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.

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 sequences 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-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 handicrafts. 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.
Molecular analysis

Genomic DNA of each genotype was extracted from young leaves. Total nuclear DNA was extracted according to Invisorb® Spin Plant Mini Kit (Invitek). 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 heterozygosis 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 MagaBACE 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 χ² 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).

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).
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 $H_e$ value for all loci is 0.63 and the mean $H_o$ 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 |
| PM9 Mean        | Soft | Semi-soft | Semi-dry | Dry | Early | Mid-season | Late |
| Mean            | 16.50 | 23.92 | 24.85 | 23.94 | 13.76 | 21.93 | 26.51 |
| s. d.           | 4.66 | 5.36 | 2.89 | 3.10 | 3.43 | 3.41 | 3.47 |
| EP3 Mean        | 56.52 | 44.66 | 35.09 | 36.25 | 61.27 | 41.47 | 40.28 |
| s. d.           | 12.88 | 9.95 | 9.01 | 4.23 | 6.4 | 13.83 | 7.24 |
| PM16 Mean       | 70.50 | 76.84 | 68.21 | 83.12 | 69.26 | 76.26 | 71.56 |
| s. d.           | 15.95 | 10.26 | 6.11 | 3.64 | 17.14 | 10.25 | 10.83 |
| PN9 Mean        | 1.61 | 1.63 | 1.45 | 1.94 | 1.47 | 1.69 | 1.66 |
| s. d.           | 0.81 | 0.49 | 0.45 | 0.46 | 0.66 | 0.70 | 0.45 |
| EP6 Mean        | 8.36 | 9.24 | 10.67 | 8.95 | 8.34 | 8.91 | 10.43 |
| s. d.           | 2.02 | 1.92 | 2.88 | 0.86 | 2.1 | 1.74 | 2.76 |
| EP8 Mean        | 57.86 | 59.10 | 49.17 | 64.00 | 56.85 | 58.95 | 53.83 |
| s. d.           | 12.44 | 9.54 | 5.62 | 16.27 | 13.40 | 12.83 | 8.97 |
| RG3 Mean        | 71.88 | 76.50 | 74.60 | 75.32 | 70.88 | 74.20 | 76.69 |
| s. d.           | 7.36 | 3.72 | 4.48 | 5.42 | 8.42 | 7.39 | 4.88 |
| EL3 Mean        | 45.95 | 44.3 | 43.16 | 43.79 | 49.16 | 43.18 | 43.31 |
| s. d.           | 7.75 | 3.88 | 3.9 | 4.55 | 7.73 | 4.62 | 3.66 |
| RB7 Mean        | 3.02 | 3.33 | 2.64 | 2.90 | 2.97 | 3.12 | 2.74 |
| 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.
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 $\chi^2$ 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.

| 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           | –0.245         | 0.336          |                  |
| RB7        | –0.295          | 0.420           |                |                  |
| EP8        | –0.245          | 0.539           |                |                  |
| PN9        | –0.093          | –0.452          |                |                  |

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

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

$H_e$: expected heterozygosity. $H_o$: observed heterozygosity. $F$: Fixation index. *: $p < 0.05$. **: $p < 0.01$. ***: $p < 0.001$. Figure 3. UPGMA dendrogram based on average distance among subpopulation means of morphological traits.
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 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     | 0.026 | 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.

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

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

**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.
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 fruiter 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 quantitative morphological 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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References

AL-KHALIFA N.S., ASKARI E., 2003. Molecular phylogeny of date palm (Phoenix dactylifera L.) cultivars from Saudi Arabia by DNA fingerprinting. Theor Appl Genet 107, 1266-1270.

BEN ABDALLAH A., STITI K., LEPOIRE P., DU JARDIN P., 2000. Identification de cultivars de palmier dattier (Phoenix dactylifera L.) par l’amplification aléatoire d’ADN (RAPD). Cah Étud Recher Francoph Agric 9, 103-107. [In French].

BENNAECEUR M., LANAUD C., CHEVALIER M.H., BOUÑAGA N., 1991. Genetic diversity of the date palm (Phoenix dactylifera L.) from Algeria revealed by enzyme markers. Plant Breed 107, 56-69.

BILLOTTE N., MARSEILLAC N., BROTIER P., NOYER J.L., JACQUEMOUD-COLLET J.P., MOREAU C., COUVREUR T., CHEVALLIER M.H., PINTAUD J.C., RISTERUCCI A.M., 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.

BRUSCHI P., VENDRAMIN G.G., BUSSOTTI F., GROSSONI P., 2003. Morphological and molecular diversity among Italian populations of Quercus petraea (Fagaceae). Ann Bot 91, 707-716.

CORNUEL B., MERCIER L., 1994. Date palm (Phoenix dactylifera L.) cultivar identification by RFLP and RAPD. Plant Sci 101, 163-172.

ELSHIBLI S., KORPELANEN H., 2008. Microsatellite markers reveal high genetic diversity in date palm (Phoenix dactylifera L.) germplasm from Sudan. Genetica 134, 251-260.

EXCOFFIER L., SMOUSE P.E., QUATTO J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479-491.

FERCHICHI A., HAMZA H., 2008. Le patrimoine génétique phoenicicole des oasis continentales tunisiennes. Institut des Régions Arides, Medenine, Tunisie. 301 pp. [In French].

HAMRICK J.L., GODT M.J.W., SHERMAN-BROYLES S.L., 1992. Factors influencing levels of genetic diversity in woody plant species. New For 6, 95-124.

HAMZA H., REJILI M., ELBEKKAY M., FERCICHI A., 2009. New approach for the morphological identification of date palm (Phoenix dactylifera L.) cultivars from Tunisia. Pak J Bot 41, 2671-2681.

IPGRI, 2005. Descripteur du palmier dattier (Phoenix dactylifera L.). Rome. 71 pp. [In French].

KOVAČ W.L., 2005. MVSP - A multivariate statistical package for windows, vers. 3.1. Kovach Computing Services, Pentraeth, Wales, UK.

LANGELLA O., 2002. Populations 1. 2. 28 Software. CNRS, UPR9034, France.

LEWONTIN R.C., 1984. Detecting population differences in quantitative characters as opposed to gene frequencies. Am Nat 123, 115-124.

LIEDLOFF A.C., 1999. Mantel nonparametric test calculator, vers 2.0. School of Natural Resource Sciences, Queensland Univ Technol, Australia.

LYNCH M., 1996. A quantitative-genetic perspective on conservation issues. In: Conservation genetics: case histories from nature (Avise J.C., Hamrick J.L., eds). Chapman and Hall, NY, USA. pp. 471-501.

MANTEL N., 1967. The detection of disease clustering and a generalized regression. Cancer Res 27, 377-394.

MARKUS S., 2000. An economic method for fluorescent labeling of PCR fragments. Nat Biotechnol 18, 233-234.

MUNIER P., 1973. Le palmier dattier. Techniques agricoles et productions tropicales. Maisonoeuvre et Larose edition, Paris. 221 pp. [In French].

MUNIER P., 1981. Origine de la culture de palmier dattier et sa propagation en Afrique. Fruits 36, 437-450. [In French].

NEI M., 1972. Genetic distance between populations. Amer Naturalist 106, 283-292.

NEI M., 1987. Molecular evolutionary genetics. Columbia Univ Press, NY, USA.

NIXON R.W., 1959. Growing dates in the United States. No. 207, US Dept Agric, Government Printing Office, Washington. 50 pp.

OULD MOHAMED SALEM A., TRIFI M., SAKKA H., ROHUMA A, MARRAKCHI M., 2001. Genetic inheritance analysis of four enzymatic systems in date palm (Phoenix dactylifera L.). Genet Resour Crop Evol 48, 361-368.

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

REED D.H., FRANKHAM R., 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 56, 1095-1103.

ROHUMA A., 2005. Le palmier dattier en Tunisie I. Le patrimoine génétique, Volume 2. IPGRI, Rome. 255 pp. [In French].

ROHUMA S., 2008. Analyse de la diversité génétique chez le palmier dattier (Phoenix dactylifera L.) Étude transcryptomique de la maladie des feuilles cassantes. University of Tunis-El Manar, Tunis. [In French].
Genetic variation among Tunisian date palm subpopulations

RHOUMA S., ZEHDI S.A., OULD MOHAMED SALEM A., RHOUMA A., MARRAKCHI M., TRIFI M., 2007. Genetic diversity in ecotypes of Tunisian date palm (Phoenix dactylifera L.) assessed by AFLP markers. The J Hortic Sci Biotechnol 82, 929-933.

SAKKA H., ZEHDI S., OULD MOHAMED SALEM A., RHOUMA A., MARRAKCHI M., TRIFI M., 2003. Tunisian date-palm (Phoenix dactylifera L.) genotypes identification mediated by plastid PCR/RFLP based DNA. J Genet Breed 57, 259-264.

SEDRA M.H., FILALI H., FRIRA D., 1993. Observation sur quelques charatéristiques phenotypiques et agronomiques du fruit des variétés et clones du palmier dattier sélectionnés. Al-Awamia 82, 105-120. [In French].

SEDRA M.H., EL FILALI H., BENZINE A., ALLAOUI M., NOUR S., BOUSSAK Z., 1996. La palmeraie dattière marrocaine: evaluation du patrimoine phoenicicole. Fruits 1, 247-259. [In French].

SEDRA M.H., LASHERMES H.P., TROUSLOT P., COMBES M.C., HAMON S., 1998. Identification and genetic diversity analysis of date palm (Phoenix dactylifera L.) varieties from Morocco using RAPD markers. Euphytica 103, 75-82.

SNOUSSI H., DU JARDIN P., BEN ABDALLAH A., LEPOIVRE P., 2001. Assessment of genetic variation within date palm (Phoenix dactylifera L.) using amplified fragment length polymorphism (AFLP) - Genotyping of apomictic seedlings as a case study. The Second International Conference on Date Palm, Al-Ain, United Arab Emirates. March 25-27, pp. 678-683.

SOKAL R.R., ROHLF F.J., 1995. Biometry, 3rd ed. WH Freeman and Company, NY, USA. 815 pp.

SPSS, 2003. SPSS for Widows. Release 12.0.0. SPSS Inc, USA.

TRIFI M., RHOUMA A., MARRAKCHI M., 2000. Phylogenetic relationships in Tunisian date palm (Phoenix dactylifera L.) germplasm collection using DNA amplification fingerprinting. Agron 20, 665-671.

ZEHDI S., TRIFI M., OULD MOHAMED SALEM A., RHOUMA A., MARRAKCHI M., 2002. Survey of inter simple sequence repeat polymorphisms in Tunisian date palms (Phoenix dactylifera L.). J Genet Breed 56, 77-83.

ZEHDI S., TRIFI M., BILLOTTE N., MARAKCHI M., PINTAUD J.C., 2004. Genetic diversity of Tunisian date palms (Phoenix dactylifera L.) revealed by nuclear microsatellite polymorphism. Hered 141, 278-287.