

Molecular and morphological analyses of date palm (*Phoenix dactylifera* L.) subpopulations in southern Tunisia

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Abstract

Phoenix dactylifera L. is widely distributed and performs an important socioeconomic role in the south of Tunisia. The objective of this work was to evaluate different methods of estimating the diversity and genetic structure of Tunisian date palm subpopulations. Nine morphological traits and five SSR loci were used to study morphological and genetic diversity. Correlations between phenotypic and genetic distances were assessed. Analyses of variance of the morphological data reveal significant differences among subpopulations for all traits measured. The Mantel test shows that morphological variation is correlated with fruit maturity period ($r=0.161$; $p=0.020$). Analysis of molecular variance reveals significant genetic variation among fruit-consistency subpopulations ($p<0.05$) and the Mantel test emphasises a correlation between genetic distance and fruit consistency ($r=0.110$; $p=0.029$). This information will be useful for germplasm collection, for conservation and for various date palm culture programmes in the Tunisian continental oases.

Additional key words: correlation; diversity; microsatellites; morphology.

Resumen

Análisis genético y morfológico de subpoblaciones de palmera datilera (*Phoenix dactylifera* L.) en el sur de Túnez

Phoenix dactylifera L. tiene una distribución amplia y una importante función socioeconómica en el sur de Túnez. El objetivo de este trabajo fue evaluar los diferentes métodos de estimación de la diversidad y la estructura genética de las subpoblaciones de palmera datilera de Túnez. Se utilizaron nueve características morfológicas y cinco loci SSR para estudiar la diversidad morfológica y genética, y se evaluó el nivel de correlación entre las distancias fenotípicas y genéticas. El análisis de varianza de los datos morfológicos reveló diferencias significativas entre las subpoblaciones para todos los caracteres medidos. La prueba de Mantel mostró que la variación morfológica se correlacionó con el período de maduración de las palmeras ($r=0,161$; $p=0,020$). El análisis de varianza molecular reveló una variación genética significativa entre las subpoblaciones clasificadas por su consistencia del fruto ($p<0,05$) y la prueba de Mantel destacó la correlación entre la distancia genética y la consistencia del fruto ($r=0,110$; $p=0,029$). Esta información será útil para la recolecciones y conservación de germoplasma y los diversos programas de cultivo de la palmera datilera en los oasis de Túnez continental.

Palabras clave adicionales: correlación; diversidad; microsatélites; morfología.

Introduction

The date palm (*Phoenix dactylifera* L., $2n=36$) is an ancient perennial plant that has been domesticated since 3000 BC in Mesopotamia (Nixon, 1959) and extensively cultivated in the Middle East and in North Africa. Being dioecious (separate male and female plants)

the date palm has high genetic diversity (Munier, 1981). In the south of Tunisia there are more than 4 million trees growing approximately on 32,000 ha of oases (Rhouma, 2005), representing more than 200 distinct cultivars (Rhouma, 2005; Ferchichi and Hamza, 2008). The most widely cultivated of these is 'Deglet Nour' which produces the major part of the commercially desirable fruit crop.

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Abbreviations used: AFLP (amplified fragment length polymorphism), ISSR (inter simple sequence repeats), RAMPO (random amplified microsatellites polymorphism), RAPD (random amplification polymorphism DNA), RFLP (restriction fragment length polymorphism), SSR (simple sequences repeat), UPGMA (unweighted pair-group method with arithmetic average).

Identification of date palm cultivars is usually not possible until fruiting (Munier, 1973). In addition the characterisation of cultivars and the evaluation of genetic diversity require large sets of phenotypic data that are often difficult to assess and are anyway sometimes variable due to environmental influences (Sedra *et al.*, 1993, 1996; Munier, 1973). In Tunisian oases, the high genetic diversity can be resolved in many ways such as by using morphological and molecular tools. Hamza *et al.* (2009) selected six stable vegetative morphological traits, without significant environment plasticity and under strong genetic control. These traits are very important adaptively and are decisive in cultivar selection and adaptation.

Molecular markers may provide a reliable tool for measuring genetic divergence. Several markers have been used in date palm studies, random amplification polymorphism DNA (RAPD) (Sedra *et al.*, 1998; Ben Abdallah *et al.*, 2000; Trifi *et al.*, 2000; Al-Khalifa and Askari, 2003), inter simple sequence repeats (ISSR) (Zehdi *et al.*, 2002), random amplified microsatellites polymorphism (RAMPO) (Rhouma, 2008) and amplified fragment length polymorphism (AFLP) (Snoussi *et al.*, 2001; Rhouma *et al.*, 2007). Restriction fragment length polymorphisms (RFLPs) have been evaluated for date palm clone identification (Corniquel and Mercier, 1994; Sakka *et al.*, 2003), but the technique is laborious and so not well suited to studies involving a large number of samples. Simple sequence repeat (SSR) is very useful for identifying date palm cultivars and high polymorphism has been detected (Zehdi *et al.*, 2004).

The purpose of this study was to examine possible relations between morphological traits and SSR marker variations in *Phoenix dactylifera* L. in different Tunisian subpopulations. The analyses were carried out in two stages. First, quantitative morphological variables and molecular markers were tested for their ability to discriminate among subpopulations and, second, correlations between genetic and morphological distances were tested.

Material and methods

Collection of material

Date palm material was collected from the continental Tunisian oases (Fig. 1). These areas represent more than 85% of the total date palm oases of Tunisia. Twenty-

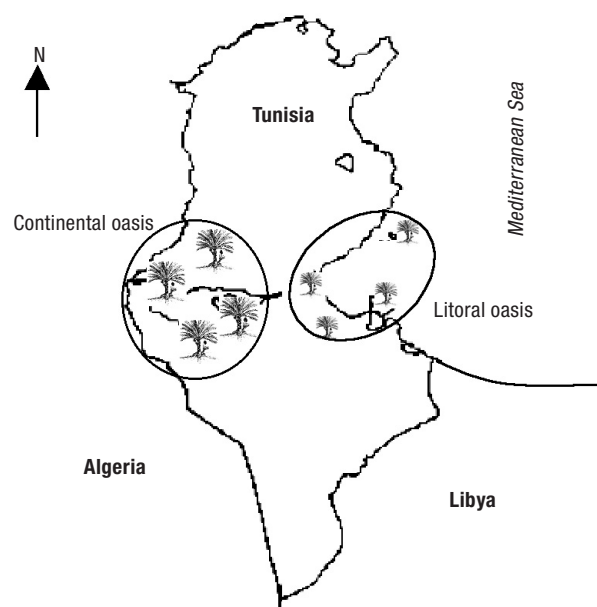


Figure 1. Locations of Tunisian oases.

six cultivars were chosen due to the commercial importance of their fruits, although almost every part of the plant is used in the rural economy for food, building, animal feed or hand crafts. In most cases in Tunisia, date palms are grown as the main constituent of mixed agricultural systems.

Analyses were performed on 78 individual trees belonging to the 26 cultivars at the rate of three replications for each cultivar. The cultivars studied were clustered into subpopulations based on fruit quality and maturity period. Three subpopulations were distinguished according to maturity period: early season (18 cultivars), mid-season (39 cultivars) and late season (21 cultivars). Based on fruit consistency (texture) the same cultivars are also clustered into four subpopulations: soft (30 cultivars), semi-soft (18 cultivars), semi-dry (18 cultivars) and dry (12 cultivars).

Morphological analysis

Six vegetative characters were measured (Table 1). These were selected for their importance in cultivar identification and also for their intra-genotype measure reproducibility (Hamza *et al.*, 2009). Three further reproductive characters describing the inflorescences and fruit were also used (Table 1). Again, these characters were selected on the principle of their low environmental plasticity and strong genetic control.

Table 1. List of morphological characters measured

	Characters	Code
Vegetative (leaves)	Spined midrib part length (%)	PM9
	Apical divergence angle (°)*	PM16
	Maximal pinnae width at the top leaf (cm)*	PN9
	Solitary spine number (%)*	EP3
	Spine length in the middle (cm)*	EP6
	Maximal spine angle (°)	EP8
Reproductive (inflorescences and fruit)	Bunch length without spikelet (%)	RG3
	Spikelet length without fruits (%)	EL3
	Fruit internal cavity ratio (L/l)	RB7

*: according to the date palm IPGRI descriptor (IPGRI, 2005).

Molecular analysis

Genomic DNA of each genotype was extracted from young leaves. Total nuclear DNA was extracted according to Invisorb® Spin Plant Mini Kit (Invitex). DNA polymorphism was detected by the polymerase chain reaction (PCR) using SSR primers. Five markers were used having been developed for *Phoenix dactylifera* L. by Billotte *et al.* (2004) and chosen for their expected high heterozygosity values.

The SSR-PCR was performed in a volume of 12.5 µL containing 50 ng of genomic DNA, 5X Green GoTaq® reaction buffer (Promega), 0.2 mM of dNTPs, 0.625 U of Taq polymerase (GoTaq, Promega), 2 mM of MgCl₂, 0.2 µM of each primer (Markus, 2000). Amplifications were carried out in a DNA amplification Thermocycler (GeneAmp® PCR System 9700). The conditions for SSR-PCR were an initial denaturation at 94°C for 3 min followed by 10 cycles of denaturation at 94°C for 20 s, annealing at primer specific melting temperature for 1 min, extension at 72°C for 40 s, followed by 25 cycles of 30 s at 94°C, 30 s at 53°C and 30 s at 72°C with a final extension at 72°C for 8 min. The amplification products were detected using electrophoresis with 1% agarose gels and by staining with ethidium bromide. For final analyses, 0.54 µL of amplified DNA and 5 µL of MegaBACE ET400-R DNA size standard were loaded. Genotyping was carried out using an automatic DNA analyser, MegaBACE 1000.

Data analysis

The nine quantitative morphological variables were analysed separately by ANOVA with post-hoc LSD

mean comparisons. The impact of selected morphological variables in subpopulation separation was assessed by discriminant analysis (Sokal and Rohlf, 1995) and the statistical program was SPSS 12.0 (SPSS, 2003). MVSP 3.1 software (Kovach, 2005) was used to compute hierarchical ascendant unweighted pair-group method arithmetic average (UPGMA) for clustering of the subpopulations based on average distance between subpopulation means of morphological traits.

Molecular data were computed with the Genalex program, version 6 (Peakall and Smouse, 2006) to estimate genetic diversity for each subpopulation, from determinations of observed and expected heterozygosity (*Ho* and *He*) (Nei, 1987). The Hardy-Weinberg equilibrium was verified and χ^2 test was used to compare observed versus expected heterozygosity at each locus. For pair-wise comparisons between groups, analysis of molecular variance (AMOVA) was tested (Excoffier *et al.*, 1992) using 999 re-sampled individuals. Populations 1.2.28 software (Langella, 2002) was used to compute Nei's genetic distances (1972) and then to build phylogenetic trees of subpopulations using the bootstrap approach with 1,000 replicates.

A Mantel's nonparametric test (Mantel, 1967) was performed to infer possible correlations between matrices of dissimilarity considering morphological variables, fruit characteristics and genetic distances. For the maturity period and fruit consistency data, binary matrices were constructed: the distance was set to 0 between individuals having identical fruit characteristics and to 1 between individuals having different fruit characteristics. These analyses were performed using the software package Mantel (version 2.0) (Liedloff, 1999).

Results

Morphological analyses

The mean values of the nine characters for the different subpopulations are listed in Table 2. ANOVA shows significant differences ($p < 0.05$) for eight characters between the fruit-consistency subpopulations and for five characters between the maturity-period ones. The percentage of spined midrib parts (PM9) for early and soft cultivars was significantly smaller than for the others and these cultivars also showed the highest percentages of solitary spines (EP3). In addition, the percentage of bunches length without spikelets (RG3) and the percentage of fructified spikelets length (EL3) for the early cultivars were, respectively, the lowest and the highest ($p < 0.05$) in comparison with the mid-season and late maturing cultivars.

Discriminant analysis of the maturity-period groups shows that the two functions represent 100% of the total variation in the data set (Table 3). The first function explains 80.6% of the total variance and separates the early maturing cultivars from the rest (Fig. 2a). This function was found to be highly correlated with the characters PM9, EP3, EL3 and RG3. The second discriminant analysis of the fruit-consistency subpopulations

shows that the first two functions explain 92.5% of total variation (Table 3). The first function accounts for 70.2% of the total variation and successfully separated the soft cultivars from the other fruit-consistency subpopulations (Fig. 2b). This function was significantly correlated with the PM9, EP3 and EL3 traits.

Further inter-relationships were investigated through the UPGMA dendrogram (Fig. 3) based on the average distances among the groups studied and measured using the means of their morphological traits. Relationship results for the different subpopulations were comparable with those for the discriminant analyses for the fruit-consistency groups. Semi-soft and dry subpopulations were clustered and the soft and semi-dry ones constituted two distinct groups. On the other hand, the mid-season cultivars were equidistant from the late and early subpopulations as shown by the discriminant analyses.

Molecular analyses

The microsatellites examined in this study were highly polymorphic possessing a great number of alleles with an average of 7.2 alleles per locus. High levels of expected and observed heterozygosity were detected, the mean *He* value for all loci is 0.63 and the mean *Ho* value is 0.70 (Table 4). The lowest expected and observed heterozygo-

Table 2. Mean values and standard deviation (s. d.) for the morphological variables analysed (see Table 1 for details).

Characters		Fruit-consistency groups				Maturity-period groups		
		Soft	Semi-soft	Semi-dry	Dry	Early	Mid-season	Late
PM9	Mean	16.50 ^b	23.92 ^a	24.85 ^a	23.94 ^a	13.76 ^c	21.93 ^b	26.51 ^a
	s. d.	4.66	5.36	2.89	3.10	3.43	3.41	3.47
EP3	Mean	56.52 ^a	44.66 ^b	35.09 ^c	36.25 ^c	61.27 ^a	41.47 ^b	40.28 ^b
	s. d.	12.88	9.95	9.01	4.23	6.4	13.83	7.24
PM16	Mean	70.50 ^{bc}	76.84 ^{ab}	68.21 ^c	83.12 ^a	69.26 ^a	76.26 ^a	71.56 ^a
	s. d.	15.95	10.26	6.11	3.64	17.14	10.25	10.83
PN9	Mean	1.61 ^{ab}	1.63 ^{ab}	1.45 ^b	1.94 ^a	1.47 ^a	1.69 ^a	1.66 ^a
	s. d.	0.81	0.49	0.45	0.46	0.66	0.70	0.45
EP6	Mean	8.36 ^b	9.24 ^b	10.67 ^a	8.95 ^b	8.34 ^b	8.91 ^b	10.43 ^a
	s. d.	2.02	1.92	2.88	0.86	2.1	1.74	2.76
EP8	Mean	57.86 ^a	59.10 ^a	49.17 ^b	64.00 ^a	56.85 ^a	58.95 ^a	53.83 ^a
	s. d.	12.44	9.54	5.62	16.27	13.40	12.83	8.97
RG3	Mean	71.88 ^b	76.50 ^a	74.60 ^{ab}	75.32 ^{ab}	70.88 ^b	74.20 ^a	76.69 ^a
	s. d.	7.36	3.72	4.48	5.42	8.42	7.39	4.88
EL3	Mean	45.95 ^a	44.3 ^a	43.16 ^a	43.79 ^a	49.16 ^a	43.18 ^b	43.31 ^b
	s. d.	7.75	3.88	3.9	4.55	7.73	4.62	3.66
RB7	Mean	3.02 ^{ab}	3.33 ^a	2.64 ^b	2.90 ^{ab}	2.97 ^a	3.12 ^a	2.74 ^a
	s. d.	0.73	0.51	0.95	0.54	0.81	0.54	0.97

Means followed by the same letter in the same row and for the same fruit characteristics are not significantly different at $p < 0.05$ according to LSD test.

Table 3. Largest absolute correlation between each variable and discriminant functions for the two discriminant analyses

Characters	Discriminant analysis of maturity-period groups		Discriminant analysis of fruit-consistency groups		
	Function 1 (80.6%)	Function 2 (19.4%)	Function 1 (70.2%)	Function 2 (22.3%)	Function 3 (7.5%)
PM9	0.926		0.733		
EP3	-0.526		-0.739		
EL3	-0.311		-0.165		
RG3	0.250			0.241	
PM16		-0.304		0.559	
EP6		0.300			0.336
RB7		-0.295		0.420	
EP8		-0.245		0.539	
PN9		-0.093			-0.452

sity values were observed in the semi-soft and the dry subpopulations. The observed heterozygosity was less than the expected within the groups studied except for the early, semi-soft and semi-dry ones (Table 4). However, the χ^2 tests indicate that no deviation from Hardy Weinberg equilibrium was observed for any locus in each subpopulation, except for locus mpdCIR093 in the mid-season and soft subpopulations (Table 4).

AMOVA tests showed no genetic differentiation among maturity-period subpopulations, however significant genetic differentiation was observed among the fruit-consistency subpopulations ($p < 0.05$) with 7% of

total genetic diversity being detected among fruit-consistency subpopulations. Pair-wise comparisons of populations (Table 5) show that significant genetic differences exist between the semi-soft subpopulation and the semi-dry and soft groups. This observation supports the morphological distinction found between the fruit-consistency subpopulations. Another significant genetic difference was detected between semi-soft groups and early-season groups. The UPGMA phenogram constructed on genetic distance (Fig. 4) reveals that soft and semi-dry cultivars are clustered while dry and semi-soft subpopulations form two separate clusters.

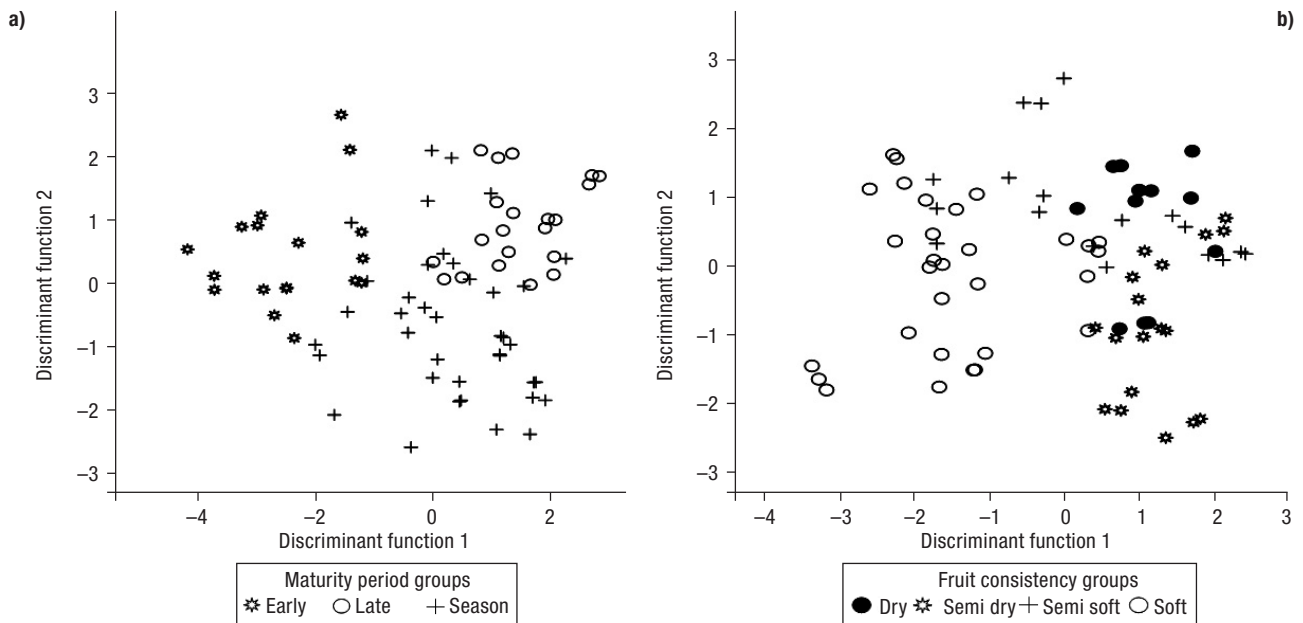


Figure 2. Morphological differences illustrated through discriminant analysis. (a) The scores of maturity-period subpopulations. (b) The scores of fruit-consistency subpopulations.

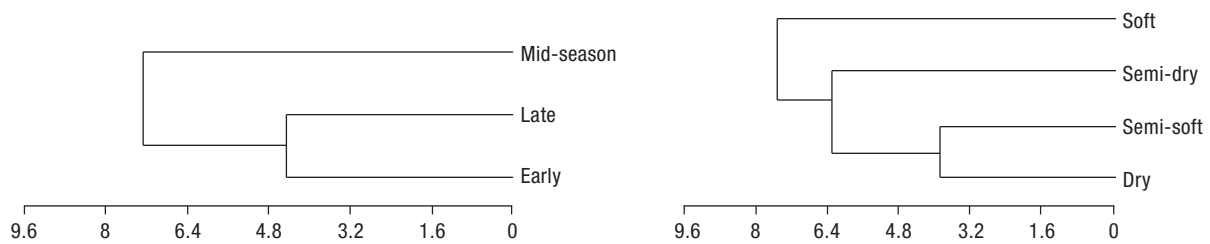


Figure 3. UPGMA dendrogram based on average distance among subpopulation means of morphological traits.

Mantel test

The Mantel statistic tests look for relationships between distance matrices representing the morphological variables, fruit characteristics and variables describing molecular diversity. The null hypothesis of no correlation between different matrices was tested (Ta-

ble 6). Distances based on quantitative morphological traits were not correlated with genetic distance. However, a significant positive correlation was found between the molecular data and consistency matrix ($r = 0.110$; $p = 0.029$) but not with the maturity matrix ($r = -0.027$; $p = 0.382$). All morphological data were correlated only with maturity matrix ($r = 0.1619$; $p = 0.020$).

Table 4. Genetic diversity for the different subpopulations

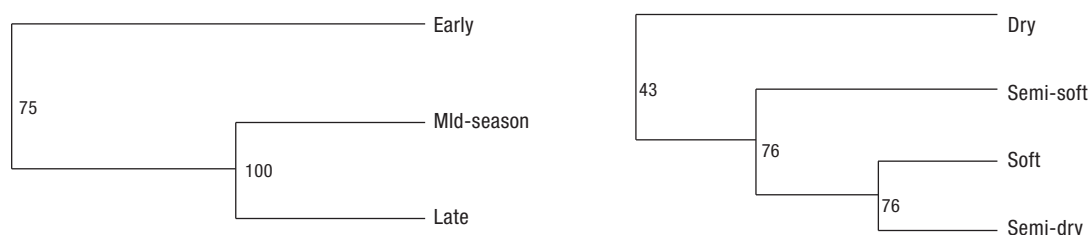
		Microsatellites					
	Locus	MPdCIR010	MPdCIR015	MPdCIR032	MPdCIR070	MPdCIR093	Mean
<i>Maturity-period subpopulations</i>							
Early	<i>He</i>	0.65	0.61	0.72	0.75	0.65	0.67
	<i>Ho</i>	1.00	1.00	0.66	0.66	0.33	0.73
	<i>F</i>	-0.532	-0.636	0.077	0.111	0.489	-0.098
Mid-season	<i>He</i>	0.79	0.67	0.71	0.61	0.64	0.68
	<i>Ho</i>	0.84	0.69	0.76	0.46	0.30	0.61
	<i>F</i>	-0.059	-0.026	-0.083	0.246	0.521**	0.120
Late	<i>He</i>	0.66	0.77	0.70	0.53	0.62	0.65
	<i>Ho</i>	0.85	0.85	0.57	0.28	0.42	0.60
	<i>F</i>	-0.292	-0.105	0.188	0.462	0.311	0.113
<i>Fruit-consistency subpopulations</i>							
Soft	<i>He</i>	0.79	0.56	0.67	0.74	0.56	0.67
	<i>Ho</i>	1.00	0.80	0.70	0.50	0.20	0.64
	<i>F</i>	-0.266	-0.416	-0.037	0.329	0.646*	0.051
Semi soft	<i>He</i>	0.61	0.72	0.69	0.27	0.48	0.56
	<i>Ho</i>	0.83	0.83	0.66	0.33	0.33	0.60
	<i>F</i>	-0.364	-0.154	0.040	-0.200	0.314	-0.073
Semi dry	<i>He</i>	0.70	0.77	0.61	0.72	0.62	0.67
	<i>Ho</i>	1.00	0.83	0.83	0.50	0.50	0.73
	<i>F</i>	-0.412	-0.071	-0.364	0.308	0.200	-0.068
Dry	<i>He</i>	0.53	0.65	0.62	0.65	0.71	0.64
	<i>Ho</i>	0.50	0.75	0.5	0.5	0.5	0.55
	<i>F</i>	0.059	-0.143	0.200	0.238	0.304	0.132
Whole population	<i>He</i>	0.75	0.70	0.72	0.69	0.65	0.63
	<i>Ho</i>	0.88	0.80	0.69	0.46	0.34	0.70
	<i>F</i>	-0.177**	-0.140	0.042	0.338	0.472***	0.107

He: expected heterozygosity. Ho: observed heterozygosity. F: Fixation index. *: $p < 0.05$. **: $p < 0.01$. ***: $p < 0.001$.

Table 5. F_{ST} values between different subpopulations based on five microsatellite loci

	Soft	Dry	Semi-dry	Semi-soft	Early	Late	Mid-season
Soft		0.104	0.329	0.013	0.447	0.149	0.450
Dry	0.042		0.244	0.142	0.222	0.438	0.409
Semi-dry	0.007	0.026		0.016	0.431	0.421	0.379
Semi-soft	0.065	0.049	0.093		0.011	0.420	0.272
Early	0.000	0.026	0.000	0.093		0.158	0.261
Late	0.020	0.000	0.000	0.000	0.026		0.436
Mid-season	0.000	0.000	0.004	0.011	0.012	0.000	

F_{ST} values below diagonal. Probability values based on 999 permutations are shown above diagonal. Negative pair-wise F_{ST} converted to zero.

**Figure 4.** UPGMA tree of the date palm subpopulations studied based on Nei's genetic distance (1972). Bootstrap values are given.

Discussion

In this study, morphological traits and SSR markers were used to characterise the 26 Tunisian date palm cultivars collected from continental oases. These cultivars were clustered into subpopulations according to fruit consistency or to maturity period.

Most of morphological characters are highly variable. The LSD tests reveal that PM9 and EP3 vegetative characters can be used outside the fruiting period to determine the maturity period and the fruit consistency characteristics of the cultivars. These variables have strong genetic control because they remain stable when edaphic or climatic factors change (Hamza *et al.*, 2009).

Table 6. Result of Mantel's test of the pair-wise correlations between dissimilarity matrices

First matrix	Second matrix	Mantel's r	Probability
PM9	SSR	-0.054	0.288
EP3	SSR	0.003	0.441
EP6	SSR	-0.014	0.111
EP8	SSR	-0.731	0.210
PN9	SSR	-0.052	0.293
PM16	SSR	-0.023	0.332
EL3	SSR	0.019	0.450
RG3	SSR	-0.015	0.435
RB7	SSR	-0.041	0.348
All morphological data	SSR	-0.007	0.496
Vegetative data	SSR	-0.012	0.500
Fruit data	SSR	-0.015	0.447
Maturity	SSR	-0.027	0.382
Fruit consistency	SSR	0.110	0.029*
Maturity period	All morphological data	0.161	0.020*
Consistency	All morphological data	0.060	0.132

*: Rejection of the null hypothesis of no correlation within a 5% confidence interval.

It is interesting that the date palm is characterised by these variables because it is a genus having high phenotypic plasticity. For this reason most farmers are unable to recognise particular cultivars outside their own oasis or outside their restricted fruiting periods (Munier, 1973; Sedra *et al.*, 1993, 1996). UPGMA analyses based on morphological data reveal a structure in which late and early-maturity groups are equal in distance with mid-season cultivars groups. On the other hand, the semi-soft and dry groups are closely associated but well differentiated from the semi-dry and soft subpopulations. The same clustering was observed in the discriminant analyses, the distribution of the maturity-period groups or fruit-consistency groups was linked to the quantitative morphological variables.

Our results indicate the presence of high genetic diversity in Tunisian date palms but less than in Sudan date palms (Elshibli and Korpelainen, 2008). This may be explained by intensive selection in Tunisian date palm oases (Zehdi *et al.*, 2004). This result agrees with other reports for Moroccan, Algerian and Tunisian date palm cultivars based on analyses using microsatellite markers (Zehdi *et al.*, 2004) and isozyme markers (Bennaceur *et al.*, 1991; Ould Mohamed Salem *et al.*, 2001). The numbers of alleles per locus detected in this study were comparable with those scored by Zehdi *et al.* (2004): for 46 Tunisian date palm accessions, 100 different alleles were identified at 14 microsatellite loci with an average of 7.14 alleles per locus.

A high genetic diversity was detected among Tunisian date palm cultivars and this was reflected in their heterozygosity values. Genetic diversity indicates good potential for further improving the agronomic and commercial characters of date palms (Elshibli and Korpelainen, 2008) and for allowing them to adapt to new environments and to climate change (Hamrick *et al.*, 1992). Mid-season and soft subpopulations show a significant deviation from Hardy-Weinberg equilibrium on the mpdCIR093 locus. This locus may be linked with genes on which selective forces have been applied over the centuries.

The differentiation detected between populations is likely the result of isolation due to geographic distance and climate differences and the difficulty of exchanging vegetative material (Zehdi *et al.*, 2004; Elshibli and Korpelainen, 2008). Another differentiation trend is revealed in this study; the F_{ST} values suggest significant genetic differentiation between subpopulations. The semi-soft subpopulation was significantly differentiated from the other fruit-consistency subpopulations

and from the early-maturity subpopulation. This genetic differentiation suggests that Tunisian date palms may not be a coherent population but instead a merged set of populations having different origins. We cannot explain this result without further information on the history of date palm culture in Tunisia.

The Mantel test shows no correlation between molecular variation and variation based on the selected morphological traits. This is reflected in the pattern of the UPGMA dendrogram based on the quantitative morphological traits and the pattern based on SSR markers. These patterns do not match. Subpopulations clustered into one subgroup based on SSR markers constitute a different cluster based on morphological traits. Many studies have found weak relationships between molecular and morphological phylogenies (Lewontin, 1984; Lynch, 1996; Reed and Frankham, 2001). Generally, natural and human selective forces acting on SSR variation differ from those acting on morphological traits. Furthermore, morphological traits are affected by the environment when they are expressed, whereas microsatellites are not and their variation is based directly on DNA sequence variation (Bruschi *et al.*, 2003).

Secondly, the Mantel test shows a significant correlation between fruit consistency and variations based on SSR markers and between fruit maturity period and variation based on quantitative morphological traits. These observations suggest that fruit consistency and maturity period are reflected, respectively, in microsatellite variation and in quantitative morphologic data. Possible explanations for this harmony are: (i) SSR or selected morphologic characters may be linked to genes coding for fruit consistency or maturity and (ii) selected characters may interfere with cultivar adaptation to local oasis conditions so as to generate early or a late maturity periods. Further researches will be needed to find the genetic and selective causes underlying these relationships.

The study reveals that the Tunisian date palm population in the continental oases is divided into subpopulations characterised by economically important features such as fruit consistency and a range of maturity periods. Their determination is made morphologically and genetically possible using reliable morphological and microsatellite markers. In the future, these findings should be able to be utilised in improving management strategies in Tunisia and also in other countries where date palms are significant economically. Additionally, the outcomes from this study should allow improve-

ments to be made in gene conservation practice in southern Tunisia where many older date palm cultivars are now endangered as the industry focuses more and more on just a handful.

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