RAPDs and ISSRs have been performed to characterize date palm genotypes (Zehdi *et al.*, 2004b) or other molecular markers like AFLP (Cao and Chao, 2002; El-Assar *et al*.,2005; Adawy *et al*., 2006).

During the last decades, microsatellite markers have demonstrated to be a powerful tool for plant diversity analysis due to their high level of polymorphism, codominant behavior, relative abundance, Mendelian inheritance, specific location and amenability to automation, and high throughput genotyping (Kalia *et al.*,2011). Therefore, data become easier to exchange among laboratories (Udupa and Baum, 2001), which allows direct comparison and use of common databases. For date palms, Microsatellite markers were initially used for the investigation of genetic diversity in date palm by Billotte *et al.* (2004), and have thereafter been extensively used for genotype and cultivar characterization in different producing countries (Zehdi *et al.*,2004a,b ; Al-Ruqaishi *et al.*,2008; Elshibli and Korpelainen,2008; Ahmed and Al-

* Corresponding author e-mail: ziane.laiadi@univ-biskra.dz; zlayadi@yahoo.fr.

Qaradawi,2009; Johnson *et al.*,2009; Akkak *et al.*,2009; Pintaud *et al.*,2010; Elmeer *et al.*,2011; Khierallah *et al.*,2011; Zehdi *et al.*,2012; Zehdi *et al.*,2015; Guettouchi *et al.*,2017; Huda *et al.*,2019). In this study, we focus on cultivars which have been mostly neglected in research and economic commercialization in spite of being traditionally important until now and common for their quality of sub-products. We report the first data on the genetic diversity and putative genetic relationships among 80 Algerian date palm cultivars of Biskra oases, using 13 microsatellite markers.

2. Materials and methods

2.1. Plant material

Plant material consisted of eighty samples, given in Supplementary Table 1, collected from cultivars traditionally cultivated in different locations in Biskra (Tolga, Biskra center, Mchounech), some of which were collected in border areas in the state (Oued Righ), and several of which are believed to be endangered autochthonous cultivars (Figure 1).Young leaves were collected from mature, randomly sampled trees, dried in silica gel and stored at room temperature until DNA extraction.

Table 1. Alleles sizes (bp) and their frequencies (%) for the thirteen loci studied.

mPdCIR010		mPdCIR015		mPdCIR016		mPdCIR025		mPdCIR035		mPdCIR044		mPdCIR048	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
121	4.80	122	2.17	128	40.91	200	13.89	162	0.63	299	64.38	158	20.67
123	13.70	124	51.45	130	53.90	213	31.94	164	1.27	301	26.71	159	0.67
126	1.37	126	10.15	132	0.65	215	3.47	165	8.23	307	1.37	175	12.67
127	2.06	130	1.45	137	4.55	217	1.39	170	1.27	317	7.53	193	27.33
132	3.43	131	0.73			219	0.69	172	6.33			195	38.67
133	13.70	136	7.25			227	22.92	175	32.28				
135	52.74	138	23.19			231	25.69	177	10.76				
138	3.43	157	3.62					182	1.90				
159	0.69							188	6.96				
163	4.11							189	26.58				
								193	1.90				
								198	0.63				
								199	0.63				
								206	0.63				

cont. Table 1

mPdCIR057		mPdCIR063		mPdCIR070		mPdCIR078		mPdCIR085		mPdCIR090	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
250	36.08	122	19.74	186	7.38	117	25.69	149	6.72	141	42.57
252	6.33	140	60.53	190	0.82	120	2.08	157	11.19	148	3.38
254	28.48	155	17.11	192	21.31	121	6.94	161	0.75	150	2.03
256	11.39	166	2.63	193	0.82	123	14.58	165	0.75	161	5.41
263	5.70			194	29.51	134	2.78	167	2.99	166	5.41
269	10.76			195	12.30	136	9.72	169	17.91	167	4.05
274	1.27			196	0.82	146	16.67	171	20.90	168	24.32
				197	16.39	152	21.53	177	17.16	169	9.46
				199	2.46			179	21.64	171	2.70
				207	1.64					172	0.68
				209	6.56						

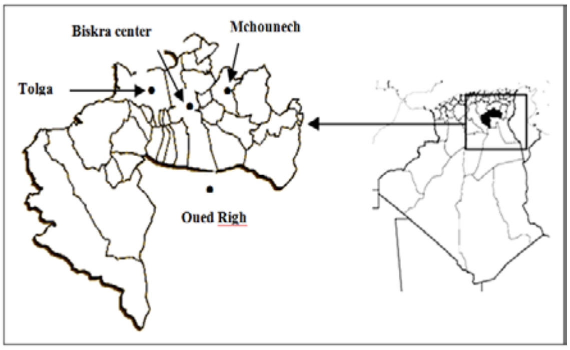

Figure 1. Map showing four Algerian oasis date palms referred in the text.

2.2. DNA isolation and microsatellite analysis

DNA isolation and microsatellite analysis was carried out according to procedure described by Saro *et al.* (2014). Total genomic DNA was extracted from 1 g of silica-dried leaf tissue from each sampled adult following Dellaporta et al. (1983), after grinding plant material with a Mixer Mill MM300 (RETSCH, Haan, Germany).

The obtained DNA solution was purified using GenEluteTM PCR Clean-Up Kit (SIGMA-ALDRICH, St. Louis, MO, USA).

The DNA concentration was checked on a spectrophotometer "Eppendorf BioPhotometer® D30" to confirm a minimum of 20 ng/µL. We amplified 13 dinucleotide (GA) microsatellite loci previously developed for *P. dactylifera* (Billotte et al., 2004): mPdCIR010, mPdCIR015, mPdCIR016, mPdCIR025, mPdCIR035, mPdCIR044, mPdCIR048, mPdCIR057, mPdCIR063, mPdCIR070, mPdCIR078, mPdCIR085 and mPdCIR090. DNA fragments were amplified in two PCR multiplex reactions with four loci each, using the Qiagen Multiplex PCR kit (QIAGEN, Valencia, CA, USA) and following the manufacturer's instructions, but accommodating reagent's proportions to a final volume of 15µL. Amplified PCR products were run on an ABI3730 capillary sequencer using an internal size standard (GS500 (-250) LIZ), and fragment sizes were manually scored using GENEMAPPER software (Applied Biosystems, Foster City, CA, USA).

2.3. Data analysis

We used « IDENTITY 1.0 » software (Wagner and Sefc, 1999) to detect all possibilities of identical genotypes, where genetic diversity was measured by estimating the average number of alleles per locus (*Na*), the average number of effective alleles (*Ne*) and the gene diversity or expected heterozygosity (*He*), the average probability of identity per locus (*PI*), the cumulative *PI,* using GENALEX 6.41 (Peakall and Smouse, 2006). Genetic distances between cultivars were calculated as the allele sharing distance (DAS) (Jin and Chakraborty, 1994). Phylogenetic tree based on the distance matrix was constructed using the neighbour-joining method (Saitou and Nei,1987) by POPULATIONS v.1.2.30 (http://bioinformatics.org, LANGELLA, unpubl.) while MEGA5.2 (Tamura *et al.*2011) was used to display it.

3. Results and discussion

3.1. Microsatellite analysis

The thirteen SSR markers were chosen for this study to identify different genotypes in the eighty analyzed samples (Supplementary Table 1). Allele sizes and frequencies of the analyzed loci are shown in Table 1. The distribution of allele frequencies for each locus allows assessing the identification ability of the markers, being more informative if this distribution is equitable (Sefc *et al.*,1999; Santana *et al.*,2007). In this study, the most frequent alleles were mPdCIR044-299,mPdCIR063-140,mPdCIR016-130 and mPdCIR010-135 with high percentage values of 64.38%, 60.53%, 53.90% and 52.74% respectively. The number of alleles per locus (Table2) varied from 4 (mPdCIR016, mPdCIR044, mPdCIR063) to 14 (mPdCIR035). A microsatellite preferably should have at least 4 alleles to be useful for the evaluation of genetic diversity as per the standard selection of microsatellites loci (Barker,1994). Total of 101 alleles were identified with an average of 7.77 alleles per locus, which agrees with the results of previous works on 49 cultivars collected from three main oases in Tunisia (Zehdi *et al.*,2004b), who detected 7.14 alleles per locus when analyzing 46 Tunisian date palm accessions by using 14 microsatellite loci where Elshibli and Korpelainen (2008) in Sudan identified a high number of alleles per locus (21.4), which is more than the number of alleles per locus detected in this study. Expected heterozygosity for each locus ranged from 50.80% (mPdCIR044) to 82.33% mPdCIR078 and 83% (mPdCIR085), with a mean of 70.71%, while observed heterozygosity varied between 23.30% (mPdCIR044) and 86.11% (mPdCIR025), with a mean of 67.40% in global Ho value is higher than expected for 9 loci vs 4 loci. Thus the higher value of Ho in the majority of loci observed in this study under Hardy-Weinberg conditions suggests high genetic variability in this population. Despite the four loci analyzed (mPdCIR044, mPdCIR035, mPdCIR070 and mPdCIR090) showed observed heterozygosity lower than Hardy-Weinberg expectations, as in previous works especially for last primers (Zehdi *et al.*,2004b ; Elshibli and Korpelainen, 2008). The most informative loci was mPdCIR085 with nine alleles (Ne=5.88) and had the lowest probability of identical genotypes (PI=0.052), followed by mPdCIR078 (Ne=5.65; PI = 0.055) and mPdCIR070 (Ne=5.40; PI = 0.058), while the opposite was the case for mPdCIR016(Ne = 2.17) and mPdCIR044(Ne = 2.03), both of which with four alleles and had the highest probability of identity (PI=0.31), for the mPdCIR044 was found to be the least informative locus. Originally, Billotte *et al.* (2004) have signalled this case as locus which produced erratic amplification and possibly referred to a mutational polymorphism at an annealing site. The probability for genotype sharing among unrelated cultivars combining the 13 loci was $5.95*10^{-13}$, lower enough to allow the check for synonymies in the sample.

Table 2. Genetic parameters obtained with thirteen SSR markers for eighty distinct cultivars.

	N	Na	Ne	I	Ho	He	F	PI
mPdCIR010	73	10	3.099	1.563	0.795	0.677	-0.173	0.130
mPdCIR015	69	8	2.976	1.404	0.710	0.664	-0.070	0.153
mPdCIR016	77	4	2.174	0.872	0.558	0.540	-0.034	0.311
mPdCIR035	79	14	4.920	1.912	0.633	0.797	0.206	0.067
mPdCIR025	72	7	4.144	1.536	0.861	0.759	-0.135	0.099
mPdCIR044	73	4	2.034	0.890	0.233	0.508	0.542	0.307
mPdCIR048	75	5	3.533	1.343	0.720	0.717	-0.004	0.130
mPdCIR057	79	7	4.111	1.606	0.772	0.757	-0.020	0.094
mPdCIR063	76	4	2.298	1.022	0.579	0.565	-0.025	0.242
mPdCIR070	61	11	5.397	1.891	0.590	0.815	0.276	0.058
mPdCIR078	72	8	5.653	1.851	0.847	0.823	-0.029	0.055
mPdCIR085	67	9	5.880	1.873	0.836	0.830	-0.007	0.052
mPdCIR090	74	10	3.859	1.701	0.622	0.741	0.161	0.098
mean	72.846	7.769	3.852	1.497	0.674	0.707	0.053	0.138
Cumulative								$5.95*10^{-13}$

Statistical results for 13 microsatellite markers used in the present study, namely: sample size N, observed number of alleles (Na), effective number of alleles (Ne), Shannon's Information index (I), observed heterozygosity (Ho), expected heterozygosity (He) and Probability of identity.

3.2. Cluster analysis

The dendrogram based on genetic distance measure (Figure2) was constructed using the weighted neighbour-Joining method for the evaluation of genetic diversity and relatedness between the investigated cultivars. The dendrogram clustered the eighty cultivars into three major groups (I, II and III).

The first minor cluster grouped ten genotypes. A first subdivision contained the well-known cultivars in Mchounech called 'Tbsrithe' closely linked to 'Lamari'and 'Abdelazaz', and were cultivated near to Biskra center. The three cultivars showed common morphological features like the color of fruit and their softness, but these two cultivars were closely linked in the color of the fruit 'brown to black', although distinct in the appearance of outer skin, smooth for the first and wrinkled skin for the second. The remaining cultivars in this group were very interesting as five cultivars shared a significant number of alleles, and they all appeared in a homogeneous color and fruit softness: ‘Bajamil’, ‘Arelou Oued Souf’, ‘Melk Lahcen’,‘Loulou’ and ’Charka’cultivated in the same area in south of the region called “Still” or “Oued Rhig” which in the border of the state (Figure 1), are very close with two other cultivars ‘DGuel Litima’ and ‘Jaouzia’, in brown color and half softness fruits. Traditionally cultivated in central areas of the region, this fact suggests that central area is a common origin of them. The second cluster contained 23 accessions representing three sub-clusters. This cluster grouped the majority of cultivars belonging to ‘Mchounech’area, and the closest relationships were detected between the following pairs of genotypes: “Hathourite, Tamazoute”, “Takarboucht, Taourekht” and “Noyet Arechti, Takerbrateth”. It should also be noted that most of them were named by using terms to refer to the color of fruit locally in chaoui dialects as one characteristic of the region. On the other hand, one sub-cluster includes “Mech degla” famous cultivar with dry fruits characterized by their resistance and low cost in terms of cultural practice, conservation and marketing of their dates which has high nutritional value; unfortunately, they are considered as secondary quality date varieties which run the risk to disappear in favor of Deglet-Nour soft variety (Amellal and Benamara, 2008). “Mech degla” shared a significant alleles with two cultivars which are closely related to each other ‘Arelou Biskra’ in Oued Righ and ‘Saout Bghal’ from Mechnouch.These last cultivars (Simozrag *et al.*,2016) showed common morphological features as the fruit color, and their leaves with Mech degla and Hamrayet hamlaoui in the same sub-cluster. The third cluster showed signs of likely genetic relationships among the largest gatherings of cultivars, including 47 cultivars which spread over three areas of oases: East (Mechounech), west (Tolga) and south of Biskra (OuedRigh). As in all date-growing areas of Sudan as reported by Elshibli and Korpelainen (2008), the farmers are using a few selected males for the pollination of female trees. In addition, they sometimes mixed the pollen grains of more than one male for pollination and finally new cultivars are a result of a continuous selection process carried out by farmers in their fields following sexual reproduction.

Altogether, these facts suggest a common origin of cultivars due to potential paternal relationships or easy exchange of plant materials among farmers by virtue of geographical zones and social well-being.

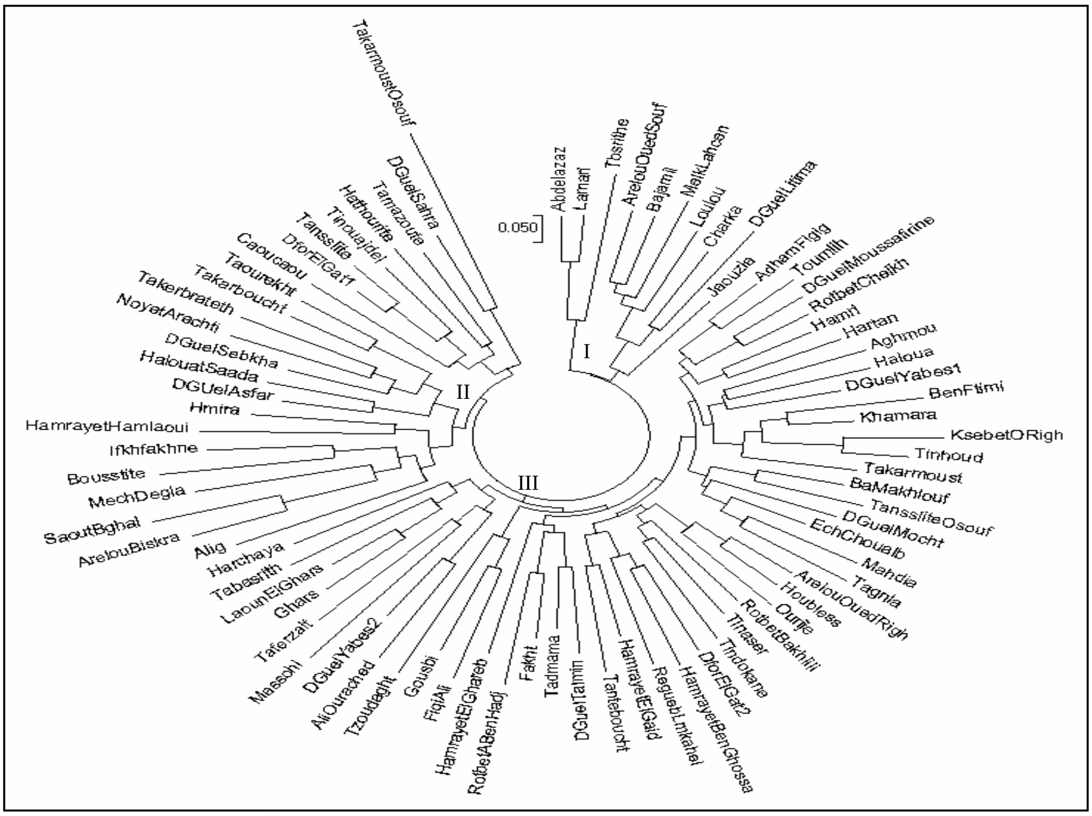

Figure 2. Neighbor-joining tree of individual genotypes based on 13 microsatellites loci. (Dendrogram generated with Populations software using shared allele distance).

4. Conclusion

Genetic relationships among cultivars from different areas in Biskra oases showed the existence of close relatedness within some groups of cultivars.

Results of the present work constitute the first study of minor varieties which are characterized by a low trade value but are still traditionally important and common for their quality of sub-products; they showed here a high genetic diversity across their traditional cultivation area.

These informations make it necessary to develop breeding strategies for economically and agronomically interesting cultivars with important traits such as fruit quality; furthermore, it is recommended to select tolerant varieties against biotic and abiotic stresses and to enrich the germplasm collections.

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Authors' contribution

Both authors have read the manuscript and agreed to its content.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ahmed TA and Al-Qaradawi AY.2009. Molecular phylogeny of Qatari date palm genotypes using simple sequence repeats markers. *Biotechnology*, **8**:126–131.

Adawy SS, Hussein EHA, Ismail SEME and El-Itriby HA. 2006. Genomic diversity in date palm (*Phoenix dactylifera* L.) as revealed by AFLPs in comparison to RAPDs and ISSRs. In: Abstracts of 3rd international date palm conference, Abu Dhabi, United Arab Emirates, 19–21 February.

AkkakA, Iscariot V,Torello-Marinoni D, Boccaccio P, Bertram C and Beta R.2009. Development and evaluation of microsatellite markers in *Phoenix dactylifera*L. and their transferability to other Phoenix species. *Biol Plantarum*,**53**:164–166.

Al-Khayri JM, Jain SM and Johnson DV.2015.**Date Palm Genetic Resources and Utilization. Volume 1: Africa and the Americas**. Springer Dordrecht Heidelberg, New York London.

Al-Ruqaishi IA, DaveyM, Alderson P andMayes S.2008.Genetic relationships and genotype tracing in date palms (*Phoenix dactylifera* L.) in Oman based on microsatellite markers. *Plant Genet Resour*,**61**:70–72.

AmellalH and Benamara S.2008. Vacuum drying of common date pulp cubes. *Dry technol*,**26**:378–382.

Barker PSF.1994. A global protocol for determining genetic distance among domestic livestock breeds. Proceeding of 5th world congress on genetic application of livestock production, Guelph and Ontario, Canada,**21**:501–508.

Bedjaoui H and Benbouza H. 2018. Assessment of phenotypic diversity of local Algerian date palm (*Phoenix dactylifera* L.) cultivars.*J Saudi Soc Agric Sci*, doi.org/10.1016/j.jssas.2018.06.002.

Billotte N, Marseillac N, Brittier P, Noyer JL, jacquemoud-Collet JP, Moreau C, Couvreur T, Chevallier MH, Pintaud JC and Risterucci AM. 2004. Nuclearmicrosatellite markers for the date palm (Phoenix dactylifera L.): characterizationand utility across the genus Phoenix and in other palm genera. *Mol Ecol Notes*,

4: 256–258.

Cao BR and Chao CT. 2002. Identification of date cultivars in California using AFLP markers. *Hortic Sci*, **37**:966–968.

Dellaporta SL, Wood J and Hicks JB. 1983. A plant DNA minipreparation: version II.*Plant Mol Biol Report*,**1**:19-21.

El-Assar AM, Krueger RR, Devanad PS and Chao CT. 2005. Genetic analysis of Egyptian date (*Phoenix dactylifera* L.) accessions using AFLP markers. *Genet Resour Evol*, **52**:601–607.

Elhoumaizi MA, Saaidi M, Oihabi A and Cilas C. 2002.Phenotypic diversity of datepalm cultivars (*Phoenix dactylifera* L.) from Morocco. *Genet Resour Crop Evol*, **49**:483–490.

ElmeerK, Sarwath H, Malek J, Baum M and Hamwieh A.2011. New microsatellite markers for assessment of genetic diversity in date palm (*Phoenix dactylifera* L.). *3 Biotech*,**1**:91-97.

ElmeerK and Mattat I.2015. Genetic diversity of Qatari date palm using SSR markers. *Genet Mol Res*,**14**: 1624-1635.

Elshibli S and Korpelainen H. 2008.Microsatellite markers reveal high genetic diversity in date palm (*Phoenix dactylifera* L.) germplasm from Sudan. *Genetica*,**134**: 251–260.

Guettouchi A, Haider N, NabulsiI and YkhlefN. 2017. Molecular characterization of Algerian date palm cultivars using circular plasmid-like DNAs. *Indian J Genet Plant Breed*,**77**(1):170.

Huda MN, Hasan M, Abdullah HM and Sarker U. 2019. Spatial distribution and genetic diversity of wild date palm (*Phoenix sylvestris*) growing in coastal Bangladesh. *Tree Genet Genomes*,**15**:3.

Jain SM, Al-Khayri JM and Johnson DV. 2011. **Date palm biotechnology**. Springer Dordrecht Heidelberg, New York, London.

Jin L and Chakraborty R. 1994. Estimation of genetic-distance and coefficient of gene diversity from single-probe multilocus DNA-fingerprinting data. *Mol Biol Evol*,**11**: 120-127.

Johnson C, Cullis TA, Cullis MA and Cullis CA. 2009.DNA markers for variety identification in date palm (*Phoenix dactylifera* L.). *J Hortic Sci Biotechnol*,**84**: 591–594.

Kalia RK, Rai MK, Kalia S, Singh R and Dhawan AK. 2011.Microsatellite markers: an overview of the recent progress in plants. *Euphytica*,**177**:309–334.

Khierallah HSM, Bader SM, Baum M and Hamwieh A.2011. Genetic diversity of Iraqi Date palms revealed by microsatellite polymorphism. *J Am Soc Hortic Sci*,**136**: 282-287.

Moussouni S, Pintaud J-C, Vigouroux Y and Bouguedoura N.2017.Diversity of Algerian oases date palm (*Phoenix dactylifera* L., *Arecaceae*): Heterozygote excessand cryptic structure suggests farmer management had a major impact on diversity. PLOS ONE |https://doi.org/10.1371/journal.pone.0175232.

Munier P.1973. **Le palmier dattier**. Ed. Maisonneuve et Larose, Paris .

Nixon RW. 1950. Imported cultivars of dates in the United States. USDA 834,144

Peakall R and SmousePE.2006. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Resour*,**6**:288-295.

Pintaud JC, Zehdi S, Couvreur T, Barrow S, Henderson S, Aberlenc-Bertossi F,Tregear J and Billotte N. 2010. Species delimitation inthe genus Phoenix (*Arecaceae*) based on SSR markers, with emphasis on the identity of the date palm (*Phoenix dactylifera* L.). In: Seberg O., Petersen G., Barfod A., Davis J. (eds) **Diversity, phylogeny, and evolution in the monocotyledons**. Arhus University Press, Denmark 267–286.

Saitou N and Nei M.1987.The neighbor-joining method - a new method for reconstructing phylogenetic trees.*Mol Biol Evol*, **4**: 406-425.

Santana JC, Hidalgo E, de Lucas AI, Recio P, Ortiz JM, Martin JP, Yuste J, Arranz C and Rubio JA. 2007. Identification and relationships of accessions grown in the grapevine (*Vitis vinifera* L.) Germplasm Bank of Castilla y Léon (Spain) and the varieties authorized in the VQPRD areas of the region by SSR-marker analysis. *Genet Resour Crop Evol*, **55**: 573-583.

Saro I, Robledo-Arnuncio JJ, González-Pérez MA and Sosa PA. 2014. Patterns of pollen dispersal in a small population of the Canarian endemic Palm (*Phoenix canariensis*). *Heredity*, **113**: 215-223.

Sedra MH, Lashermes P, Trouslot M and Hamon S.1998. Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties of Morocco using RAPD markers. *Euphytica*,**103**:75–82.

Sefc KM, Regner F, Turetschek E, Glossl J and Steinkellner H. 1999. Identification of microsatellite sequence in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome,* **42**: 367-373.

Simozrag A, Chala A, Djerouni A and Bentchikou ME. 2016. Phenotypic diversity of date palm cultivars (Phoenix dactylifera L.) from Algeria. *Gayana Bot*,**73** (1), 42-53.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M andKumar S.2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*,**28**: 2731-2739.

Udupa SM and Baum M. 2001. High mutation rate and mutational bias at (TAA)n microsatellite loci in chickpea (Cicerarietinum L.). *MolGenet Genomics*, **265** :1097-1103.

Wagner HW and SefcKM.1999. Identity 1.0. Centre for Applied Genetics, University of Agricultural Science, Vienna.

Zehdi S, Sakka H, Rhouma A, Salem, AOM, Marrakchi M and Trifi M.2004a. Analysis of Tunisian date palm germplasm using simple sequence repeat primers. *Afr J Biotechnol,* **3**:215–219.

Zehdi S, Trifi M, Billotte N, Marrakchi M and Pintaud JC.2004b. Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. *Hereditas*,**141**:278–287.

Zehdi S, Cherif E, Rhouma S, Santoni S, Salhi HAand Pintaud JC.2012. Molecular polymorphism and genetic relationships in date palm (*Phoenix dactylifera* L.): The utility of nuclear microsatellite markers. *Sci Horticult,* **148**:255-263.

Zehdi S, Cherif E, Moussouni S, Gros-Balthazard M, Abbas-Naqvi Sand Ludeña B. 2015. Genetic structure of the date palm (*Phoenix dactylifera* L.) in the Old World reveals a strong differentiation between eastern and western populations.*Ann Bot*,**116**: 101-112.

Supplementary Table 1. some morphological characters and genetic profiles of eighty Algerian date palm (*Phoenix dactylifera* L.) cultivars analyzed at 13 microsatellite loci

N°	Cultivar	Origine	Fruit color	Fruit shape	Fruit consistency	mPdCIR010		mPdCIR015		mPdCIR016		mPdCIR035		mPdCIR025		mPdCIR044		mPdCIR048	
1	Abdelazaz	Biskra center	black	straight	soft	133	135	122	126	130	130	175	175	227	231	299	299	158	195
2	Adham Figig	Oued Righ	brown	oval	soft	133	135	124	124	128	130	164	175	227	231	299	299	158	193
3	Aghmou	Oued Righ	brown	oval	semi-dry	135	135	124	138	128	128	175	188	/	/	299	299	158	193
4	Ali Ourached	Oued Righ	brown	triangular	semi-dry	127	/	/	/	128	137	175	175	227	227	317	317	193	195
5	Alig	Oued Righ	yellow	oval	semi-dry	135	163	/	/	128	130	172	175	231	231	299	301	158	175
6	Arelou Oued Righ	Oued Righ	amber	oval	soft	/	/	124	138	128	130	175	177	200	213	299	299	195	195
7	Arelou Oued souf	Oued Righ	amber	oval	soft	126	135	124	130	130	130	189	189	213	231	301	301	193	195
8	Arelou Biskra	Biskra center	brown	oval	soft	/	/	/	/	130	130	172	188	213	227	301	301	158	175
9	Ba Makhlouf	Oued Righ	red	triangular	semi-dry	123	135	124	157	128	130	175	188	227	231	299	299	158	193
10	Bajamil	Oued Righ	amber	oval	soft	121	135	124	126	130	130	189	189	213	231	301	301	193	195
11	Ben Ftimi	Oued Righ	brown	oval	soft	133	135	124	126	130	130	172	177	215	227	299	299	158	195
12	Bousstite	Oued Righ	brown	straight	semi-dry	135	135	126	138	128	130	189	189	200	217	301	301	175	175
13	Caoucaou	Biskra center	brown	oval	semi-dry	/	135	/	/	130	130	175	182	213	213	299	301	/	/
14	Charka	Oued Righ	amber	straight	semi-dry	135	135	136	138	128	130	189	189	213	231	301	301	195	195
15	Dfor El Gat1	Oued Righ	yellow	oval	semi-dry	/	135	/	/	130	130	175	175	213	215	299	299	193	195
16	Dfor El Gat2	Oued Righ	black	straight	semi-dry	123	135	131	138	128	128	172	175	213	227	299	317	158	195
17	DGuel Mocht	Oued Righ	//	//	//	133	135	/	/	130	130	175	175	213	227	299	301	193	195
18	DGuel Sahra	Oued Righ	//	//	//	121	135	124	124	130	130	175	177	213	213	299	299	175	195
19	DGUel Asfar	Oued Righ	amber	straight	soft	133	135	124	138	128	130	175	175	/	/	299	301	/	/
20	DGuel Litima	Biskra center	amber	straight	semi-dry	163	135	136	138	130	130	165	172	215	231	301	301	195	195
21	DGuel Moussafirine	Oued Righ	brown	oval	semi-dry	133	135	124	157	128	130	162	175	200	213	299	299	158	193
22	DGuel Sebkha	Oued Righ	brown	straight	semi-dry	133	135	124	124	128	130	175	188	200	231	299	299	158	193
23	DGuel Talmin	Oued Righ	brown	triangular	semi-dry	135	135	/	/	130	137	188	188	/	/	299	301	193	195
24	DGuel Yabes1	Oued Righ	yellow	triangular	dry	135	135	124	138	128	130	172	175	213	231	299	299	158	175
25	DGuel Yabes2	Oued Righ	yellow	triangular	dry	123	135	124	124	128	137	165	175	227	231	299	317	193	195
26	Ech Chouaib	Tolga	yellow	straight	semi-dry	123	135	124	138	130	137	/	/	/	/	299	317	175	193
27	Fakht	Oued Righ	brown	straight	dry	123	135	124	126	128	137	175	177	213	231	299	299	193	195
28	Fiqi Ali	Oued Righ	brown	straight	soft	123	135	124	138	128	137	175	188	200	227	301	301	195	195
29	Ghars	Biskra center	brown	triangular	soft	132	135	124	126	128	130	189	189	200	231	299	301	195	195
30	Gousbi	Oued Righ	yellow	straight	semi-dry	123	135	124	138	128	128	165	175	200	227	299	299	195	195
31	Haloua	Tolga	yellow	oval	semi-dry	135	135	124	138	128	130	189	189	213	213	299	299	195	195
32	Halouat Saada	Tolga	amber	oval	semi-dry	132	135	124	138	128	130	189	189	213	227	299	299	159	193

33	Hamrayet Ben Ghossa	Oued Righ	black	ovoid	soft	135	135	136	138	128	128	175	177	213	227	301	301	158	195
34	Hamrayet El Gaid	Oued Righ	black	triangular	semi-dry	135	135	124	124	/	/	175	177	213	227	299	301	158	195
35	Hamrayet El Ghareb	Oued Righ	black	triangular	soft	121	135	124	138	/	/	165	177	213	231	299	299	195	195
36	Hamrayet Hamlaoui	Oued Righ	black	straight	semi-dry	121	135	124	124	128	130	172	175	200	231	307	307	175	193
37	Hamri	Tolga	black	oval	dry	123	135	124	138	128	130	175	177	213	231	299	299	158	193
38	Harchaya	Biskra center	brown	straight	dry	132	135	124	124	128	128	189	189	213	213	299	301	193	195
39	Hartan	Oued Righ	brown	oval	semi-dry	135	135	124	138	128	128	189	189	213	231	299	299	158	193
40	Hathourite	M'chouneche	brown	triangular	soft	123	135	124	126	130	130	170	175	213	231	/	/	195	195
41	Hmira	Biskra center	amber	straight	soft	123	135	124	136	128	130	189	193	231	231	299	301	175	193
42	Houbless	Oued Righ	brown	oval	semi-dry	133	135	124	124	128	130	175	177	227	227	299	299	195	195
43	Ifkhfakhne	M'chouneche	brown	oval	semi-dry	135	135	122	138	128	130	165	172	213	217	301	317	158	175
44	Jaouzia	Biskra center	brown	oval	semi-dry	123	135	124	124	128	130	165	175	227	231	301	301	158	195
45	Khamara	Oued Righ	black	oval	soft	133	135	124	138	128	130	172	177	/	/	299	299	158	195
46	Ksebet ORigh	Oued Righ	amber	oval	semi-dry	133	135	/	/	128	130	189	189	227	231	299	299	158	175
47	Lamari	M'chouneche	brown	oval	semi-dry	135	/	122	126	130	130	189	189	227	231	299	299	158	195
48	Laoun El Ghars	Biskra center	brown	straight	soft	133	135	/	/	128	130	165	175	200	231	299	299	195	195
49	Loulou	Oued Righ	black	oval	soft	132	135	124	136	130	130	189	189	213	231	301	301	158	193
50	Mahdia	Oued Righ	yellow	oval	dry	135	138	124	136	130	130	189	189	200	227	299	299	158	193
51	Massohi	Oued Righ	red	oval	dry	123	123	124	126	130	130	189	206	227	231	299	317	175	193
52	Mech Degla	Biskra center	yellow	straight	dry	/	/	124	/	130	130	170	182	213	231	/	/	175	175
53	Melk Lahcen	Oued Righ	brown	triangulaire	semi-dry	123	126	124	124	130	130	189	189	215	231	301	301	193	195
54	Noyet Arechti	Biskra center	brown	oval	semi-dry	133	138	124	124	128	128	175	175	200	231	/	/	193	193
55	Ourije	Oued Righ	brown	straight	semi-dry	133	135	138	138	128	130	175	177	200	227	299	301	/	/
56	Regueb Lmkahel	Oued Righ	amber	straight	dry	123	135	138	138	128	128	177	182	213	227	299	299	175	195
57	Rotbet ABenHadj	Biskra center	yellow	oval	semi-dry	123	127	124	126	128	137	165	175	213	231	317	317	175	193
58	Rotbet Bakhlili	Biskra center	brown	oval	semi-dry	135	135	124	138	130	130	175	175	200	213	299	299	195	195
59	Rotbet Cheikh	Biskra center	amber	straight	soft	133	135	124	124	128	130	175	177	200	213	299	299	158	193
60	Saout Bghal	M'chouneche	black	straight	semi-dry	123	163	126	138	128	130	189	199	213	227	301	301	158	175
61	Tansslite Osouf	Oued Righ	black	straight	soft	121	123	124	157	128	130	175	175	213	227	299	299	158	193
62	Tabasrith	M'chouneche	brown	straight	soft	135	163	124	126	128	128	189	193	227	231	299	301	195	195
63	Tadmama	Oued Righ	red	oval	soft	133	135	124	157	128	130	175	188	200	213	299	299	193	195
64	Taferzait	Oued Righ	brown	oval	semi-dry	135	163	124	138	128	130	189	193	215	227	301	301	193	193
65	Tagnia	M'chouneche	brown	oval	semi-dry	123	135	138	138	130	130	189	189	200	213	299	299	158	193
66	Takarboucht	Biskra center	black	round	semi-dry	133	135	124	136	128	130	165	175	/	/	/	/	193	193
67	Takarmoust	Biskra center	black	round	soft	132	135	124	138	128	130	189	189	213	231	299	299	158	195
68	Takarmoust	Oued	black	round	soft	127	127	124	136	/	/	175	188	/	/	299	301	175	195

	Osouf	Righ																	
69	Takerbrateth	M'chouneche	black	round	semi-dry	138	159	124	124	128	128	175	177	200	231	299	299	/	/
70	Tamazoute	M'chouneche	amber	oval	dry	123	135	124	124	130	130	165	165	200	213	299	299	/	/
71	Tansslite	Oued Righ	black	straight	soft	121	135	124	136	130	130	189	189	213	213	299	299	193	195
72	Tanteboucht	M'chouneche	black	round	soft	135	163	124	130	130	130	189	189	213	227	299	299	158	195
73	Taourekht	M'chouneche	brown	oval	soft	133	135	124	124	128	130	175	188	200	213	/	/	193	193
74	Tbsrithe	M'chouneche	brown	straight	semi-dry	135	138	126	138	128	130	165	165	227	231	317	317	158	195
75	Tinaser	M'chouneche	amber	oval	soft	121	135	/	/	128	130	172	175	213	231	299	299	193	195
76	Tindokane	Oued Righ	black	round	soft	135	135	136	138	128	130	175	177	219	227	299	299	195	195
77	Tinhoud	Oued Righ	yellow	oval	semi-dry	133	135	126	138	128	130	175	177	227	231	299	299	158	175
78	Tinouajdel	Oued Righ	brown	straight	semi-dry	135	135	124	157	130	130	175	189	/	/	/	/	193	195
79	Toumlih	Oued Righ	brown	oval	soft	133	135	124	124	128	128	164	177	213	213	299	299	158	193
80	Tzoudaght	M'chouneche	black	straight	soft	133	138	124	124	128	132	188	198	200	227	/	/	195	195

Supplementary Table 1. some morphological characters and genetic profiles of eighty Algerian date palm (*Phoenix dactylifera* L.) cultivars analyzed at 13 microsatellite loci

N°	Cultivar	Origine	Fruit color	Fruit shape	Fruit consistency	mPdCIR057		mPdCIR063		mPdCIR070		mPdCIR078		mPdCIR085		mPdCIR090	
1	Abdelazaz	Biskra center	black	straight	soft	254	254	140	140	194	199	/	/	157	169	141	166
2	Adham Figig	Oued Righ	brown	oval	soft	250	254	122	140	192	194	117	123	/	/	168	168
3	Aghmou	Oued Righ	brown	oval	semi-dry	250	252	140	155	194	195	120	152	157	171	161	168
4	Ali Ourached	Oued Righ	brown	triangular	semi-dry	250	256	140	140	/	/	121	123	179	179	141	168
5	Alig	Oued Righ	yellow	oval	semi-dry	252	254	155	155	/	/	134	146	167	179	141	169
6	Arelou Oued Righ	Oued Righ	amber	oval	soft	250	250	122	140	194	/	152	152	157	179	168	168
7	Arelou Oued souf	Oued Righ	amber	oval	soft	254	269	140	140	192	192	117	136	169	177	141	171
8	Arelou Biskra	Biskra center	brown	oval	soft	250	252	122	140	209	209	117	146	167	169	148	166
9	Ba Makhlouf	Oued Righ	red	triangular	semi-dry	250	254	140	155	193	195	152	152	171	177	141	168
10	Bajamil	Oued Righ	amber	oval	soft	254	256	140	140	186	197	136	146	169	177	141	141
11	Ben Ftimi	Oued Righ	brown	oval	soft	250	250	155	155	/	/	117	123	171	179	148	168
12	Bousstite	Oued Righ	brown	straight	semi-dry	250	254	122	122	192	192	134	146	149	165	/	/
13	Caoucaou	Biskra center	brown	oval	semi-dry	254	269	140	140	/	/	146	146	177	179	150	167
14	Charka	Oued Righ	amber	straight	semi-dry	250	263	122	140	192	192	117	152	177	179	141	141
15	Dfor El Gat1	Oued Righ	yellow	oval	semi-dry	254	263	140	140	/	/	136	146	169	179	141	141
16	Dfor El Gat2	Oued Righ	black	straight	semi-dry	250	254	140	155	/	/	117	123	149	161	168	168
17	DGuel Mocht	Oued Righ	//	//	//	250	254	140	155	/	/	134	152	171	177	141	150
18	DGuel Sahra	Oued Righ	//	//	//	252	256	122	140	192	197	117	136	169	171	141	148
19	DGUel Asfar	Oued Righ	amber	straight	soft	250	274	122	140	195	209	117	146	171	177	141	166
20	DGuel Litima	Biskra center	amber	straight	semi-dry	250	269	140	140	197	197	136	152	177	179	141	141

21	DGuel Moussafirine	Oued Righ	brown	oval	semi-dry	250	254	155	155	194	194	/	/	/	/	141	161
22	DGuel Sebkha	Oued Righ	brown	straight	semi-dry	269	269	122	140	/	/	117	117	/	/	141	166
23	DGuel Talmin	Oued Righ	brown	triangular	semi-dry	250	254	140	140	194	195	117	152	177	179	/	/
24	DGuel Yabes1	Oued Righ	yellow	triangular	dry	250	250	122	140	/	/	152	152	171	177	141	168
25	DGuel Yabes2	Oued Righ	yellow	triangular	dry	250	256	140	166	186	194	123	152	179	179	168	168
26	Ech Chouaib	Tolga	yellow	straight	semi-dry	250	254	140	155	/	/	/	/	/	/	/	/
27	Fakht	Oued Righ	brown	straight	dry	250	254	140	140	192	192	117	152	177	179	141	172
28	Fiqi Ali	Oued Righ	brown	straight	soft	256	269	/	/	192	/	117	117	149	171	/	/
29	Ghars	Biskra center	brown	triangular	soft	263	269	122	140	194	194	121	123	157	179	141	167
30	Gousbi	Oued Righ	yellow	straight	semi-dry	256	269	140	140	192	195	120	152	157	179	161	168
31	Haloua	Tolga	yellow	oval	semi-dry	250	250	122	140	/	/	117	152	157	171	161	169
32	Halouat Saada	Tolga	amber	oval	semi-dry	250	274	140	140	195	209	117	146	171	177	141	171
33	Hamrayet Ben Ghossa	Oued Righ	black	ovoid	soft	250	269	140	155	197	197	123	136	/	/	141	168
34	Hamrayet El Gaid	Oued Righ	black	triangular	semi-dry	250	254	140	155	194	197	152	152	169	179	141	168
35	Hamrayet El Ghareb	Oued Righ	black	triangular	soft	254	256	140	140	/	/	123	152	/	/	168	168
36	Hamrayet Hamlaoui	Oued Righ	black	straight	semi-dry	263	263	140	140	186	192	146	152	169	169	141	171
37	Hamri	Tolga	black	oval	dry	254	256	140	155	194	194	117	123	171	177	141	166
38	Harchaya	Biskra center	brown	straight	dry	256	269	122	140	194	195	117	123	177	179	141	167
39	Hartan	Oued Righ	brown	oval	semi-dry	250	254	140	155	186	194	123	146	171	177	141	167
40	Hathourite	M’chouneche	brown	triangular	soft	254	254	122	140	190	195	146	152	169	179	141	166
41	Hmira	Biskra center	amber	straight	soft	250	263	140	140	192	192	117	146	169	177	141	141
42	Houbless	Oued Righ	brown	oval	semi-dry	250	250	140	140	194	194	/	/	/	/	168	168
43	Ifkhfakhne	M’chouneche	brown	oval	semi-dry	254	254	122	140	192	192	117	146	/	/	141	141
44	Jaouzia	Biskra center	brown	oval	semi-dry	250	269	122	140	/	/	117	152	/	/	141	168
45	Khamara	Oued Righ	black	oval	soft	250	252	/	/	192	194	/	/	171	179	141	141
46	Ksebet ORigh	Oued Righ	amber	oval	semi-dry	250	252	155	155	/	/	123	136	171	171	169	169
47	Lamari	M’chouneche	brown	oval	semi-dry	254	254	122	140	194	199	117	152	157	169	141	167
48	Laoun El Ghars	Biskra center	brown	straight	soft	263	269	140	140	/	/	121	123	157	179	/	/
49	Loulou	Oued Righ	black	oval	soft	250	256	140	140	197	197	136	152	169	171	141	141
50	Mahdia	Oued Righ	yellow	oval	dry	250	254	140	155	192	192	117	152	171	177	169	169
51	Massohi	Oued Righ	red	oval	dry	256	263	166	166	194	207	117	146	171	179	141	171
52	Mech Degla	Biskra center	yellow	straight	dry	250	252	122	140	192	209	146	146	169	171	168	168
53	Melk Lahcen	Oued Righ	brown	triangulaire	semi-dry	254	256	122	140	197	197	117	136	157	169	141	169
54	Noyet Arechti	Biskra center	brown	oval	semi-dry	250	269	122	140	194	195	121	146	157	177	141	166
55	Ourije	Oued Righ	brown	straight	semi-dry	250	250	140	155	194	194	123	123	171	179	141	168
56	Regueb Lmkahel	Oued Righ	amber	straight	dry	250	269	140	140	197	197	121	152	/	/	141	168

57	Rotbet ABenHadj	Biskra center	yellow	oval	semi-dry	250	254	140	140	/	/	117	146	171	177	141	161
58	Rotbet Bakhlili	Biskra center	brown	oval	semi-dry	250	269	140	140	/	/	121	136	/	/	141	168
59	Rotbet Cheikh	Biskra center	amber	straight	soft	250	269	140	155	194	194	117	123	149	149	141	168
60	Saout Bghal	M’chounec he	black	straight	semi-dry	250	252	122	122	209	209	117	146	167	167	150	167
61	Tansslite Osouf	Oued Righ	black	straight	soft	250	254	/	/	196	197	/	/	169	171	141	168
62	Tabasrith	M’chounec he	brown	straight	soft	250	254	122	140	194	194	121	123	169	179	169	169
63	Tadmama	Oued Righ	red	oval	soft	250	254	140	140	192	194	/	/	177	179	168	168
64	Taferzait	Oued Righ	brown	oval	semi-dry	256	256	140	166	186	194	123	152	171	179	141	169
65	Tagnia	M’chounec he	brown	oval	semi-dry	250	254	140	155	194	195	121	152	157	171	161	169
66	Takarboucht	Biskra center	black	round	semi-dry	254	256	140	140	186	195	117	146	157	177	141	161
67	Takarmoust	Biskra center	black	round	soft	250	252	140	155	192	195	123	152	171	179	141	141
68	Takarmoust Osouf	Oued Righ	black	round	soft	256	256	122	122	197	197	120	136	157	169	148	148
69	Takerbrateth	M’chounec he	black	round	semi-dry	250	/	/	/	195	195	117	117	/	/	168	168
70	Tamazoute	M’chounec he	amber	oval	dry	254	256	122	140	186	192	117	146	169	179	141	168
71	Tansslite	Oued Righ	black	straight	soft	254	263	140	140	197	209	136	146	169	179	141	141
72	Tanteboucht	M’chounec he	black	round	soft	250	250	140	155	197	197	152	152	169	179	141	169
73	Taourekht	M’chounec he	brown	oval	soft	254	254	140	140	186	195	/	/	177	177	141	166
74	Tbsrithe	M’chounec he	brown	straight	semi-dry	254	254	122	140	194	207	117	134	169	169	169	169
75	Tinaser	M’chounec he	amber	oval	soft	250	269	122	140	194	194	117	121	149	149	141	141
76	Tindokane	Oued Righ	black	round	soft	250	254	122	140	192	194	123	136	149	149	141	141
77	Tinhoud	Oued Righ	yellow	oval	semi-dry	250	252	140	155	194	199	123	136	171	171	168	168
78	Tinouajdel	Oued Righ	brown	straight	semi-dry	254	254	140	155	186	197	117	146	/	/	141	141
79	Toumlih	Oued Righ	brown	oval	soft	250	254	122	140	192	194	117	152	157	171	/	/
80	Tzoudaght	M’chounec he	black	straight	soft	250	254	140	155	192	194	117	121	157	169	161	168