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Molecular based assessment of genetic diversity within Barbary fig (*Opuntia ficus indica* (L.) Mill.) in Tunisia

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Abstract

In this work, we report for the first time on the analysis of genetic diversity within a set of 36 Tunisian *Opuntia ficus indica* (L.) Mill. ecotypes using RAPD markers.

Random decamer primers were screened to assess their ability to detect polymorphisms in this plant crop. Thirty-nine RAPD markers were revealed and used to survey the genetic diversity at the DNA level and to establish relationships.

Consequently, considerable genetic diversity was detected and the UPGMA analysis permitted the discrimination of all the genotypes and enabled their sorting into thirteen groups. The accession 'R Sbiba inerme' was significantly divergent from all tested genotypes. In addition, as shown by the clustering the tested genotypes did not significantly diverge, though originating from different localities.

Since RAPD markers proved to be useful for germplasm discrimination as well as for discovery of patterns of variation in Barbary fig, the opportunity of this study was discussed in relation to the setting up of rational decisions concerning the management of a national reference collection that ought to allow the preservation of all studied genotypes.
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Keywords: *Opuntia ficus indica*; RAPD; Genetic diversity; Preservation

1. Introduction

The *Cactaceae* are a dicotyledonous angiosperm of about 2260 accepted taxa. They are most plentiful in the arid and semiarid regions between 35°N and 35°S (Taylor, 1997).

Cacti have been exploited since the pre-Columbian times and are mainly present in North and South America but also in the Mediterranean basin, Middle-East, South Africa, India, Thailand and Australia (Britton and Rose, 1919; Anderson, 2001). For instance, this species reached the Mediterranean basin during the 16th century (Le Houérou, 1992; Barbera, 1995). Nevertheless, only towards the end of the 20th century have large plantations been established.

Fruits are eaten raw, cooked or fermented into alcoholic beverages and some species produce substances of pharmaceutical or industrial interest (Boyle and Anderson, 2001).

The absence of updated statistical data from countries in which this species is cultivated allows only a rough estimate of the worldwide land area. For instance, in Tunisia about 500,000 ha are cultivated (Nefzaoui and Ben Salem, 2001).

In Tunisia, *Opuntia ficus indica* has proved to be an important fodder crop mainly for sheep, particularly during periods of drought and seasons of low feed availability (Nefzaoui and Ben Salem, 2000, 2001) in central and southern regions of the country. The cladodes can be fed either as fresh forage, or stored as silage for later feeding (Castra et al., 1977).

Cactus is also planted to reduce water and wind erosion, rangeland degradation, sand movement and to enhance the restoration of the vegetation cover.

Recently, large plantations have been established near the region of Kasserine (Inglese et al., 2001); the most specialized cactus-growing region, where the highest morphological diversity of this plant crop is maintained. Nevertheless, this species does not play a basic role in the human diet as it is, for the animals. In fact, only late ripening fruits produced through scollozatura (removal of spring flush of flowers and cladodes) are promoted, as in the Island of Sicily (Barbera et al., 1992).

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Despite from these aforementioned advantages, no collections of Barbary fig were set up in the country in order to preserve this crop. In fact, the conservation of biodiversity is one of the major issues facing humankind.

Due to the multiple uses and the ability of cacti to thrive in arid and semiarid environments, it has become increasingly important to describe and characterize these valuable resources. The latter is a challenging goal since up to now; knowledge regarding the amount of genetic variation and genetic relationship by means of molecular tools is missing in Tunisian *Opuntia ficus indica*. In fact, though this crop is widely cultivated in the country, the majority of research works were especially oriented towards the characterization of the nutritional value of the cladodes as an important fodder crop in arid areas, independently from their genetic potential (Nefzaoui and Ben Salem, 2000, 2001). In addition, most of the published information available on biodiversity of the cultivated cacti in the world comes from allozyme studies (Chessa et al., 1997; Uzun, 1997; Boyle and Anderson, 2001).

At present, molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Powell et al., 1996; Russell et al., 1997). The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques (Saïki et al., 1998). These molecular markers had been successfully used in *Opuntia* genus for detecting genetic diversity and relationships (Wang et al., 1998; Arnholdt-Schmitt et al., 2001; Labra et al., 2003). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material, etc.

The aims of this study were to produce suitable markers for the investigation of DNA polymorphism in Tunisian Barbary fig ecotypes useful in the analysis of genetic diversity and ecotype identification, and to set up rational decisions concerning the establishment of a national reference collection. Indeed, though this crop is widely cultivated in the country, collection repositories are missing.

2. Materials and methods

2.1. Plant material

Thirty-six Barbary fig (*Opuntia ficus indica*) accessions, well representative of the *Opuntia ficus indica* germplasm in Tunisia were used in this study (Zemni, personal communication). They were sampled from 15 localities in the regions of Kairouan and Kasserine (Fig. 1); the most well known regions sheltering various different morphological sorts of Barbary fig in the country (Table 1).

2.2. DNA extraction

Within Barbary fig, Isolation of DNA from cladodes is difficult, especially due to the presence of high amounts of mucilage that interfere with DNA extraction.

In this study, a DNA method extraction technique for cacti which helps to overcome the difficulties caused by mucilage has been used. Thus, for each accession, an external slice of the cladode was taken for analysis. The cuticle was removed and a piece of about 1 g of the chlorenchyma (mesophyll cells; which is the interior of the cladode, between the upper and the lower layers of epidermis) was cut using a scalpel and taking care not to include areolar meristems (light to dark coloured bumps, out of which grow clusters of spines).

The protocol of DNA extraction used here is that of Bowers et al. (1993) later modified by This et al. (1997) and Zoghlami et al. (2001).

DNA was quantified by visual comparison with lambda DNA molecular marker on ethidim bromide stained agarose gels.

2.3. Primers and PCR assays

Twenty-two universal decamer oligonucleotides, purchased from the University of British Colombia were used for the amplification of random DNA banding patterns (Table 2). They were tested on three ecotypes (labeled 5, 10 and 29 in Table 1) for their ability to produce polymorphic, unambiguous and stable RAPD markers (Table 2).

PCR reactions were performed in a 10 μl reaction mixture containing: 2.5 ng of template DNA, 2.0 μl of Go Taq buffer (Promega), 0.4 M dNTPs (0.1 M of each: dATP, dGTP, dTTP and dCTP (Promega), 0.35 μl of 25 mM $MgCl_2$, 2.5 μM of primer and 0.5 U of Go Taq DNA polymerase (Promega).

The PCR was performed in a Thermoblock thermocycler (Genius), as described by Burrow et al. (1996).

Products of the PCR were separated by electrophoresis in 1.6% agarose gels with 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) using a volt range of 3 V cm^{-1} during 3 h. Lambda DNA *Eco*RI/*Hin*dIII digested (Boehringer Mannheim, Germany) was used as a molecular size standard.

Amplifications were performed at least twice and only reproducible (stable) products were taken into account for further data analysis.

2.4. Data analysis

Photographs were taken with a Biometra Bio-doc II^{TM} system.

In order to ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designated by their primer code and their size in base pairs.

Polymorphic DNA bands were scored as discrete variables: 1 for the presence and 0 for the absence of a similar band.

For each primer, the number of bands and the polymorphic ones were calculated as well as the percentage of polymorphic bands (PPB). The latter was determined as the percentage of polymorphic bands over the total number of the yielded bands. The number of RAPD banding profiles (profiles generated by all the accessions per primer) has been also calculated.

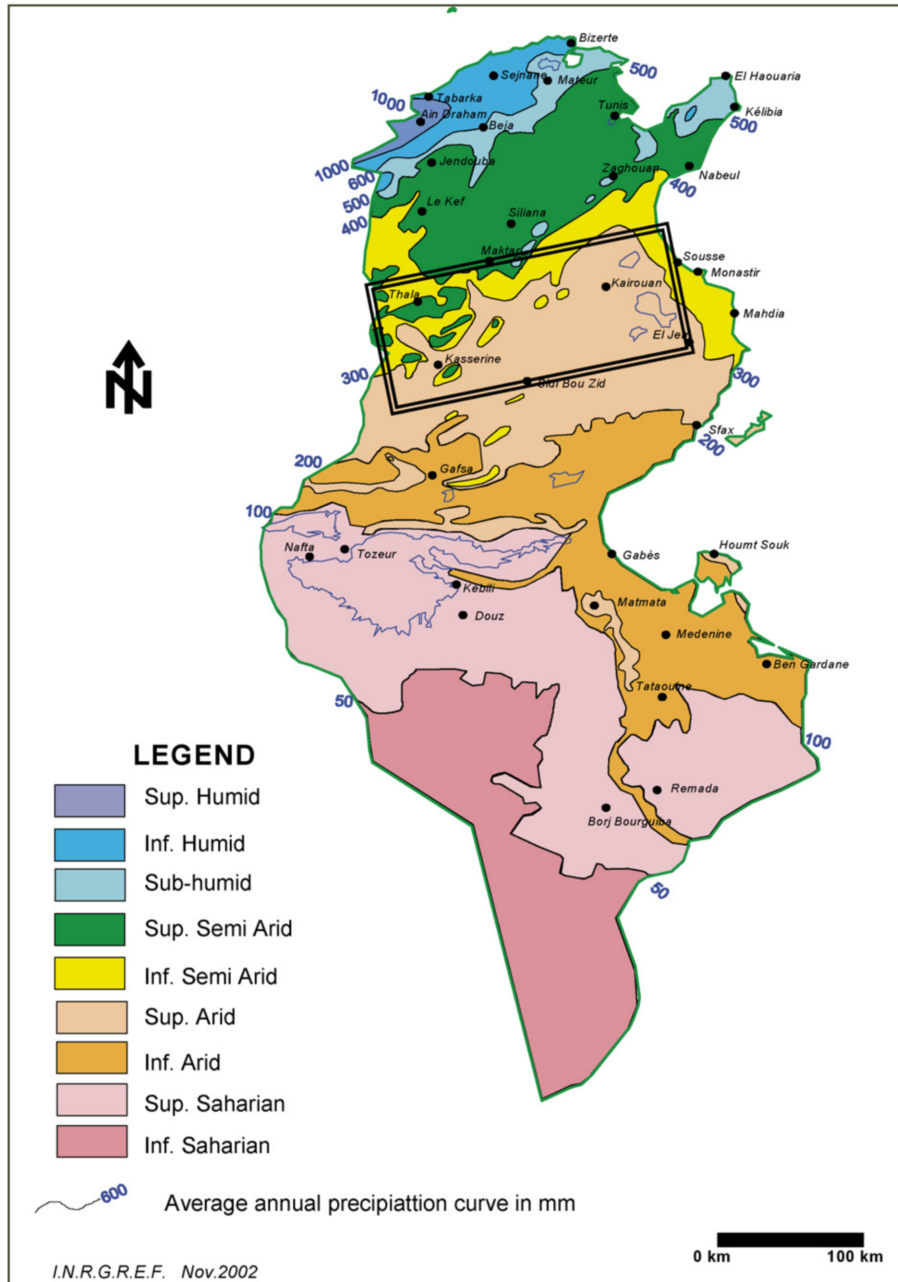

Fig. 1. Area of prospecting *Opuntia ficus indica* in Tunisia (squared).

The ability of the most informative primers to differentiate between accessions was assessed by the estimation of their resolving power (Rp) (Prevost and Wilkinson, 1999). The Rp has been described to correlate strongly with the ability to distinguish between the accessions according to the following Gilbert et al. formula (1999):

$$\mathrm{Rp} = \sum \mathbf{I}\mathrm{b}$$

where $\mathbf{I}\mathrm{b} = 1 - (2 \times |0.5 - p|)$ where p is the accessions' proportion containing the **I** band.

RAPD bands were transformed into a binary character matrix. Then, data were processed by DARwin software (Version 5.0.148) http://darwin.cirad.fr/darwin to produce a genetic distance matrix using the formula of Sneath and Sokal (1973), which assesses the similarity between any two accessions on the basis of the number of generated bands. The resulting matrix was computed with the unweighed pair group method with arithmetic averaging algorithm (UPGMA) in order to construct phylogenetic diagrams.

Doubts in clustering were solved by analysis of bands with low frequency (LFBs), i.e. bands shared by only 25% or less varieties (Bourquin, 1993).

The origin of the accessions under investigation was taken into account in order to examine their potential effect on the genetic clustering.

Table 1
Opuntia ficus indica accessions included in this study with their main area of origin in Tunisia

Label	Accession name	Locality of origin	Region
1	Zelfene épineux 1	Zelfene	Kasserine
2	Zelfene inerme	Zelfene	Kasserine
3	Snad haddad épineux 1	Snad Haddad	Kasserine
4	R Thala Kasserine inerme 1	Thala	Kasserine
5	R Thala Kasserine épineux	Thala	Kasserine
6	Sidi Zid épineux	Sidi Zid	Kasserine
7	Saddine	Saddine	Kasserine
8	R Sbiba inerme	Sbiba	Kasserine
9	Zouagha	Zouagha	Kasserine
10	Téoucha inerme	Téoucha	Kasserine
11	Lokki inerme 1	Zelfene	Kasserine
12	Beldi épineux	Zelfene	Kasserine
13	Sokri	Zelfene	Kasserine
14	Snad Haddad inerme	Snad Haddad	Kasserine
15	Snad Haddad épineux 2	Snad Haddad	Kasserine
16	R Thala Kasserine inerme 2	Thala	Kasserine
17	Marjaa Ayouem 1	Marjaa Ayouem	Kasserine
18	Sbiba chaouki Ahmar	Sbiba	Kasserine
19	Sbiba épineux 1	Sbiba	Kasserine
20	Lokki Zelfene	Zelfene	Kasserine
21	Zelfene épineux 2	Zelfene	Kasserine
22	Sbiba Thala très épineux	Sbiba	Kairouan
23	Marjaa Ayouem 2	Marjaa Ayouem	Kasserine
24	Sbitla 22	Sbitla	Kasserine
25	Sbiba épineux 2	Sbiba	Kasserine
26	Henchir Ejjouf	Henchir Ejjouf	Kasserine
27	Aïn Ghrab	Aïn Ghrab	Kairouan
28	Sidi Saad inerme 1	Sidi Saad	Kairouan
29	Lokki el Ala	El Ala	Kairouan
30	Bir Echaouch épineux 1	Bir Echaouch	Kairouan
31	Sidi Saad épineux	Sidi Saad	Kairouan
32	El Ala inerme	El Ala	Kairouan
33	Bir Echaouch Tubulaire	Bir Echaouch	Kairouan
34	El Ala ordinaire	El Ala	Kairouan
35	Bir Echaouch épineux 2	Bir Echaouch	Kairouan
36	Sidi Saad inerme 2	Sidi Saad	Kairouan

3. Results

3.1. DNA extraction

Photometric measurements at 260 and 280 nm of the nucleic acid extracts of the various accessions of Barbary fig indicated a quotient (260/280) between 1.82 and 2.02. Thus, the obtained DNA is of high quality. In addition, DNA yield was of 0.1 μg/μl for all samples.

3.2. Primers and resolving power

A total of 22 primers were screened for their ability to generate consistently amplified band patterns and to assess polymorphism in the tested ecotypes (Table 2). Among these primers, only 8 have revealed unambiguously scorable polymorphic bands. These are identified as UBC 204, UBC 212, UBC 226, UBC 211, UBC 231, UBC 232, UBC 235 and UBC 241. In fact, these mentioned primers generated multiple banding with 3–8 polymorphic amplified DNA bands ranging in size from 200 to 2020 pb.

Sixty-nine bands were obtained, which is an average of 8.6 bands per primer: 30 bands were common in all accessions and 39 were polymorphic (Table 2). The polymorphic markers yielded 79 different electrophoretic banding profiles. Hence, we may assume that a large genetic diversity at the DNA level characterises the Tunisian Barbary fig germplasm.

Estimation of the Rp values exhibited a collective rate of 15.56 and varied from 0.68 for the UBC-212 primer to 3.36 for the UBC-231 one with a mean of 1.94 (Table 2). In addition, the UBC-231, UBC-232, UBC-235 and UBC-212 primers seem to be the most efficient to assess the genetic diversity, since they presented relatively high Rp rates.

Twenty-five low frequency bands were detected (Table 2). The accessions called (Zelfene épineux 1 and Zelfene inerme) (Marjaa Ayouem 1 and Sidi Saad inerme 1), (el Ala and Bir Echaouch épineux) and (Sokri and Lokki Zelfene) shared identically the same LFBs. Thus, the hypothesis of genetic closeness between these pairs of genotypes could be forwarded.

3.3. Genetic distances and ecotypes relationships

Between the accessions analysed, genetic distance matrix shows an average distance range from 0.13 to 0.89 with a mean of 0.51 (Table 3). Thus, the ecotypes tested in this study are highly divergent at the DNA level. The smallest distance value of 0.13 was observed between the ecotypes Marjaa Ayouem 1 (17) and Sidi Saad inerme 1 (28). Whereas, the maximum distance value of 0.89, suggesting great dissimilarities, was observed between the ecotypes Henchir Ejjouf (26) and Bir Echaouch Tubulaire (33). All the remaining accessions display different intermediate levels of similarity.

The UPGMA analysis (Fig. 2) confirmed the genetic divergence described above (Table 3). In fact, the distinctiveness of the clusters identified in the UPGMA derived dendrogram exhibited 13 distinct groups (a, b, c, d, e, f, g, h, i, j, k, l and m) (Fig. 2). In addition, all tested genotypes were differentiated. This result suggests the high level of genetic diversity within Barbary fig in Tunisia.

Groups labelled a, b, d, h, and i were composed each by one ecotype. Thus, the preservation of the latter would be strongly recommended as representing each, an independent source of genetic variation.

The accession R Sbiba inerme (8), housed in group a, was significantly divergent from all tested genotypes. This has been better illustrated in Fig. 3. In fact, the aforementioned ecotype has been identified not only as an out group of analysis but also, as the most genetically distinct one.

Besides, a high level of variability was scored in the case of group m, as sheltering the highest number of distinct genotypes (10 ecotypes). The opposite situation is observed in the case of groups, e, g and k, with only 2 ecotypes.

On the other hand, the dendrogram (Fig. 2) illustrates ecotype clustering made independently from the region of origin, since the genotypes housed in all the groups did not significantly diverge, though originating from different localities (Table 1).

Table 2
Primers and RAPD banding patterns obtained among 36 Tunisian Barbary fig ecotypes

Label	Primer	Sequence (5′–3′)	Total bands	Polymorphic bands	PPB	Rp	RAPD banding profiles	LFBs
1	UBC-204	TTCGGGCCGT						
2	UBC-212	GCTGCGTGAC	7	4	57.1	1.76	8	4
3	UBC-226	GGGCCTCTAT	8	3	37.5	0.68	8	2
4	UBC-227	CTAGAGGTCC	9	5	55.5	2.02	11	3
5	UBC-231	AGGGAGTTCC	7	4	57.1	1.36	1	3
6	UBC-232	CGGTGACATC	9	7	77.7	3.36	9	3
7	UBC-235	CTGAGGCAAA	10	8	80	2.76	11	5
8	UBC-241	GCCCGACGCG	12	5	50	2.18	11	4
9	UBC-162	AACTTACCGC	7	3	42.8	1.44	10	1
10	UBC-211	GAAGCGCGAT	–	–	–	–	–	–
11	UBC-213	CAGCGAACTA	Smear	–	–	–	–	–
12	UBC-225	CGACTCACAG	Smear	–	–	–	–	–
13	UBC-230	CGTCGCCCAT	–	–	–	–	–	–
14	UBC-238	CTGTCCAGCA	Smear	–	–	–	–	–
15	UBC-239	CTGAAGCGGA	Smear	–	–	–	–	–
16	UBC-243	GGGTGAACCG	Smear	–	–	–	–	–
17	UBC-245	CGCGTGCCAG	–	–	–	–	–	–
18	UBC-246	TATGGTCCGG	–	–	–	–	–	–
19	UBC-248	GAGTAAGCGC	–	–	–	–	–	–
20	UBC-259	GGTACGTACT	–	–	–	–	–	–
21	UBC-261	CTGGCGTGAC	–	–	–	–	–	–
22	UBC-264	TCCACCGAGC	–	–	–	–	–	–

The pairs of the accessions Marjaa Ayouem 1 (17) and Sidi Saad inerme 1 (28) and Lokki Zelfene (20) and Sokri (13), clustered, respectively, in groups m and l, were the most genetically close to each other, with genetic distances of 0.13 and 0.14 (Table 4). The hypothesis for these for their being synonymous could be forwarded, since they also shared the same specific markers (LFBs) (Table 4).

4. Discussion

The genome characterization of *Opuntia ficus indica* is regarded as an important tool in research on genetic diversity and on the breeding potential of this species (Wang et al., 1998; Mondragon and Bordelon, 1996). Indeed, the RAPDs technique was proved to be useful as genetic markers. They were successfully applied to verify the somatic origin within some Mexican cactus accessions (Mondragon, 1999) and to establish genetic polymorphisms and relationships (Wang et al., 1998).

In this study, we fingerprinted a set of Tunisian Barbary fig accessions by means of RAPD markers in order to obtain molecular data of the national gene pool. In fact, the characterization of material based on morphological traits (Weniger, 1984) and common local names often leads to duplicated accessions, many of which are synonymous, homonymous or false attributions (Gibson and Nobel, 1986).

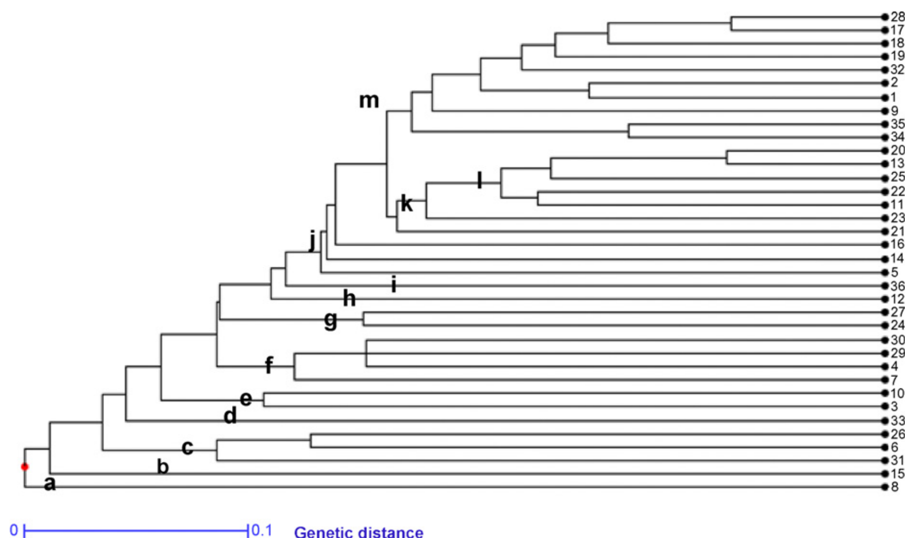

Fig. 2. UPGMA cluster analysis within 36 Tunisian accessions of *Opuntia ficus indica* established by means of 39 RAPD markers: the letters (from a to m) stand for the groups individualized.

Table 3
Genetic distances matrix among 36 Tunisian Barbary Fig ecotypes based on RAPD data and computed using Sneath and Sokal's formula

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	0																																			
2	0.26	0																																		
3	0.64	0.68	0																																	
4	0.29	0.47	0.62	0																																
5	0.51	0.5	0.68	0.54	0																															
6	0.68	0.71	0.76	0.71	0.66	0																														
7	0.54	0.65	0.68	0.5	0.65	0.6	0																													
8	0.84	0.79	0.82	0.8	0.71	0.82	0.77	0																												
9	0.48	0.4	0.71	0.43	0.47	0.73	0.63	0.7	0																											
10	0.63	0.55	0.56	0.66	0.55	0.81	0.72	0.68	0.52	0																										
11	0.48	0.4	0.66	0.57	0.4	0.69	0.63	0.7	0.44	0.45	0																									
12	0.58	0.51	0.82	0.66	0.62	0.85	0.82	0.8	0.65	0.68	0.55	0																								
13	0.47	0.38	0.74	0.61	0.52	0.76	0.71	0.76	0.55	0.57	0.35	0.4	0																							
14	0.57	0.5	0.75	0.61	0.56	0.73	0.66	0.68	0.54	0.56	0.47	0.57	0.45	0																						
15	0.75	0.73	0.88	0.74	0.77	0.81	0.8	0.86	0.71	0.81	0.71	0.79	0.7	0.7	0																					
16	0.54	0.45	0.72	0.63	0.58	0.7	0.77	0.75	0.56	0.58	0.42	0.54	0.48	0.59	0.8	0																				
17	0.31	0.3	0.7	0.42	0.38	0.63	0.55	0.72	0.25	0.51	0.25	0.59	0.42	0.45	0.7	0.41	0																			
18	0.31	0.38	0.7	0.42	0.46	0.68	0.55	0.72	0.43	0.63	0.43	0.63	0.48	0.52	0.7	0.55	0.25	0																		
19	0.44	0.43	0.73	0.54	0.55	0.66	0.52	0.75	0.47	0.61	0.31	0.62	0.46	0.5	0.77	0.52	0.3	0.38	0																	
20	0.48	0.4	0.76	0.57	0.53	0.77	0.72	0.74	0.51	0.59	0.36	0.32	0.14	0.38	0.66	0.5	0.43	0.5	0.47	0																
21	0.52	0.57	0.75	0.55	0.57	0.76	0.66	0.73	0.48	0.57	0.32	0.52	0.47	0.51	0.7	0.54	0.4	0.47	0.44	0.41	0															
22	0.44	0.35	0.68	0.54	0.5	0.75	0.69	0.79	0.73	0.55	0.31	0.44	0.3	0.5	0.73	0.45	0.38	0.46	0.43	0.4	0.44	0														
23	0.44	0.5	0.73	0.6	0.6	0.78	0.69	0.82	0.53	0.66	0.47	0.57	0.3	0.61	0.68	0.63	0.46	0.46	0.5	0.4	0.51	0.43	0													
24	0.58	0.57	0.79	0.61	0.66	0.72	0.61	0.8	0.65	0.72	0.6	0.63	0.63	0.68	0.75	0.65	0.53	0.53	0.57	0.65	0.63	0.51	0.66	0												
25	0.53	0.38	0.74	0.61	0.52	0.72	0.71	0.76	0.5	0.57	0.35	0.53	0.25	0.45	0.7	0.48	0.42	0.48	0.38	0.35	0.47	0.3	0.46	0.63	0											
26	0.71	0.69	0.68	0.75	0.73	0.51	0.57	0.77	07.6	0.76	0.63	0.82	0.71	0.6	0.8	0.73	0.66	0.66	0.59	0.72	0.71	0.69	0.73	0.71	0.66	0										
27	0.6	0.52	0.76	0.63	0.68	0.7	0.62	0.82	0.66	0.7	0.5	0.69	0.6	0.69	0.72	0.61	0.55	0.6	0.45	0.61	0.65	0.52	0.68	0.47	0.55	0.62	0									
28	0.31	0.3	0.7	0.42	0.46	0.68	0.55	0.76	0.35	0.57	0.25	0.53	0.34	0.45	0.7	0.41	0.13	0.25	0.2	0.35	0.4	0.3	0.38	0.47	0.34	0.66	0.48	0								
29	0.55	0.6	0.62	0.51	0.69	0.71	0.58	0.8	0.63	0.66	0.63	0.71	0.66	0.71	0.82	0.63	0.56	0.56	0.6	0.68	0.61	0.54	0.65	0.48	0.66	0.7	0.63	0.5	0							
30	0.5	0.55	0.56	0.44	0.55	0.73	0.51	0.78	0.59	0.55	0.59	0.72	0.63	0.62	0.81	0.7	0.51	0.57	0.61	0.65	0.68	0.55	0.66	0.57	0.63	0.72	0.64	0.51	0.44	0						
31	0.69	0.68	0.77	0.68	0.68	0.58	0.55	0.75	0.71	0.7	0.66	0.77	0.69	0.64	0.77	0.75	0.65	0.65	0.57	0.71	0.65	0.68	0.76	0.69	0.65	0.62	0.66	0.65	0.62	0.7	0					
32	0.42	0.41	0.68	0.52	0.55	0.61	0.51	0.76	0.45	0.61	0.45	0.66	0.51	0.48	0.73	0.51	0.26	0.36	0.41	0.52	0.56	0.48	0.55	0.5	0.51	0.58	0.57	0.26	0.52	0.54	0.68	0				
33	0.58	0.63	0.78	0.55	0.68	0.83	0.74	0.85	0.54	0.7	0.71	0.74	0.73	0.7	0.83	0.76	0.59	0.65	0.68	0.71	0.74	0.68	0.72	0.74	0.73	0.89	0.8	0.59	0.73	0.64	0.8	0.62	0			
34	0.42	0.32	0.68	0.52	0.41	0.71	0.7	0.76	0.45	0.46	0.27	0.56	0.44	0.48	0.68	0.43	0.26	0.44	0.48	0.45	0.5	0.32	0.55	0.61	0.44	0.7	0.57	0.36	0.65	0.54	0.72	0.47	0.62	0		
35	0.47	0.27	0.66	0.57	0.52	0.74	0.73	0.82	0.5	0.58	0.42	0.6	0.48	0.52	0.72	0.48	0.41	0.48	0.52	0.5	0.6	0.37	0.58	0.65	0.41	0.68	0.61	0.41	0.68	0.58	0.75	0.51	0.66	0.23	0	
36	0.5	0.55	0.68	0.59	0.55	0.75	0.7	0.8	0.63	0.66	0.52	0.56	0.57	0.61	0.85	0.51	0.51	0.51	0.5	0.58	0.56	0.41	0.6	0.56	0.57	0.74	0.68	0.44	0.59	0.54	0.76	0.54	0.68	0.54	0.51	0

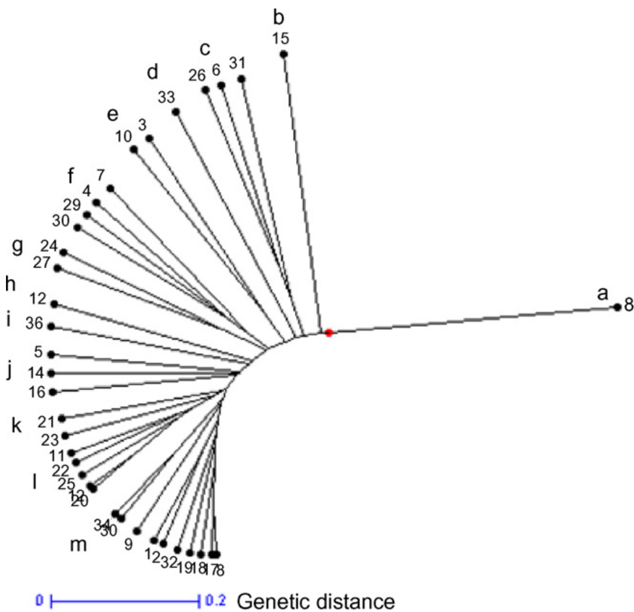

Fig. 3. Phenogram representing the genetic relationships between 36 Tunisian Barbary fig accessions (*Opuntia ficus indica* (L.) Mill.), established by means of 39 RAPD markers: letters (from a to m) stand for the groups individualized.

We have thus developed a reliable DNA method extraction technique which helps to overcome the difficulties caused by high amounts of mucilage that interfere with DNA extraction. Indeed, the yield of DNA was comparable to those published by De La Cruz et al. (1997), Wang et al. (1998) and Mondragon et al. (2000) for Barbary fig.

On the other hand, we have demonstrated the reliability of RAPD analysis to detect DNA polymorphisms and relationships within *Opuntia ficus indica* (L.) Mill. in Tunisia. In fact, the selected primers are characterised by relatively high collective Rp rate of 15.56 and a high number of electrophoretic banding patterns, though no single primer allowed the differentiation of the accessions under investigation. The primers generated 39 polymorphic out of 63 bands with a mean of 8.62.

Fourteen primers did not allow the amplification of DNA. In fact, in RAPD literature, the presence of primers that do not let amplification to occur (Caetano-Annoles, 1994), with others yielding faint banding profiles (Moreno et al., 1995; Ortiz et al., 1997) was reported. Thus, accordingly to the results forwarded by Devos and Gale (1992), Penner et al. (1993) and This et al. (1997), some primers are more efficient than others in producing stable and reproducible profiles.

Moreover, the needed number of accessions to select primers was also questioned. For instance, Vidal et al. (1999) used only two varieties. In our case, the selection of primers was performed on three varieties and eight primers were retained from 22.

Using eight universal primers tested in 36 Barbary fig accessions, we registered a mean of 4.37 markers per primer. This is significantly higher than one reported by Wang et al. (1998) for *Opuntia* accessions originating from Texas, Mexico and Chile, which is an average of 4.31 RAPD markers per primer. Thus, we may assume that the Tunisian Barbary fig germplasm is characterized by a high genetic diversity at the DNA level. This assumption is strongly supported with regard to the scored genetic distances among the ecotypes studied (0.13–0.89).

Table 4
Genetic distances and LFBs shared by genetically close *Opuntia ficus indica* accessions sampled in Tunisia

Pairs of genetically close accessions	Genetic distance	Shared LFBs
Marjaa Ayouem 1 and Sidi Saad inerme 1	0.13	3
Sokri and Lokki Zelfene	0.14	3

The genetic divergence of the ecotypes under investigation was confirmed at the DNA level. In fact, the UPGMA cluster analysis permitted the discrimination of all the genotypes and their sorting into 13 main groups. This has permitted to precise the genetic diversity organisation of Barbary fig in Tunisia and the genetic background of the ecotypes on one hand and helped the setting up of rational decisions concerning the management of this important fruit crop on the other hand. Indeed, the establishment of a national Barbary fig reference collection ought to preserve all the ecotypes; fingerprinted in the current study. In addition, special interest ought to be attributed to the ecotype R Sbiba inerme (8), clustered in group a (Fig. 3), as being genetically distinct from all of the studied accessions. This has shown in fact, how important this technique is, in germplasm gene banking and also how to assist the selection of genotypes by molecular tools.

The LFBs provided interesting information to understand the clusters obtained. For instance, these markers are specific to the varieties in which they are displayed and are shared by few varieties (Vidal et al., 1999) (Table 4).

Finally, as illustrated by the dendrogam (Fig. 2), clustering was made independently from the region of origin of the ecotypes. In fact, the tested genotypes did not significantly diverge, though originating from different localities (Table 1). Thus, no groups of genotypes were assigned to any region. In the same way, Wang et al. (1998) did not managed to differentiate cactus accessions by reference to their geographic origin.

5. Conclusion

In this study, we used molecular tools to investigate for the first time the genetic background of the national Barbary fig gene pool. Thus, all tested genotypes were significantly divergent at DNA level and ought to be preserved in a reference collection. The reasonable preservation of such crops would aid firstly the setting up of a definitive taxonomic sorting, which could be of great interest for breeders and second, the selection of interesting ecotypes that ought to be included in human and animal diets. For instance, the selection of glochid (specialized short hair like spines) free cultivars with reduced seed content is a major challenge for future investigations.

In addition, we further aim to include wild germplasm in order to select cultivars of economically importance and to transfer important genetic traits from wild to cultivated species by marker-assisted-selection.

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