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Investigation of genetic variation in Tunisian date palm (*Phoenix dactylifera* L.) cultivars using ISSR marker systems and their relation with fruit characteristics

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Abstract: *Phoenix dactylifera* L. was introduced in Tunisia a very long time ago and plays an important socioeconomic role, especially in the south of the country. Genetic diversity and relationships among 26 cultivars were assessed with 7 ISSR primers. A total of 43 amplified bands were obtained. Principal component analyses based on Nei genetic distances showed no geographical separation with the exception of the Dhahbi cultivar, which has a limited geographical distribution. A group of cultivars that are also phonetically clustered was distinguished. These cultivars have a common maturity period and a common fruit consistency. The Mantel test emphasizes a significant correlation between genetic distance and fruit consistency ($r = -0.120$; $P = 0.026$). A significant differentiation was observed between the soft and dry subpopulations (*PhiPT* = 0.126; $P = 0.007$). Discriminant analyses highlight the association of markers with fruit consistency groups. Fruit consistency is an economically important feature. In the future, these findings may be utilized for improving management strategies in Tunisia and other countries where date palms are economically significant.

Key words: *Phoenix dactylifera* L., ISSR, diversity, fruit consistency, maturity period

Introduction

Date palm belongs to the family Arecaceae and genus *Phoenix*, which includes 12 species; *Phoenix dactylifera* L. is the only one that is cultivated. This species colonizes worldwide in arid and semiarid regions (1). Nixon (2) reported that the domestication of *Phoenix dactylifera* L. has likely taken place since approximately 3000 BC in Mesopotamia. It is tolerant to very low and high temperatures (under 0 °C and over 50 °C) (3), whereas rainfall can damage the crop. The roots penetrate to great depths in the soil and the species can grow in wide range of soils. The date palm is recognized for fruit production, although almost every part of the plant is used in the rural economy for food, building, animal feed, handcrafts, windbreaks, and sand stabilizers (4).

The date palm out-crossers model (5) is the origin of the high genetic diversity that creates potential for the plant to grow in several regions. The Middle East and North Africa constitute the 2 centers of this diversity. Many cultivars have been widely introduced in several countries around the world for date production and they have the ability to bear poor soils and stress (1). In Tunisia, the date palm is grown over about 32,000 ha of land that covers littoral and continental oases (6). More than 200 distinct

cultivars are grown in Tunisian oases (7,8). These cultivars have different ripening times. As a result, 3 different classes of date palm cultivars are identified based on fruit maturation period: early, mid-season, and late. Moreover, fruit consistency is the most important parameter determining the quality of fruit and its commercial value. Formerly, this trait was segregated into 3 different classes of date: soft, semi-dry, and dry (1); however, the heterogeneity of semi-dry fruit cultivars was established. As a result, fruits have been divided into 2 new clusters: semi-soft and semi-dry, with approved morphological and genetic distinction (9,10).

The genetic richness of the date palm is threatened by the erosion caused by the wide cultivation of Deglet Nour, which produces the majority of commercially desirable fruit crops. This situation is aggravated by the threat of many stresses, namely the destructive Bayoud diseases caused by the fungus *Fusarium oxysporum f.* sp. *albedinis* (11,12). This constitutes a danger to biodiversity and affects the livelihood of the population that depends on farming as the main store of capital income.

In support of heritage protection and preservation, many studies have been undertaken to characterize date palm cultivars, such as morphological and molecular characterization. Phenotypic identification of date palm cultivars is usually not possible until fruits are produced (1). In addition, the characterization of cultivars and evaluation of genetic diversity require a large set of phenotypic data that are often difficult to assess and vary, due to environmental influences (1,13,14). Hamza et al. (9) selected a set of steady characters with no significant environmental effect. Such morphological traits have aided in the identification of fruit characteristics such as maturity period and fruit consistency.

Several molecular markers have been used for measuring date palm genetic diversity: RAPD (14-17), ISSR (18), RAMPO (19), AFLP (20,21), RFLPs (22,23), and SSR (24). A discrepancy has usually appeared between morphological and molecular tools. However, Hamza et al. (10) highlighted a significant relationship between SSR data and date palm fruit consistency.

The aims of this study were to investigate the structure and the distribution of genetic variability in *Phoenix dactylifera* cultivars of Tunisian continental oases using ISSR primers and to assess the efficiency of ISSR in determining fruit characteristics.

Material and methods

Sample materials

Cultivars were chosen for the importance of their fruit and their presence in continental Tunisian oases (Figure 1). These areas represent more than 85% of the total date palm oases of Tunisia. Analyses were performed on 52 individual trees belonging to the 26 cultivars (Table 1), with 2 replications for each cultivar. The replications were performed to confirm the intra-cultivar stability underscored in previous studies (18,24). The studied cultivars have 3 different maturity periods: early, mid-season, and late. The fruit consistencies were: soft, semi-soft, semi-dry, and dry.

Molecular analysis

Total nuclear DNA was extracted from young leaves by Invisorb® Spin Plant Mini Kit (Invitek). DNA polymorphism was detected by polymerase chain reaction (PCR) using ISSR primers. We used 7 ISSR primers in this study (Table 2).

PCR was carried out at 20 µL final volume using 25 ng of genomic DNA containing 4 µL of 5 × Green GoTaq® (pH 8.5, 7.5 mM MgCl2), 100 µM dNTPs, 150 pmol random primer, and 1.2 units of Taq DNA polymerase. The mixture was brought up to 20 µL by adding sterilized distilled water. The mixture was amplified in a thermal cycler (GeneAmp® PCR System 9700) that was programmed for 1 cycle of initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 1 min, followed by specific annealing temperature for 55 s, and ending with an extension at 72 °C for 1 min; and a final extension cycle at 72 °C for 7 min. The PCR machine was adjusted to hold the product at 4 °C. The PCR products and 1 kb DNA ladder were electrophoresed on 2% agarose gel (stained with EtBr). The separated fragments were visualized with an ultraviolet (UV) transilluminator.

Data analysis

Fragments of the same molecular weight were considered to have the same locus. The numbers of bands produced for each primer were scored

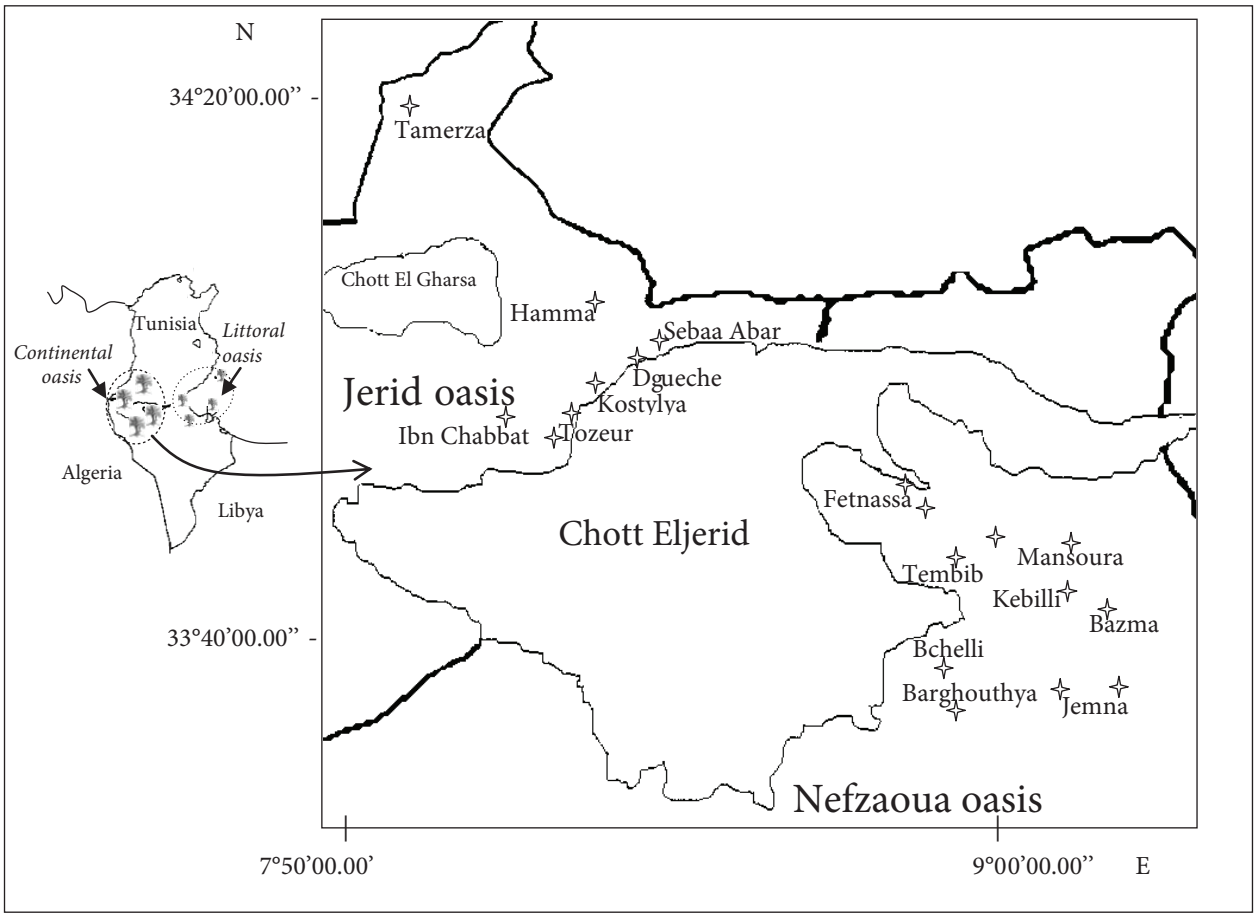

Figure 1. Sites of the continental Tunisian oases.

manually for presence (1) or absence (0), and a binary matrix was generated and used for analysis. The date palm cultivar scores were coordinated in a bi-dimensional space using principal component analysis (PCA) by computing a matrix based on the Nei genetic distances (25) using GenALEx 6.1 software (26). A Mantel's nonparametric test (27) was performed using the Mantel software package, version 2.0 (28). This test was carried out to examine correlations between matrices of dissimilarity in fruit characteristics and genetic distances. Binary matrices were constructed for maturity period and fruit consistency data; the distance was set at 0 between individuals having identical fruit characteristics and at 1 between individuals having different fruit characteristics. AMOVA (29) pair-wise comparison between groups was tested using 999 resampled individuals. The effect of fruit characteristics on genetic variation in date palm populations was analyzed by canonical discriminant function analysis (SPSS 12.0) using ISSR loci as independent variables.

Results

A high level of polymorphism was observed using ISSR marker systems. The 7 primers used gave a total of 43 polymorphic fragments, ranging from 250 to 2500 bp. An example of banding patterns in the different date palm cultivars using ISSR primer D12 is shown in Figure 2. The maximum number of fragments was 8 bands, which were produced by the D9 primer with 62.5% polymorphism. The minimum number of fragments was 5 bands, which were produced by primers D12 and PO6 with 80% and 60% polymorphism, respectively (Table 2). The average was 6.1 bands per primer.

Table 1. Name, origin, and main characteristics of date-palm genotypes studied.

No.	Name	Code	Geographical distribution	Fruit characteristics (at Tamer stage)		
				Color	Consistency	Maturity period
1	Alig	Alg	Nefzoua & Jerid	Dark brown	Semi-dry	Late
2	Ammary	Amm	Nefzoua & Jerid	Black	Soft	Early
3	Bejjou	Bjj	Nefzoua & Jerid	Brown	Dry	Late
4	Bissr Helou	Bsh	Nefzoua & Jerid	Pale brown	Dry	Season
5	Choddakh	Cdk	Nefzoua & Jerid	Dark Amber	Semi-soft	Season
6	Choddakh Ben Jbir	Cbj	Nefzoua & Jerid	Dark Amber	Semi-soft	Season
7	Dhahbi	Dhb	Temerza	Amber	Semi-soft	Late
8	Deglet Nour	Dnr	Nefzoua & Jerid	Amber	Semi-soft	Late
9	Fermla	Frm	Nefzoua	Brown	Semi-dry	Season
10	Fezzani	Fez	Nefzoua & Jerid	Amber	Semi-dry	Season
11	Gondi	Gnd	Nefzoua & Jerid	Amber	Semi-soft	Season
12	Gosbi	Gsb	Nefzoua & Jerid	Black	Soft	Early
13	Ghars Souf	Gsf	Nefzoua & Jerid	Dark brown	Soft	Season
14	Hissa	His	Nefzoua & Jerid	Honey	Soft	Early
15	Hlwa	Hlw	Nefzoua	Honey	Semi-dry	Late
16	Hamra	Hmr	Nefzoua & Jerid	Amber	Semi-dry	Season
17	Horra	Hor	Nefzoua & Jerid	Amber	Dry	Season
18	Kintichi	Knt	Jerid	Reddish	Dry	Late
19	Loghrabi	Lgr	Jerid	Dark brown	Semi-soft	Season
20	Om Leghlez	Olg	Jerid	Amber	Soft	Early
21	Rtob Houdh	Rth	Nefzoua & Jerid	Amber	Soft	Season
22	Rtotbayet elmansoura	Rtm	Nefzoua	Brown	Soft	Season
23	Rotbayet yagouta	Rty	Nefzoua	Dark amber	Soft	Early
24	Tronja	Trj	Nefzoua & Jerid	Dark brown	Semi-dry	Late
25	Tezerzayet Kahla	Tzk	Nefzoua & Jerid	Black	Soft	Season
26	Tezerzayet Safra	Tzs	Jerid	Dark brown	Soft	Early

Table 2. List of ISSR primers and their characteristics.

Name	Sequence of primer (5'-3')	Tm (°C)	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands
D9	$(CT)_{10}G$	59.8	8	5	62.5%
D12	$(GA)_6CC$	44.0	5	4	80%
UBC 888	$(GCT)(AGT)(GCT)(CA)_7$	51.9	6	5	83.3%
UBC 890	$(AGC)(ACT)(AGC)(GT)_7$	51.9	7	7	100%
UBC 891	$(ACT)(AGC)(ACT)(TG)_7$	51.1	6	6	100%
PO6	$(AG)_{10}C$	59.8	5	3	60%
PO7	$(AG)_{10}T$	57.9	6	4	66.6%

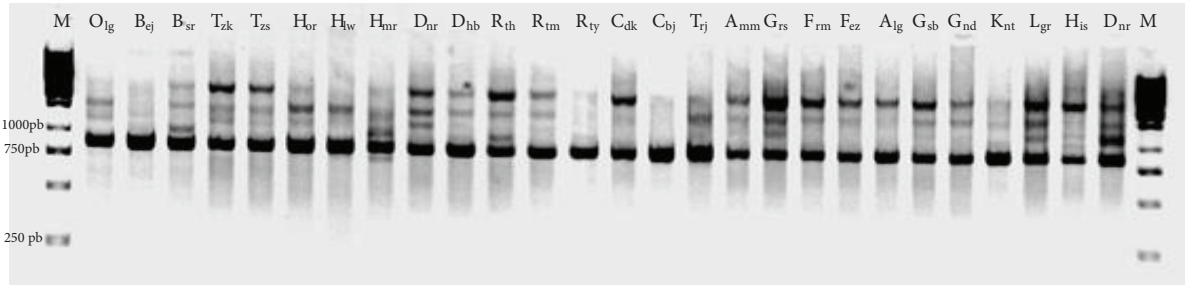

Figure 2. Banding patterns in different date palm cultivars by ISSR primer D12 (M: 1 Kb ladder).

Figure 3 shows the degree of variation for each cultivar. Om Leghlez, Horra, Hamra, and Deglet Nour cultivars showed the highest variability (75.53% of their bands are polymorphic), while the Dhahbi cultivar had the lowest variability (50% of its bands are polymorphic). Based on ISSR data the Nei genetic distances ranged from 0.05 and 0.48. Gondi and Choddakh Ben Jbir cultivars showed the highest genetic distance (0.48), while the Tezerzyet Safra and Tezerzyet Kahla showed the lowest distance (0.05).

Nei genetic distances were used to analyze the variability of the studied cultivars by principal component analysis. The first 3 axes of PCA accounted for 62.51% of the total variability. Distribution of cultivars on plan 1-2 and on plan 1-3 (Figure 4), which reflect 46.84% and 45.39% of the total variability, respectively, shows the differentiation of the Dhahbi cultivar. This cultivar was noted only in Tamerza oasis (Figure 1). No other geographical group was distinguished; the different cultivars are classified independent of their oases. However, we can distinguish some clusters of cultivars according to their fruit characteristics. In fact, PCA results show the grouping of Ammary, Choddakh Ben Jbir, Hissa, Rotbyet Yagouta, Rotbayet elmansoura, Rtob Houdh, Ghars Souf, Tezerzayet Safra, and Tezerzayet Kahla cultivars (Figure 4), which all have soft or semi-soft fruits and early or mid-season maturities.

This observation allows us to test the relationships between distance matrices representing the fruit characteristics and variables describing molecular diversity by Mantel statistical tests. The null hypothesis

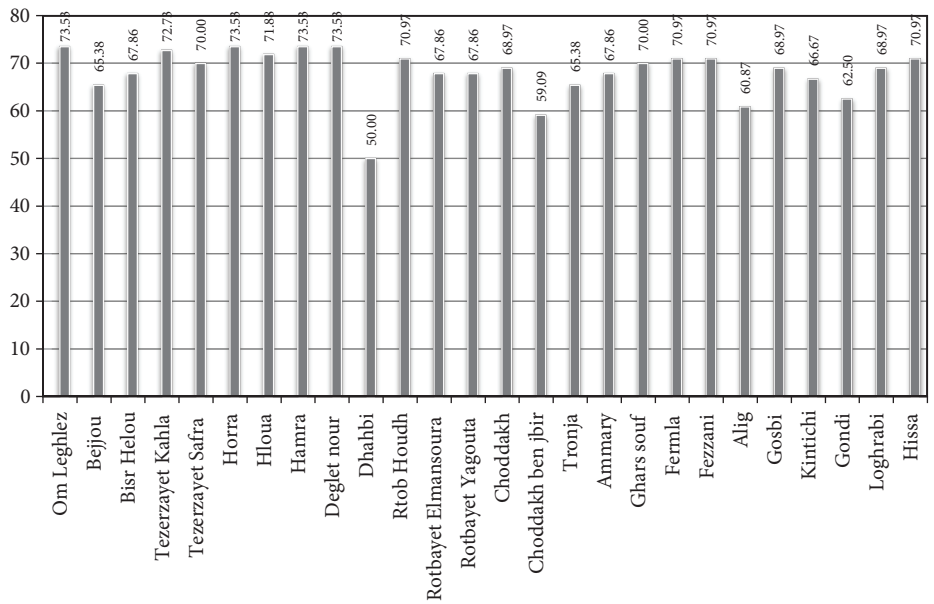

Figure 3. Percentage of polymorphic bands for each cultivar.

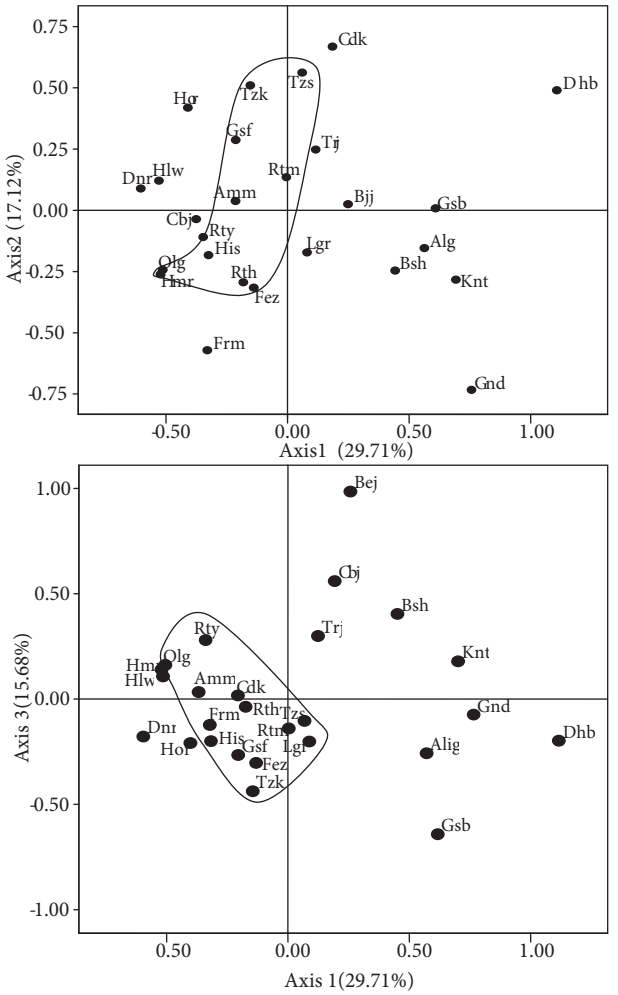

Figure 4. Cultivar distribution on plan 1-2 and plan 1-3 of PCA, based on ISSR amplified loci.

of no correlation between different matrices was tested (Table 3). A significant correlation was found between molecular data and fruit consistency matrix (r = −0.1205; P = 0.026) but not with the maturity period matrix (r = −0.0546; P = 0.225). The studied cultivars were grouped into subpopulations according to fruit qualities. Genetic distances were examined for all pairwise comparisons between subpopulations (Table 4). The distances range from 0.01, between soft fruits subpopulations and early maturity, to 0.12, between cultivars with soft fruits and those with dry ones. In addition, large genetic distances were observed between dry subpopulations and other groups such as early and semi-soft. AMOVA tests showed no significant genetic differentiation (P > 0.05) among fruit characteristic subpopulations, although 3% of total genetic diversity was detected among fruit-consistency subpopulations. Pairwise comparisons of subpopulations (Table 5) showed that the soft subpopulation and the dry subpopulation are significantly differentiated. On the other hand, no relationship was detected between ISSR data and fruit color; the grouping of cultivars did not show clusters of cultivars with the same fruit color.

Canonical discriminant analysis (Figure 5) of molecular data and date palm cultivars showed that several molecular markers are associated with fruit consistency. Amplicons PO6-3, PO7-3, PO7-1, and UBC890-4 are highly correlated with the first function, which explains 90.5% of total variance and separates soft and semi-dry cultivars from the others. The second function explains 6.9% of total variance; was highly correlated with the amplicons UBC890-3, UBC890-6, PO7-4, PO7-2, UBC890-7, D9-6, UBC890-1, UBC891-5, and D12-4 and separated the cultivars with dry fruits from semi-dry cultivars.

Discussion

A level of polymorphism among the cultivars tested is necessary for the reliability of a molecular marker technique. In comparison with RAPD techniques, ISSR techniques have high reproducibility (30). Among date palms, ISSR markers are more informative than the RAPD markers (31). According

Table 3. Result of Mantel's test of pair-wise correlations between dissimilarity matrices.

Matrix 1	Matrix 2	Mantel's r	P
Maturity period	ISSR	−0.054	0.225
Fruit consistency	ISSR	−0.120	0.026*

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

Table 4. Nei genetic distances among several date palm subpopulations.

	Early	Late	Mid-season	Dry	Semi-dry	Semi-soft	Soft
Early	0.00						
Late	0.08	0.00					
Mid-season	0.03	0.03	0.00				
Dry	0.11	0.06	0.07	0.00			
Semi-dry	0.03	0.06	0.03	0.01	0.00		
Semi-soft	0.08	0.02	0.03	0.10	0.08	0.00	
Soft	0.01	0.07	0.02	0.12	0.03	0.07	0.00

to the literature, many ISSR primers were tested and applied to date palm genotypes (18,31) or other monocotyledon species such as genus *Poa* (32) and durum wheat (33). A total of 7 primers were screened for ISSR-PCR analysis. They were useful for characterizing the samples and produced strongly amplified polymorphic bands. The selected primers generated an appropriate amplification pattern with clear, consistent, and reproducible bands. In the present study the number of polymorphic bands (34 bands) and the average number of fragments produced per primer (6.14) were lower than numbers obtained in previous studies of date palm (18,31).

In this study, 26 cultivars were characterized. Tezerzayet Safra and Tezerzayet Kahla showed the lowest genetic distance. The same result was found by Rhouma-Chatti et al. (34) in a study that grouped these 2 cultivars jointly in a dendrogram based on AFLP data. In addition, a high degree of independence between geographical origin and molecular data was indicated. The only distinction is for Dhahbi cultivar, which is from a specific variety of continental mountain oasis (Tamerza) (Figure 1), where it is intensively cultivated. SSR, RAMPO, and AFLP data applied to Tunisian date palm (10,34) showed that the studied cultivars were clustered independently from their geographic origin. This favors the hypothesis proposed by Wrigley (35) that suggests a common genetic base among cultivars of the Tunisian continental oasis. The lack of basic geographic differentiation is explained by the fact that communication in the studied oases facilitates the exchange of plant material.

Molecular studies have proved the efficiency of molecular markers in assessing genetic diversity between date-palm genotypes. However, few studies have shown a correlation between molecular and phenotypic markers. Mirta et al. (31) underscored the discrimination of date palm cultivars on the basis of tree sex with RAPD and ISSR markers.

Table 5. *PhiPT* values among different subpopulations based on 7 ISSR primers.

	Early	**Late**	**Mid-season**	**Dry**	**Semi-dry**	**Semi-soft**	**Soft**
Early	0.000	0.443	0.460	0.168	0.475	0.417	0.432
Late	0.000	0.000	0.443	0.421	0.410	0.493	0.174
Mid-season	0.000	0.000	0.000	0.254	0.437	0.419	0.443
Dry	0.057	0.000	0.033	0.000	0.081	0.450	**0.007***
Semi-dry	0.000	0.000	0.000	0.096	0.000	0.496	0.440
Semi-soft	0.000	0.000	0.000	0.000	0.000	0.000	0.458
Soft	0.000	0.036	0.000	**0.126**	0.004	0.001	0.000

PhiPT values below diagonal; negative *PhiPT* values converted to zero; probability values based on 999 permutations are shown above diagonal. Negative pair-wise *PhiPT* converted to zero

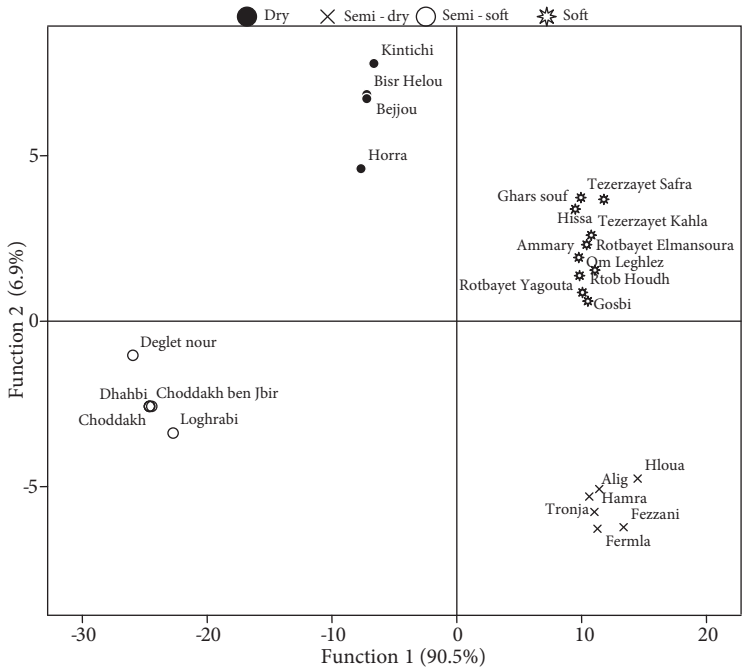

Figure 5. Distribution of fruit consistency groups on matrix derived from discriminant function analysis using ISSR markers as independent variables.

However, Rhouma-Chatti et al. (34) did not show significant discrimination of male trees using AFLP and RAMPO markers. The current results revealed a significant correlation between genetic data and fruit consistency as shown by Mantel test, which is partly supported by PCA analysis. However, no significant correlation between genetic distance and the maturity period matrix has been detected. Similar results were highlighted in a more convincing manner by morphological and SSR markers (9,10) than via ISSR results. The discrepancy between ISSR markers and maturity period supports the idea that maturity period may be affected by the local environment, whereas ISSR markers are not; their variation is based directly on DNA sequence variation (36). These results allowed us to compare genetic structure between groups based on fruit characteristics. Nei genetic distances showed that dry subpopulations are more distant from the others. Dry and soft cultivars have different fruit consistency, which is reflected in ISSR data. No genetic differentiation was observed among the maturity period subpopulation. However, when the fruit consistency subpopulations were compared significant differentiation was detected. Indeed, soft and dry subpopulations are genetically differentiated ($PhipT$ = 0.126; $P < 0.05$). The correlation observed between overall genetic variability and fruit characteristics is reflected by canonical analysis in which several markers were capable of separating fruit consistency groups. This relationship was not implicated in linkage between the markers and genes that participate in fruit consistency; however, it points to the possible common origin of cultivars of a given consistency.

ISSR tools are very important for explaining genetic diversity and population structures. The observations and interpretations of the current investigation are interesting as a preliminary exploration and analysis. In the future the use of ISSR should be broadened in order to investigate the relationships between molecular markers and fruit characteristics more fully. This could increase the selection efficiency of date palm cultivars derived through sexual reproduction. In fact, cultivar selection could begin at the seedling stage since the fruit appears only after 5 years. Furthermore, this study should contribute to cultivar conservation in southern Tunisia where many older date palm cultivars are now endangered and need to be conserved in the name of sustainable harvesting.

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References

1. Munier P. Le Palmier Dattier. Techniques Agricoles et Productions Tropicales. Maisonoeuvre et Larose Edition. Paris; 1973.
2. Nixon RW. Growing Dates in the United States. No. 207, US Dept Agric, Government Printing Office. Washington; 1959.
3. Chao CT, Krueger RR. The date palm (*Phoenix dactylifera* L.): overview of biology, uses and cultivation. Hort Science 42: 1077-1082, 2007.
4. Elbekr A. Le Palmier Dattier: Passé et Présent et Nouveauté Dans son Agronomie, Industrie et Commerce. Bagdad; 1972.
5. Munier P. Origine de la culture de palmier dattier et sa propagation en Afrique. Fruits 36: 437-450, 1981.
6. Sghaier M. Les agro systèmes de production oasiens en Tunisie, fonctionnement, rôle et adaptation aux changements écologiques et socio-économiques. In: Tonneau JP, Rhouma A. eds. Agriculture Oasienne: Quelles Recherches? Actes du Séminaire Franco-Tunisien, Degache, Tunisie. Montpellier CIRAD; 1994: pp. 85-99.
7. Rhouma A. Le Palmier Dattier en Tunisie I. Le Patrimoine Génétique, Volume 2. IPGRI, Rome; 2005.
8. Ferchichi A, Hamza H. Le Patrimoine Génétique Phoenicicole des Oasis Continentales Tunisiennes. Institut des Régions Arides Tunisie, Medenine; 2008.
9. Hamza H, Rejili M, Elbekkay M et al. New approach for the morphological identification of date palm (*Phoenix dactylifera* L.) cultivars from Tunisia. Pak J Bot 41: 2671-2681, 2009.
10. Hamza H, Elbekkay M, Ben Abederrahim MA et al. Molecular and morphological analyses of date palm (*Phoenix dactylifera* L.) subpopulations in southern Tunisia. Span J Agric Res 9: 484-493, 2011.
11. Louvet J, Toutain G. Recherches sur les fusarioses VIII. Nouvelles observations sur la fusariose du palmier dattier et precisions concernant la lutte. Ann Phytopathol 4: 35-52, 1973.
12. Baaziz M. Date palm culture in the Maghreb countries: constraints and scientific research. The Date Palm International Symposium. Namibia; 2000.
13. Sedra MH, El Filali H, Benzine A et al. La palmeraie dattière marrocaine: evaluation du patrimoine phoenicicole. Fruits 1: 247-259, 1996.
14. Sedra MH, Lashermes HP, Trouslot P et al. Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica 103: 75-82, 1998.
15. Ben Abdallah A, Stiti K, Lepoivre P et al. Identification de cultivars de palmier dattier (*Phoenix dactylifera* L.) par l'amplification aléatoire d'ADN (RAPD). Cah Étud Recher Francoph/Agric 9: 103-107, 2000.
16. Trifi M, Rhouma A, Marrakchi M. Phylogenetic relationships in Tunisian date palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. Agronomie 20: 665-671, 2000.
17. Al-Khalifa NS, Askari E. Molecular phylogeny of date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting. Theor Appl Genet 107: 1266-1270, 2003.
18. Zehdi S, Trifi M, Ould Mohamed Salem A et al. Survey of inter simple sequence repeat polymorphisms in Tunisian date palms (*Phoenix dactylifera* L.). J Genet Breed 56: 77-83, 2002.
19. Rhouma S. Analyse de la diversité génétique chez le palmier dattier (*Phoenix dactylifera* L.). Étude transcriptomique de la maladie des feuilles cassantes, PhD, University of Tunis-El Manar, 2008.
20. Rhouma S, Zehdi SA, Ould Mohamed Salem A et al. Genetic diversity in ecotypes of Tunisian date palm (*Phoenix dactylifera* L.) assessed by AFLP markers. J Hortic Sci Biotechnol 82: 929-933, 2007.
21. Snoussi H, Du Jardin P, Ben Abdallah A et al. 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: 678-683, 2001.
22. Corniquel B, Mercier L. Date palm (*Phoenix dactylifera* L.) cultivar identification by RFLP and RAPD. Plant Sci 101: 163-172, 1994.
23. Sakka H, Zehdi S, Ould Mohamed Salem A et al. Tunisian date-palm (*Phoenix dactylifera* L.) genotypes identification mediated by plastid PCR/RFLP based DNA. J Genet Breed 57: 259-264, 2003.

24. Zehdi S, Trifi M, Billotte N et al. Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. Hereditas 141: 278-287, 2004.

25. Nei M. Genetic distance between populations. Amer Naturalist 106: 283-292, 1972.

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

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

28. Liedloff AC. Mantel Nonparametric Test Calculator, Version 2.0. School of Natural Resource Sciences, Queensland University of Technology, Australia; 1999.

29. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491, 1992.

30. Williams JGK, Kubelik AR, Livak KJ et al. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6231-6235, 1990.

31. Mitra C, Kharb P, Uppal S et al. Genetic diversity analysis in date palm (*Phoenix dactylifera* L.): a comparative assessment using ISSR and RAPD marker assays. J Hortic Sci Biotechnol 86: 398-402, 2011.

32. Arslan E, Tamkoç A. The application of ISSR-PCR to determine the genetic relationship and genetic diversity between narrow leaved bluegrass (*Poa angustifolia*) and rough bluegrass (*Poa trivialis*) accessions. Turk J Biol 35: 415-423, 2011.

33. Pasqualone A, Lotti C, Bruno A et al. Use of ISSR markers for cultivar identification in durum wheat**.** Option Méditerranéennes, Série A 40: 157-161, 2000.

34. Rhouma-Chatti S, Baraket G, Dakhlaoui-Dkhil S et al. Molecular research on the genetic diversity of Tunisian date palm (*Phoenix dactylifera* L.) using the random amplified microsatellite polymorphism (RAMPO) and amplified fragment length polymorphism (AFLP) methods. Afr J Biotechnol 10: 10352-10365, 2011.

35. Wrigley G. Date-palm (*Phoenix dactylifera* L.). In: Smartt J, Simmonds NW. eds. The Evolution of Crop Plants. Longman; 1995: pp. 399-403.

36. Bruschi P, Vendramin GG, Bussotti F et al. Morphological and molecular diversity among Italian populations of *Quercus petraea* (Fagaceae). Ann Bot 91: 707-716, 2003.