

Tunisian fig (*Ficus carica* L.) genetic diversity and cultivar characterization using microsatellite markers

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Abstract — Introduction. *Ficus carica* L., well adapted to the Mediterranean climate, is ubiquitous in Tunisia. In spite of the large possibilities of its adaptation to the Tunisian climate, its cultivation remains traditional. In Tunisia, this species is represented by a large number of varieties which are facing genetic erosion. To save these genetic resources, we studied some of the Tunisian varieties using molecular markers. The aim of this analysis was to study the genetic diversity of some cultivars and to characterize them. **Materials and methods.** Six microsatellites were used to characterize 16 cultivars (*Ficus carica* L.) belonging to two fig tree collections in the south of Tunisia. **Results.** The molecular markers used appeared highly polymorphic in common fig trees since 4–12 alleles per locus and a mean of heterozygosity of 0.656 were scored. The resolving power (R_p) of the six microsatellites tested ranged from 2.12 to 3.87 for the 16 cultivars studied, showing a significant genetic diversity ($H_t = 0.762$). Genetic differentiation between geographical groups was low ($G_{st} = 0.032$). The factorial correspondence analysis showed no well-defined relation between the 16 cultivars and their geographical origin. The genotype patterns allowed us to discriminate all of the cultivars. **Conclusion.** The characterization of the accessions belonging to different varieties was possible, showing the power and efficiency of the molecular tools used.

Tunisia / *Ficus carica* / genetic resources / identification / microsatellites / genetic markers

Diversité génétique et caractérisation des cultivars tunisiens de figuier (*Ficus carica* L.) par marqueurs microsatellites.

Résumé — Introduction. *Ficus carica* L., bien adapté au climat méditerranéen, est omniprésent en Tunisie. Malgré ses grandes possibilités d'adaptation au climat tunisien, sa culture demeure traditionnelle. En Tunisie, cette espèce est représentée par un grand nombre de variétés qui sont exposées à l'érosion génétique. Afin de préserver ces ressources génétiques, nous avons étudié certaines variétés tunisiennes en utilisant des marqueurs moléculaires. Le but de ces analyses a été d'étudier la diversité génétique et de caractériser ces cultivars. **Matériel et méthodes.** Six microsatellites ont été utilisés afin de caractériser 16 cultivars de figuiers appartenant à deux collections du sud tunisien. **Résultats.** Les marqueurs moléculaires utilisés se sont révélés hautement polymorphes chez le figuier commun, puisque 4 à 12 allèles par locus et une hétérozygotie moyenne de 0,656 ont été enregistrés. Le pouvoir de résolution (R_p) des six microsatellites testés a varié de 2,12 à 3,87 pour les 16 cultivars étudiés qui ont montré une importante diversité génétique totale ($H_t = 0,762$). La différenciation génétique entre collections a été faible (< 5 %) ($G_{st} = 0,032$). L'analyse factorielle des correspondances n'a pas mis en évidence de relations entre les cultivars et leur origine géographique. Les profils génotypiques ont permis de discriminer la totalité des cultivars. **Conclusion.** La caractérisation des accessions appartenant à différentes variétés de figuiers a été possible ce qui témoigne de la puissance et de l'efficacité des outils moléculaires utilisés.

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Tunisie / *Ficus carica* / ressource génétique / identification / microsatellite / marqueur génétique

1. Introduction

Ficus carica L. (2n = 26 chromosomes) [1] is one of the 750 species of the genus *Ficus* [2]. Phylogeny studies have shown that the common fig has a monoecious ancestor [3]. It evolved later in gynodioecious species with bisexual trees (functional male figs or caprifigs) and unisexual female trees. The “male” caprifig has separate male (staminate) flowers and female (pistillate) flowers. It is functionally male because it produces pollen. Edible figs contain only long-style female flowers. Since functional male trees have hermaphrodite flowers, *Ficus carica* L. is usually considered gynodioecious rather than dioecious. In wild populations of the Mediterranean region, caprifig and female trees occur in similar frequencies [4].

Ficus carica L., well adapted to the Mediterranean climate, is ubiquitous in Tunisia. In spite of the large possibilities of its adaptation to the Tunisian climate, its cultivation remains traditional. In Tunisia, this species is represented by a large number of varieties which are facing genetic erosion. Many studies have reported the morphological characterization of fig cultivars and proved the sustainability of the use of morphological parameters to evaluate and to establish a description of genotypes [5–10]. Thus, it has been assumed that the most discriminant parameters were those related to leaves and fruits. However, these characters are sensitive to environmental conditions; the discriminant ones are limited in number and do not allow the separation of the phenotypes into distinct groups [11].

Many studies have been interested in the development of other methods for cultivar identification. For instance, protein polymorphisms in common figs were suitable for characterizing fig varieties [7, 10]. However, the success of varietal identification depends on the number of isoenzymatic systems and alleles studied. Unfortunately, this number is often limited, which leads to insufficient polymorphism among closely related genotypes. Various DNA profiling methods are currently available [12] and consist of two main categories according to the information provided: specific locus and codominant markers and arbitrary and dominant

markers. The use of dominant markers such as RAPD and ISSR allowed the detection of significant inter-cultivar polymorphism [13, 14]. PCR amplified fragments polymorphism can be obtained using restriction enzymes or *via* conformational analysis. RFLPs and microsatellites are the most commonly used codominant molecular markers [14–16].

Microsatellite loci usually involve many alleles. Microsatellite markers, also called Simple Sequence Repeats (SSRs) or Short Tandem Repeat (STRs), are the most informative markers currently available [17]. They consist of tandem repeats with a basic repeat unit of two to eight base pairs. Variation in the number of repeats can be detected with PCR by developing primers complementary to the conserved DNA sequence flanking the SSR. As molecular markers, SSRs combine many desirable marker properties including high level of polymorphism and information content, unambiguous designation of alleles, even dispersal, selective neutrality, high reproducibility, codominance, and rapid and simple genotyping assays. Thus, microsatellites have become the molecular markers of choice for a wide range of applications in genetic mapping, genome analysis, genotype identification and variety protection, seed purity evaluation, germplasm conservation, diversity studies, paternity determination and pedigree analysis gene, quantitative trait locus analysis and marker-assisted breeding [18, 19]. In figs, eight specific primers were identified recently, and microsatellite amplifications have become possible and reproducible [19].

In this paper, we report the use of such markers in order to assess genetic diversity and to establish identification of Tunisian fig cultivar groups.

2. Materials and methods

2.1. Plant material and DNA extraction

Fig leaves were sampled from two collections located in southern Tunisia (*table 1*): one in a CRDA (Commissariat Régional au Développement Agricole) experimental orchard in Gafsa, the other supported by the

CRPh (Centre de Recherches Phoenicicoles) in Degache. For each collection eight individuals were analyzed, which were chosen according to their fruit quality and because they are the main cultivars exploited in the prospected regions. A caprifig sample, called Dhokkar and originating from the Gafsa groves, was included in the study. All the remaining ones are common figs.

DNA was extracted from 5 g of material (leaves) according to Dellaporta *et al.* [20]. DNA concentrations were estimated using an agarose gel stained with ethidium bromide.

2.2. Primers and PCR assays

Six microsatellites that previously showed clear polymorphisms in *Ficus carica* [19, 21] were targeted in this study. These are labeled as MFC2, MFC3, MFC5, MFC6, MFC7 and MFC8.

PCR amplification was performed in a total volume of 25 µL including (20 to 30) ng of genomic DNA, 10 pM of each primer, 20 mM of each dNTP, 1 U of Taq DNA polymerase (QBIogène, France), 2,5 µL of Taq DNA polymerase buffer (10x), 2,5 mM MgCl₂ and H₂O sterile water in sufficient quantity to make up 25 µL. Amplification was then performed in a Crocodile III thermocycler (QBIogène, France) according to the following conditions: after an initial denaturation at 94 °C for 4 min, samples were processed through 35 cycles, each consisting of a denaturation step at 94 °C for 30 s, an annealing step at (50 or 55) °C (according to the primers) for 45 s and an extension step at 72 °C for 1 min. An elongation step of 4 min was programmed at the last cycle.

Amplifications were checked on 2% agarose gels visualized by ethidium bromide staining under UV light. SSR bands were then resolved on non-denaturing polyacrylamide gels (10%) and revealed by ethidium bromide staining according to Sambrook *et al.* [22].

2.3. Data analysis

The ability of primers to differentiate between cultivars was assessed by calculating the resolving power (R_p) [23]. This index was determined using Gilbert *et al.* formula [24]:

Table I.

Origin and names of the fig cultivars from the south of Tunisia, used to study Tunisian fig genetic diversity with microsatellite markers.

Collection location	Cultivar name	Geographic origin		
Gafsa	Assal Boudchiche	Gafsa ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓		
	Bither Abiadh			
	Dhokkar			
	Gaa Zir			
	Khadhour			
	Mlouki			
	Sawoudi			
	Soltani			
	Degache		Bouslames	Degache
			Chetoui	Mahdia
Ghabri		Ouerdanine		
Khadhri		Sfax		
Khortoumi		Béja		
Mokh Bagri		Gafsa		
Wahchi		Béja		
Zergui		Béja		

$R_p = Ib$ and $Ib = 1 - [2 \times (0.5 - p)]$ where p is the proportion of accessions sharing the I band.

Genetic polymorphism in each population was evaluated by the mean number of alleles, the observed (H_{ob}) and expected (H_e) heterozygosity, and the genetic diversity ($H_K = 1 - \sum p_i^2$), where H_K corresponds to the genetic diversity at the locus K , and P_i the allele frequencies [25]. According to Nei's formula [26], the total genetic diversity (H_T) was portioned into intra-collection (H_C) and inter-collection differentiation (G_{ST}) components. The Wright inbreeding coefficient (F_{IS}) was computed according to Weir and Cockerham [27]. A positive value of F_{IS} indicates a deficit in heterozygotes in comparison with the Hardy-Weinberg equilibrium expectations. Relationships among cultivars were revealed with a multivariate factorial analysis of correspondences performed using Genetix 4.01 [28]. A synthetic picture was then obtained representing cultivars' and alleles' correlation.

The cultivars' identification key was established as described in Ould Mohamed Salem *et al.* [29].

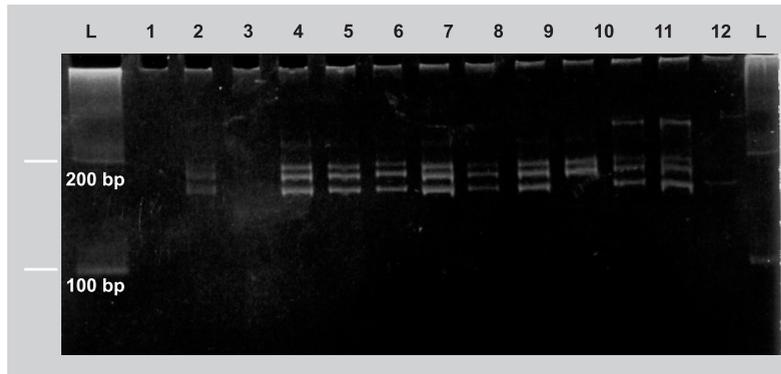


Figure 1. Example of microsatellite polymorphisms revealed in locus MFC2 in cultivars of two Tunisian common fig collections. Lanes: 1–12, PCR products corresponding to 12 fig cultivars; L, 100–bp ladder (Gbico BRL, France).

Table II.

Expected and observed heterozygosities for the six microsatellite loci examined to study Tunisian fig genetic diversity among 16 fig cultivars exploited in the south of Tunisia (regions of Gafsa and Degache).

Locus	Number of alleles	Collection location				Total	
		Gafsa		Degache		H_e	H_{ob}
		H_e	H_{ob}	H_e	H_{ob}		
MFC2	6	0.79	0.87	0.78	0.62	0.78	0.75
MFC3	12	0.83	0.37	0.85	0.75	0.84	0.56
MFC5	4	0.73	0.62	0.65	1.00	0.69	0.81
MFC6	4	0.60	0.37	0.55	0.25	0.58	0.62
MFC7	6	0.80	0.87	0.76	1.00	0.78	0.94
MFC8	8	0.70	0.50	0.79	0.62	0.75	0.56
Total	40	0.74	0.60	0.73	0.71	0.74	0.67

3. Results

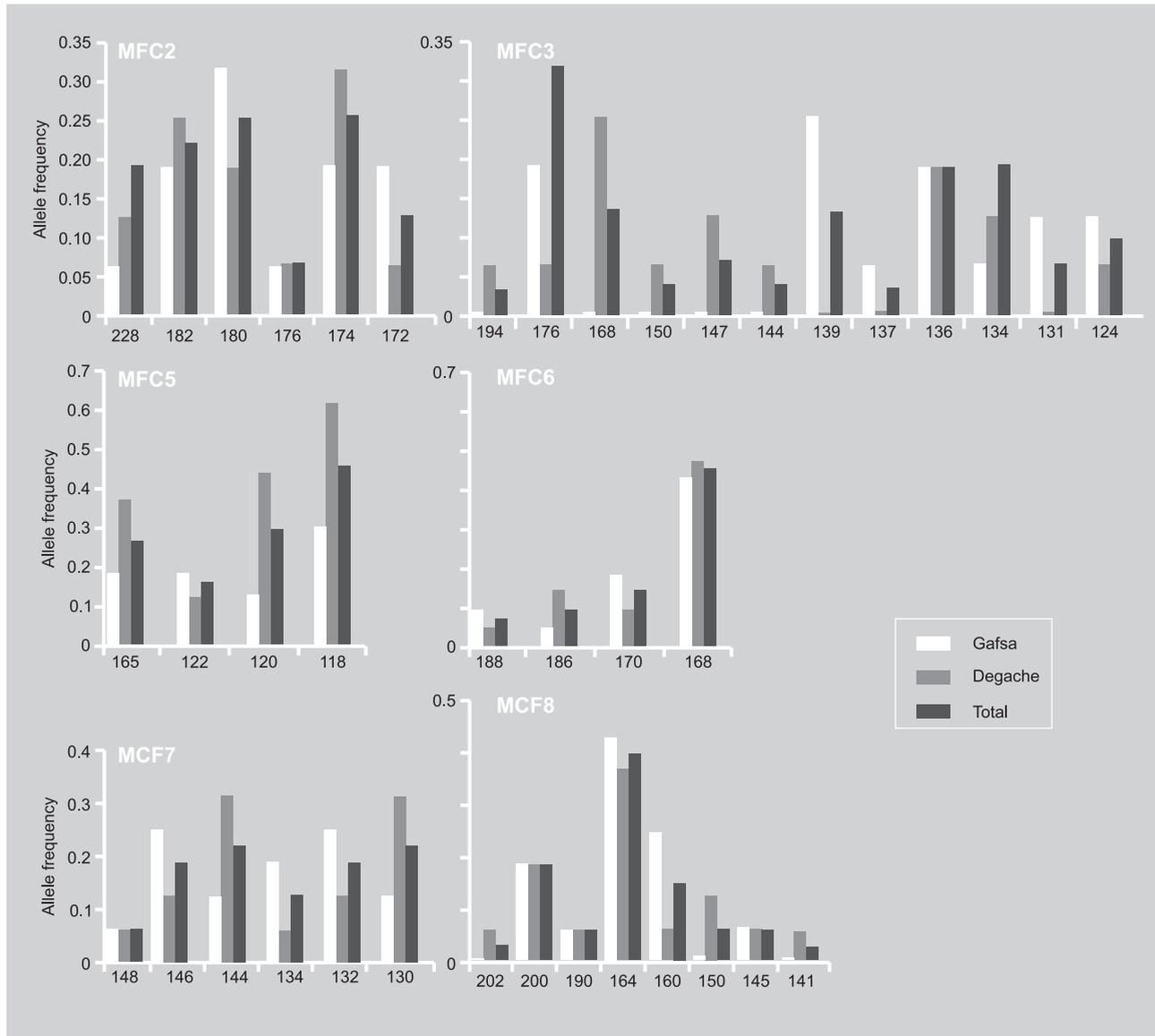
3.1. Microsatellite polymorphism

The reported six primer pairs were screened in the 16 Tunisian accessions of *Ficus carica* L. in order to examine their ability to generate banding patterns. Microsatellite polymorphisms were resolved on non-denaturing polyacrylamide gel (figure 1 for the locus MFC2, for example). On the whole,

amplifications yielded banding patterns ranging from 118 (MFC5) to 228 bp (MFC2) in size and a number of alleles ranging from four alleles for MFC5 and MFC6 to twelve for MFC3 with a mean of 6.66 alleles per locus (II). This result suggests the presence of a high level of polymorphism of microsatellites. In addition, taking into account the collection site, the mean number of alleles is 5.33 per locus for the fig trees of the Gafsa site and 6.16 alleles for the Degache site. Consequently, we may assume that the fig trees of the latter are more diversified than those of Gafsa.

The expected heterozygosity was 0.58 for the MFC6 locus to 0.84 for the MFC3 one. The observed heterozygosity, at the six loci, varied from 0.56 (MFC3 and MFC8) to 0.94 (MFC7). In addition, the results provide evidence of a differentiation between the collections studied since the heterozygosity differed from one collection to another. In fact, the Degache collection is characterized by a heterozygosity ranging from 0.25 for the locus MFC6 to 1 for both MFC7 and MFC5, whereas the collection of Gafsa exhibits a heterozygosity of 0.37 (MFC3 and MFC6) to 0.87 (MFC7 and MFC2) (table II). This result is in agreement with the hypothesis suggesting a relatively higher degree of genetic diversity in Degache's collection, as a consequence of larger intra-collection variability. Moreover, the MFC7 locus seems to contribute the most to the surveying of genetic diversity since it exhibits the highest number of heterozygotes observed in the two collections.

Allele frequencies ranged from 0.037 for alleles 202 and 141 of the MFC8 locus to 0.593 for alleles 118 of MFC5 and 168 of MFC6 (figure 2). Comparison of these distributions of allele frequencies at the six polymorphic loci indicates some differences between the two collections studied. Some alleles occurred exclusively in one collection such as the MFC3 alleles 131, 137 and 139 in the Gafsa collection, and the MFC8 141, 150 and 202 alleles and the MFC3 144, 147, 150, 168 and 194 ones in the Degache collection. Hence, we may assume that these alleles characterize the collections studied. However, regarding the relatively small number of cultivars examined and the



low frequencies [30], only the 139 allele of MFC3 could be considered as fixed in the Gafsa collection and the 168 MFC3 allele in the Degache collection. In addition, application of Wright's parameters showed a significant deviation from Hardy-Weinberg equilibrium ($P < 10^{-4}$). According to the Wright inbreeding coefficient (F_{IS}) values, this result could be explained by an excess of heterozygotes, taking into account the MFC5 ($F_{IS} = -0.02$) and MFC7 ($F_{IS} = -0.13$) loci. For the remaining loci (*i.e.*, MFC2,

MFC3, MFC6 and MFC8) positive F_{IS} values indicating heterozygote deficit were registered (0.11, 0.39, 0.51 and 0.31, respectively).

3.2. Resolving power

The resolving power of each primer was estimated in order to determinate the most informative ones for the discrimination between cultivars (*table III*). The results show that, the tested primer pairs are characterized by

Figure 2. Distribution of allele frequencies for the loci of six microsatellites studied to characterize 16 accessions present in two Tunisian common fig (*Ficus carica* L.) collections in the south of Tunisia.

Table III.

Resolving power (R_p) of the six primers used for studying Tunisian fig genetic diversity with microsatellite markers among 16 fig cultivars exploited in the south of Tunisia (regions of Gafsa and Degache).

Locus	Resolving power for all accessions
MFC2	3.50
MFC3	3.12
MFC5	2.62
MFC6	2.12
MFC7	3.87
MFC8	2.37
Total	17.62

Table IV.

Genotypes in 16 common fig (*Ficus carica* L.) cultivars revealed by the use of six microsatellites tested to study Tunisian fig genetic diversity present in the south of Tunisia (regions of Gafsa and Degache). For each microsatellite, alleles are hierarchically ordered according to their size and then labelled from A to L.

Variety	MFC2	MFC3	MFC5	MFC6	MFC7	MFC8
Assal boudchiche	CF	JJ	CC	CC	BE	BG
Bither Abiadh	BE	KK	DD	DD	CF	BD
Bouslames	CC	CH	AC	DD	AD	AF
Chetoui	CF	CD	AC	BC	CF	CE
Dhokkar	CF	GG	AB	BD	DD	DD
Gaa Zir	CE	BH	BC	AD	BE	BE
Ghabri	EE	AI	AC	DD	CF	DD
Khadhour	BB	GG	AC	AD	AD	DD
Khadhri	AE	BL	BC	DD	CF	BF
Khortoumi	BD	CK	AC	BB	BE	BG
Mlouki	CF	BI	BD	DD	BE	CE
Mokh Bagri	BE	EE	AC	DD	CF	DD
Sawoudi	CF	BI	DD	CC	CF	EE
Soltani	AD	HH	AC	CC	BE	DD
Wahchi	AE	FC	BD	AC	CF	BH
Zergui	BB	HH	AC	DD	BE	DD

a considerable global resolving power value of 17.62, suggesting their reliability for assessing the genetic diversity in the two

collections studied. In addition, since they exhibited the highest values of resolving power (3.87), the MFC7 primers are the most efficient for surveying genetic diversity in the 16 cultivars of the two collections studied, and the MFC6 ones ($R_p = 2.12$) are relatively less efficient at proving genetic diversity.

3.3. Cultivar characterization

In order to establish a cultivar identification key, we took into account the cultivars' multilocus fingerprints. Thus a total of 48 genotypes were identified in our study (table IV). Note that these loci were hierarchically classified according to the number of genotypes provided. The precise diagram was then drawn in order to discriminate the 16 cultivars according to their SSR profiles (figure 3). This identification key permitted the unambiguous discrimination of all the accessions studied and confirmed the reliability of microsatellites for fingerprinting genotypes.

3.4. Genetic diversity

The sixteen cultivars were pooled to examine genetic diversity and differentiation between collections. Analysis of molecular variance shares highly significant differences ($P < 10^{-3}$) among the two collections. In addition, according to the G_{st} values, 96.79% of the total diversity is attributable to variation among individuals within collections and only 3.2% to collection differences (table V). Moreover, the results of the multivariate factorial correspondence analysis data is in agreement with this consideration since no evident clusters correlated with the geographic origin were observed. In fact, according to the first plan of the factorial correspondence analysis that accounted for 38.18% of total variability, cultivars are randomly arranged, suggesting a typically continuous genetic diversity in this crop (data not shown). Nevertheless, the factorial correspondence analysis permitted the characterization of several cultivars since the simultaneous representation of variables (different alleles) and varieties proved correlation between alleles and cultivars (table VI).

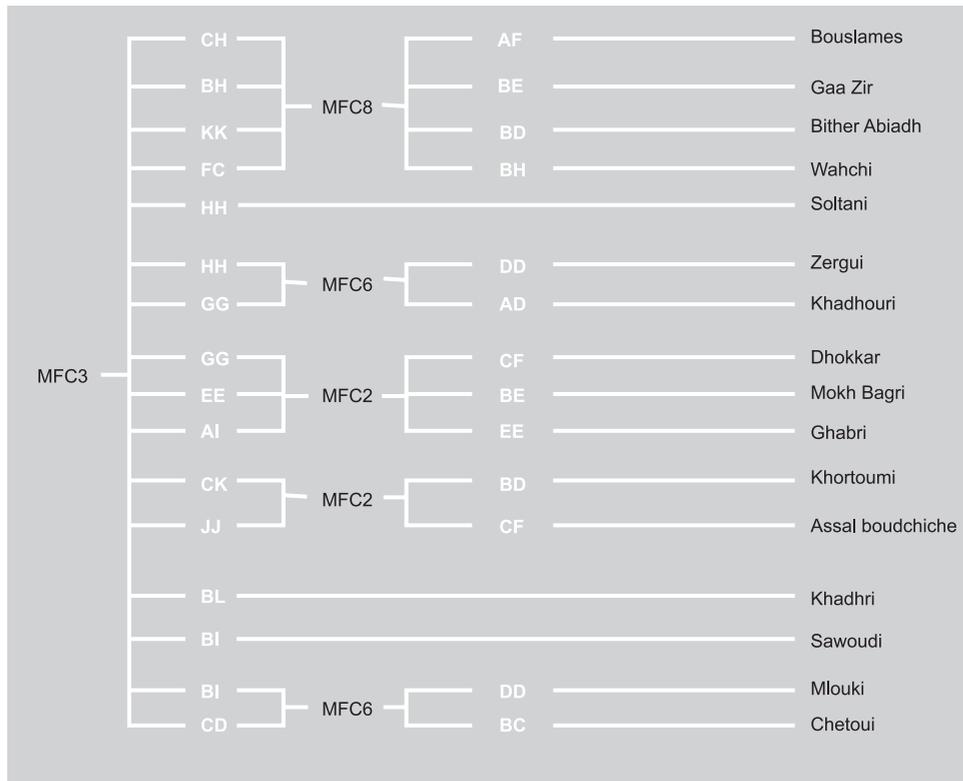


Figure 3. Identification key of 16 cultivars of common figs (*Ficus carica* L.) present in two south-Tunisian collections, based on multilocus genotypes by the use of four microsatellite (same codes as in table IV).

4. Discussion

In this study, we used microsatellites to examine the genetic diversity of, and to fingerprint Tunisian fig cultivars. Starting from a set of appropriate primers, we proved that the targeted loci are characterized by a relatively higher number of alleles per locus than those reported in French and Moroccan figs, for which only 5.74 and 3.83 alleles per locus have been revealed, respectively [21]. Moreover, the total number of identified alleles in the sixteen Tunisian cultivars (40) is larger than those observed in French (23) and Moroccan ones (23) (table VII). In addition, comparison between the evidenced alleles in the three countries illustrates the presence of either common or country-specific alleles. Thus, we may assume that the alleles 172 of MFC2, 124 of MFC3 and 144 of MFC7 are common in the different countries, while the alleles 118, 120, 122 and 165 of MFC5 and the alleles 168, 170, 186 and 188 of MFC6 are specific to Tunisian cultivars. These results suggest a large diversity that

Table V. Nei genetic diversity index [25] estimated for the six microsatellite loci used to study Tunisian fig genetic diversity among 16 fig cultivars exploited in the south of Tunisia (regions of Gafsa and Degache).

Locus	H_s	H_t
MFC2	0.78	0.80
MFC3	0.84	0.89
MFC5	0.69	0.72
MFC6	0.58	0.59
MFC7	0.79	0.81
MFC8	0.75	0.76
Total	0.74	0.76

characterize Tunisian figs. Similar data have been reported in other plant species such as barley [31], *Pinus taeda* L. [32], rice [33], and fruit species such as peach (*Prunus persica*) [34], cherry (*Prunus avium*) [35] and apricot

Table VI.

Repartition, between cultivars, of the locus alleles of six microsatellites examined to study Tunisian fig genetic diversity among fig cultivars exploited in the south of Tunisia (regions of Gafsa and Degache), according to the results of a factorial correspondence analysis.

Cultivar	MFC2	MFC3	MFC5	MFC6	MFC7	MFC8
Bither Abiadh	–	–	122	–	–	–
Chetoui	–	–	–	–	–	200
Dhokkar	–	–	–	–	132	164
Ghabri	–	–	–	168	–	150
Khadhourri	–	–	–	–	134–148	–
Khadhri	–	134	–	–	130	–
Khortoumi	–	136	–	186	–	–
Mlouki	–	–	122	–	–	–
Mokh Bagri	–	147	–	–	–	–
Sawoudi	–	144	118	–	–	–
Soltani	–	136	–	–	–	–
Wahchi	–	176	–	–	–	–
Zergui	182	–	–	–	–	–

Table VII.

Comparison of number and length of microsatellite alleles detected in Tunisian, Moroccan and French fig cultivars, according to Khadari *et al.* [21].

Locus	Tunisian cultivars		French cultivars		Moroccan cultivars	
	Number of alleles	Size of alleles	Number of alleles	Size of alleles	Number of alleles	Size of alleles
MFC2	6	172–174–176 –180–182–228	4	158–160 –166–172	4	158–160 –166–172
MFC3	12	124–131–134 –136–137–139 –144–147–150 –168–176–194	5	122–124 –126–128 –138	5	122–124 –126–128 –138
MFC5	4	118–120–122 –165	4	130–134 –140–150	3	130–140 –150
MFC6	4	168–170–186 –188	4	291–301 –303–311	6	291–295 –301–303 –309–311
MFC7	6	130–132–134 –144–146–148	3	144–150 –156	3	144–150 –156
MFC8	8	141–145–164 –150–160–190 –200–202	3	171–175 –179	2	175–179

(*Prunus armeniaca*) [36, 37]. This relatively high level of genetic diversity could be explained by high heterozygosity and/or the main proportion of the intra-collection component ($H_S = 0.74$). Furthermore, fig diversity is intensified by its dioecious nature. This consideration is strongly supported both by the low values of inter-collection differentiation ($G_{ST} = 0.032$) and by the outcrossing mating system of this crop as suggested by Hamrick in other outcrossing species [38]. In this case, the G_{ST} value is higher than the observed one in other species where gene flow occurs freely. This is well illustrated in the case of Mediterranean oak (*Quercus* ssp.) trees [39] since only 7% of the genetic diversity could be explained by inter-population differences due to the contiguous nature and frequent gene flow among the populations studied.

On the other hand, the data proved that no geographical trends were observed among the cultivar groupings. A hypothesis based on the presence of common origin of the cultivars analyzed could be advanced to explain this result. This is in agreement with the monoecious origin of the common fig (*Ficus carica* L.) that evolved later into a dioecious plant [3]. Moreover, the caprifig (Dhokkar), which represented the only male fig sample, did not significantly diverge from the female trees, and suggested a sex-independent clustering either by factorial correspondence analyses or by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrograms [13, 14]; it also confirms the monoecious origin. Similar results have been reported in common figs since an unstructured genetic variability between cultivars has been registered using other molecular markers [13, 14, 40].

In addition to taking advantage of the SSR markers to fingerprint genotypes, we established a cultivar identification key. The resultant diagram proved the powerfulness of the revealed markers to discriminate common fig cultivars. In this case, only four microsatellites were successfully applied to unambiguously identifying the cultivars studied. The remaining ones would provide multilocus genotypes suitable for discriminating between any additional common fig cultivars grown in Tunisia and in any areas of

the world. Thus, we assume that the microsatellite markers constitute an efficient tool in the identification of fig cultivars according to Bailey criteria [41]. Opportunely, the present study provided evidence of the feasibility of the designed procedure as a powerful alternative to precisely survey the genetic diversity in Tunisian figs. It is obviously necessary to enlarge the number of cultivars to obtain a deeper insight into the genetic diversity in the Tunisian fig germplasm. A prerequisite for the achievement of this work is the establishment of reliable fingerprinting in order to improve selective programs and to increase the efficiency of this crop evaluation and conservation management.

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References

- [1] Falistocco E., Antonielli L., Molecular cytogenetics of *Vitis vinifera* L. and *Ficus carica* L.: location of rDNA sequences, in: Fippone E. (Ed.), Proc. XLVI Italian Society of Agricultural Genetics, SIGA Annual Congr., Giardini, Italy, 2002.
- [2] Berg C.C., Classification and distribution of *Ficus*, *Experientia* 45 (1989) 605–611.
- [3] Machado C.A., Jousselein E., Kjellberg F., Compton S.G., Herre E.A., Phylogenetic relationships, historical biogeography and character evolution of fig pollinating wasps, *P. Roy. Soc. Lond. B Bio.* 268 (2001) 7–10.
- [4] Valdeyron G., Llyod D.G., Sex differences and flowering phenology in the common fig, *Ficus carica* L., *Evolution* 33 (2) (1979) 673–685.
- [5] Condit I.J., Fig characteristics useful in the identification of varieties, *Hilgardia* 14 (1) (1941).
- [6] Mars M., Chebli T., Marrakchi M., Multivariate analysis of fig (*Ficus carica* L.) germplasm in southern Tunisia, *Acta Hortic.* 480 (1998) 75–81.
- [7] Hedfi J., Trifi M., Salhi-Hannachi A., Ould Mohamed Salem A., Marrakchi M., Morphological and isoenzymatic polymorphism in Tunisian fig (*Ficus carica* L.) collection, *Acta Hortic.* 605 (2003) 319–325.
- [8] Salhi-Hannachi A., Mars M., Chatti K., Marrakchi M., Trifi M., Specific genetic markers for Tunisian fig germplasm: evidence of morphological traits, random amplified polymorphic DNA and inter simple sequence repeats markers, *J. Genet. & Breed.* 57 (2) (2003) 125–136.
- [9] Chatti K., Salhi-Hannachi A., Mars M., Marrakchi M., Trifi M., Analyse de la diversité génétique de cultivars tunisiens de figuier (*Ficus carica* L.) à l'aide de caractères morphologiques, *Fruits* 59 (1) (2004) 49–61.
- [10] Chessa I., Niedu G., Serra P., Fig germplasm characterization using isozymes analysis, *Acta Hortic.* 480 (1998) 43–148.
- [11] Valdeyron G., Sur le système génétique du figuier (*Ficus carica* L.), Essai d'interprétation évolutive, *Ann. Inst. Natl. Agron.* 5 (1976).
- [12] Santoni S., Faivre-Rampant P., Prado E., Prat D., Marqueurs moléculaires pour l'analyse des ressources génétiques et amélioration des plantes, *Cah. Agric.* 9 (4) (2000) 311–327.
- [13] Salhi-Hannachi A., Trifi M., Zehdi Si-Hannachi A., Trifi M., Zehdi S M., Inter Simple Sequence Repeat fingerprintings to assess genetic diversity in Tunisian fig (*Ficus carica* L.), *Genet. Res. Crop Ev.* (2004) 51 269–275.
- [14] Salhi-Hannachi A., Chatti K., Mars M., Marrakchi M., Trifi M., Comparative analysis of genetic diversity in two collections of fig cultivars based on random amplified polymorphic DNA and inter simple sequence repeats fingerprints, *Genet. Res. Crop Ev.* (2005) (in press).
- [15] Russell J., Fuller J., Young G., Thomas B., Taramino G., Macaulay M., Waugh R., Powell W., Discriminating between barley genotypes using microsatellite markers, *Genome* 40 (1997) 442–450.

- [16] Tautz D., Renz, M., Simple sequences are ubiquitous repetitive components of eukaryotic genomes, *Nucleic Acids Res.* 12 (1984) 4127–4138.
- [17] Li C.D., Fatkoun C.A., Ubi B., Scoles G.J., Determining genetic similarities and relationship among cowpea breeding lines and cultivars by microsatellite markers, *Crop Sci.* 41 (2001) 189–197.
- [18] Ciofi C., Funk S.M., Coote T., Genotyping with microsatellite markers, in: Karp A., Isaac P.G., Ingram D.S. (Eds.), *Molecular tools for screening biodiversity: plants and animals*, Chapman and Hall, London, UK, 1998.
- [19] Khadari B., Hochu I., Santoni S., Kjellberg F., Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus *Ficus*, *Mol. Ecol. Notes* 1 (2001) 191–193.
- [20] Dellaporta S.L., Wood J., Hicks J.B., A plant DNA preparation ,Version II, *Plant Mol. Biol Rep.* 4 (1983)19–21.
- [21] Khadari B., Hochu I., Bouzid L., Roger J.P., Kjellberg F., The use of microsatellite markers for identification and genetic diversity evaluation of the fig collection in CBNMP, *Acta Hortic.* 605 (2003) 77–86.
- [22] Sambrook J., Frithsch E.F., Maniatis T., *Molecular cloning: a laboratory manual*, 2nd ed., Cold spring Harbord Laboratory, Cold spring Harbord, New York, USA, 1989.
- [23] Prevost A., Wilkinson M.J., A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars, *Theor. Appl. Genet.* 98 (1999) 107–112.
- [24] Gilbert J.E., Lewis R.V., Wilkinson M.J., Caligari P.D.S., Developing an appropriate strategy to assess genetic variability in plant germplasm collections, *Theor. Appl. Genet.* 98 (1999) 1125–1131.
- [25] Nei M., *Molecular evolutionary genetics*, Columbia university Press, New York, USA, 1987.
- [26] Nei M., Estimation of average heterozygosity and genetic distance from small number of individuals, *Genetics* 89 (1978) 583–590.
- [27] Weir B.S., Cockerham C.C., Estimating F-statistics for the analysis of population structure, *Evolution* 38 (1984) 1358–1370.
- [28] Belkhir K., Genetix version 4.0, Conception genome & populations, Programmation Belkir Biosoft, Laboratoire Génome, populations, interactions, CNRS UPR 9060, Univ. Montpellier II, Montpellier, France, 2001.
- [29] Ould Mohamed Salem A., Trifi M., Salhi-Hannachi A., Rhouma A., Marrakchi M., Genetic variability analysis of Tunisian date-palm (*Phoenix dactylifera* L.) cultivars, *J. Genet. & Breed.* 55 (2001) 269–278.
- [30] Dumas V., Hunder S., Bebb A., Cadoux-Barnabé C., Bellec C., Cuny G., Polymorphic microsatellites in *Simulium damnosum* S.I. and their use for differentiating two Savannah populations: implications for epidemiological studies, *Genome* 41 (1998) 154–161.
- [31] Struss D., Plieske J., The use of microsatellite markers for detection of genetic diversity in barley populations, *Theor. Appl. Genet.* 97 (1998) 308–315.
- [32] Williams C.G., Elsik C.G., Barnes R.D., Microsatellite analysis of *Pinus taeda* L. in Zimbabwe, *Heredity* 84 (2000) 261–268.
- [33] Ni J., Colowit P.M., Mackill D.J., Evaluation of genetic diversity in rice subspecies using microsatellite markers, *Crop Sci.* 42 (2002) 601–607.
- [34] Wunsch A., Hormaza J.I., Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences, *Heredity* 89 (1) (2002) 56–63.
- [35] Dirlewanger E., Cosson P., Tavaud M., Aranzana J., Poizat C., Zanetto A., Arùs P., Laigret F., Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.), *Theor. Appl. Genet.* 105 (1) (2002) 127–138.
- [36] Hormaza J.I., Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats, *Theor. Appl. Genet.* 104 (2–3) (2002) 321–328.
- [37] Aranzana M.J., Carbo J., Arùs P., Microsatellite variability in peach [*Prunus persica* (L.) Batsch]: cultivar identification, marker mutation, pedigree inferences and population structure, *Theor. Appl. Genet.* (2003)106 (8) 1341–1352.
- [38] Hamrick J.L., Isozymes and the analysis of genetic structure in plant populations, in: Soltis E.D., Soltis P.S. (Eds.), *Isozymes in plant biology*, Chapman and Hall, London UK, 1990, 87–90.

- [39] Kremer A., Petit R.J., Gene diversity in natural populations of oak species, *Ann. Sci. For.* 50 (1993) 1865–2025.
- [40] Khadari B., Lashermes P.H., Kjellberg F., RAPD fingerprints for identification and genetic characterization of fig (*Ficus carica* L.) genotypes, *J. Genet. & Breed.* 49 (1995) 77–86.
- [41] Bailey D.C., Isozymic variation and plant breeder rights, in: Tanksley S.D., Orton T.J., *Isozymes in plant genetics and breeding, part A*, Elsevier, Amsterdam, The Netherlands, 1983, 425–440.

Diversidad genética y caracterización de los cultivares tunecinos de higuera (*Ficus carica* L.) mediante marcadores de microsatélite.

Resumen — Introducción. *Ficus carica* L., bien adaptado al clima mediterráneo, se encuentra omnipresente en Túnez. A pesar de sus grandes posibilidades de adaptación al clima tunecino, su cultura sigue siendo tradicional. En Túnez, esta especie se representa por un amplio número de variedades que se exponen a la erosión genética. Con el fin de salvar estos recursos genéticos, estudiamos algunas variedades tunecinas utilizando marcadores moleculares. El objetivo de estos análisis fue estudiar la diversidad genética además de caracterizar estos cultivares. **Material y métodos.** Se utilizaron seis microsatélites con el fin de caracterizar 16 cultivares de higuera que pertenecían a dos colecciones del sur tunecino. **Resultados.** Los marcadores moleculares utilizados resultaron ser altamente polimorfos en la higuera común, puesto que se registraron entre 4 a 12 alelos por locus y una heterozigosis media de 0,656. El poder de resolución (R_p) de los seis microsatélites testados varió de 2,12 a 3,87 para los 16 cultivares estudiados que mostraron una importante diversidad genética total ($H_t = 0,762$). La diferenciación genética entre colecciones fue escasa ($< 5\%$) ($G_{st} = 0,032$). El análisis factorial de las correspondencias no puso en evidencia las relaciones entre los cultivares y su respectivo origen geográfico. Los perfiles genotípicos permitieron discriminar la totalidad de los cultivares. **Conclusión.** La caracterización de los clones que pertenecen a distintas variedades de higuera fue posible, lo que acredita la potencia y la eficacia de las herramientas moleculares utilizadas.

Túnez / *Ficus carica* / recursos genéticos / identificación / microsatélites / marcadores genéticos