

Optimization of the Management of an Ex-situ Germplasm Bank in Common Fig with SSRs

Esther Giraldo and Margarita Lopez-Corrales

Department of Hortofruticulture, Servicio de Investigación y Tecnología,
Finca La Orden-Valdesequera, 06187 Guadajira, Badajoz, Spain

Jose Ignacio Hormaza¹

Estación Experimental la Mayora, CSIC, 29750 Algarrobo-Costa, Málaga, Spain

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ABSTRACT. Common fig (*Ficus carica* L.) is an underused fruit crop cultivated in Mediterranean countries since antiquity. In this study, 20 simple sequence repeat (SSR) loci were used to characterize 209 fig accessions conserved in an ex-situ field germplasm collection. A total of 78 fragments were amplified with the 20 pairs of SSR primers, with an average of 3.9 alleles per locus and a size between 120 and 376 bp. The mean expected and observed heterozygosities were 0.36 and 0.41, respectively. The total value for the probability of identity was 6.8×10^{-4} . The SSRs studied resulted in identification of 98 unique genotypes (46.86% of all accessions preserved in the bank), indicating a high number of synonyms. Unweighted pair group method with arithmetic averages (UPGMA) cluster analysis did not show clear groups based on geographic distance, although some specific groups related to fruit type were observed. The results confirm the usefulness of microsatellites for the identification of genetic diversity and potential value of germplasm management for fig.

Common fig ($2n = 26$) belongs to the Moraceae, a family with >1400 species classified into ≈ 40 genera (Watson and Dallwitz, 2004). The genus *Ficus* L. contains ≈ 700 species, mainly in the tropics, currently divided into six subgenera (Berg, 2003). Common fig is considered, together with grape (*Vitis vinifera* L.) and olive (*Olea europaea* L.), one of the three classical fruit trees associated with the beginning of horticulture in the Mediterranean Basin (Zohary and Spiegel-Roy, 1975), and consequently it is one of the earliest domesticated fruit tree species (Janick, 2005; Khadari et al., 2005; Zohary and Hopf, 2000). In fact, recent studies (Kislev et al., 2006) indicate that common fig is probably the first domesticated crop of the Neolithic revolution. Fig cultivation has extended throughout other regions of the world with mild weather. Current total world fig production has reached >1 million tonnes, with a few countries (Turkey, Egypt, Greece, Iran, Morocco, Spain, and the United States) accounting for $\approx 70\%$ of the production, with Turkey being the main producer with $\approx 25\%$ of the world production (Food and Agricultural Organization of the United Nations, 2005).

Common fig is a gynodioecious species with two different morphs: female trees that produce syconia with female flowers that will develop into edible seeded figs (syconia with multiple one-seed fruit or drupelets) and caprifigs that produce syconia with male and female flowers with a shorter style than female flowers of female trees. Because only caprifigs produce pollen, the reproductive system is functionally dioecious (Kjellberg et al., 1987). Three types of female figs are grown commercially (Storey, 1976): common type that develops fruit parthenocarpically, the Smyrna type that requires pollination with pollen from caprifigs (caprification) to develop fruit, and the San Pedro

type that produces a first crop (brevas) parthenocarpically and a second crop (fig) only after pollination. Common-type figs can produce one (unifera types) or two crops (bifera types).

The long history of fig cultivation has resulted in hundreds of cultivars available worldwide, and consequently, appropriate germplasm characterization and diversity studies are very valuable for efficient management of fig genetic resources. Synonyms and homonyms are especially common in vegetatively propagated fruit tree species, such as fig, for which the cost of maintaining unwanted duplicate genotypes in ex-situ germplasm collections can be a limiting factor. Fig cultivar identification has been usually carried out using traditional methods based on phenotypic traits [International Plant Genetics Resources Institute (IPGRI) and International Center for Advanced Mediterranean Studies (CIHEAM), 2003]. As an example, Condit (1955) described >600 cultivars of fig. However, traditional characterization using morphologic and agronomic parameters is expensive and often ineffective in distinguishing genotypes due to the influence of the environment, the limited number of discriminating traits, and the need to study a high number of characters in organs that are not always present. Therefore, recently, as with other fruit tree species (Wünsch and Hormaza, 2002), molecular identification of fig cultivars has been carried out with different molecular systems, such as isozymes (Cabrita et al., 2001; Chessa et al., 1998; Elisario et al., 1998), RAPDs (Cabrita et al., 2001; Chessa and Nieddu, 2005; De Masi et al., 2005; Galderisi et al., 1999; Khadari et al., 1995; Papadopoulou et al., 2002; Salhi-Hannachi et al., 2005; Sadder and Ateyyeh, 2006), AFLPs (Cabrita et al., 2001), or ISSRs (Khadari et al., 2004; Salhi-Hannachi et al., 2004, 2005). However, microsatellites or SSR (SSRs) have become the markers of choice for fingerprinting and analysis of genetic diversity in most plant species (Gupta and Varshney, 2000) due to their high level of polymorphism, codominant Mendelian inheritance, reproducibility, and easy detection through PCR and electrophoretic methods.

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¹Corresponding author. E-mail: ihormaza@eelm.csic.es.

Until recently, the use of SSR markers has been limited due to the high costs associated with the construction and screening of genomic libraries to find sequences flanking the repeated regions. However, the development of genomic libraries enriched with SSRs sequences has allowed the use of these markers on a larger scale. Thus, >50 microsatellites have been recently isolated and characterized in fig (Giraldo et al., 2005; Khadari et al., 2001), and some of them have been used to identify fig genotypes (Giraldo et al., 2005; Khadari et al., 2004).

The objective of this work is to use the SSRs developed in fig to fingerprint and study the genetic similarity among 209 genotypes conserved in the Spanish fig germplasm bank located in Badajoz.

Materials and Methods

PLANT MATERIAL. Two-hundred nine fig accessions from diverse geographical areas maintained at the Finca La Orden of the Junta de Extremadura, Badajoz, Spain, were analyzed in this study. Among those accessions, 196 were collected from different regions in Spain and 13 were obtained from other countries (Table 1).

GENOMIC DNA EXTRACTION AND PCR AMPLIFICATION. Genomic DNA extraction was performed on dormant winter buds following the protocol described in Giraldo et al. (2005). The DNA was quantified spectrophotometrically and diluted to 10 ng- μL^{-1} in modified TE buffer (10 mM Tris-HCl, 0.1 mM EDTA). The extracted DNA was amplified by PCR, using 20 pairs of microsatellite primers (Table 2), 16 developed by Giraldo et al. (2005) and four by Khadari et al. (2001). These SSRs were chosen due to the high quality of amplification and reproducibility of the results. PCR reactions were performed in an iCycler (Bio-Rad Laboratories, Hercules, CA) as reported by Giraldo et al. (2005). Size evaluation of the amplified fragments was performed using a CEQ 8000 capillary DNA analysis system (Beckman Coulter, Fullerton, CA). Reverse primers of each primer pair were labeled with WellRED fluorescent dyes D2, D3, and D4 (Prologo, Paris, France). The analyses were repeated at least twice to assure the reproducibility of the results.

DATA ANALYSIS. Genetic relationships among the fig accessions were represented by UPGMA cluster analysis of the similarity matrix obtained from the proportion of shared amplification fragments (Nei and Li, 1979). The cophenetic correlation coefficient was estimated by comparing with the Mantel test the cophenetic matrix obtained from the dendrogram with the original similarity matrix. All of these analyses were computed with NTSYSpc 2.11 (Exeter Software, Stauket, NY). Bootstrap support values were obtained from 2000 replicates using TREECON 1.3b (Van de Peer and De Wachter, 1994).

For the SSRs that amplified a single locus, different indices of genetic variability were calculated: number of alleles per locus and accession, observed heterozygosity (H_o , direct count, calculated as the number of heterozygous genotypes over the total number of genotypes analyzed for each locus), expected heterozygosity ($H_e = 1 - \sum p_i^2$, where p_i is the frequency of the i th allele in the cultivar; Nei 1973), effective number of alleles [$N_e = 1/(1 - H_e)$], Wright's fixation index ($F = 1 - H_o/H_e$) (Wright 1951), and the probability of identity [$PI = 1 - \sum p_i^2 + \sum \sum (2p_i p_j)^2$, where p_i and p_j are the frequency of the i th and j th alleles, respectively] that measures the probability of two

accessions sharing the same alleles (Paetkau et al., 1995). Total probability of identity, defined as the probability of two cultivars sharing the same genetic profile by chance, was also calculated from individual PI values. All of these parameters were calculated with IDENTITY 1.0 (Wagner and Sefc, 1999) and POPGENE 1.32 (Yeh and Boyle, 1997).

When synonyms were found, the selection of the reference cultivar names was made according to the apparent seniority or frequency of the names used, historical records, or bibliographic references (Condit, 1955; Estelrich, 1910; Priego Jaramillo and Sanchiz, 1934; Rosselló et al., 1996).

Results and Discussion

SSR POLYMORPHISM AND GENETIC DIVERSITY. The total number of alleles detected with the selected 20 SSRs was 78, ranging between two and nine alleles per locus, with an average of 3.9 alleles per locus and a size between 120 and 376 bp. Eight out of the 20 SSRs (LMFC12, LMFC13, LMFC17, LMFC19, LMFC30, LMFC32, MFC1, and MFC4) showed three amplification fragments in some genotypes, and one SSR (LMFC28) produced up to four fragments indicating possible amplification of more than one locus. The remaining 11 SSRs showed one or two bands per genotype, suggesting amplification of a single locus, although fragment segregation in the progeny obtained from a cross is the only way to assign alleles to a particular locus. Consequently, for these 11 loci the accessions studied were considered homozygous or heterozygous when one or two fragments are present per locus, respectively (Callen et al., 1993). For these 11 loci, genetic parameters were computed. Allelic frequencies ranged from 0.0024 to 0.95, with a mean of 0.33. A total of 36.4% of the alleles analyzed were considered rare ($P < 0.1$). Only 9.1% of the alleles were fixed in most of the genotypes ($P > 0.9$), and seven alleles were each found in only one of the 209 accessions: allele 334 of LMFC19 is present only in 'Smyrna', allele 242 of LMFC24 is present only in accession 176, allele 250 of LMFC30 is present only in accession 179, allele 133 of MFC5 is present only in 'Moisoniere', and three unique alleles are amplified with LMFC32 (223 bp in 'Pezonuda', 217 bp in 'Smyrna', and 211 bp in 'Cordobis'). These rare alleles were verified and validated after two replicates.

Expected heterozygosity ranged from 0.09 in LMFC23 to 0.74 in MFC2 (mean of 0.36) (Table 3). Observed heterozygosity ranged from 0.10 in LMFC23 to 0.80 in MFC2 (mean of 0.41). For all single-locus SSRs except two (LMFC15 and LMFC21), the observed heterozygosity was higher than the expected. Consequently, most of the F values are negative with an average overall single locus SSRs of -0.12 , showing homozygote deficiency. Regarding the probability of identity, the maximum (0.84) was detected in LMFC23 and the minimum (0.20) in MFC2, with a total PI of 6.8×10^{-4} .

The number of alleles per locus (3.9) and H_e (0.38) are relatively low compared with other works in fig with a lower number of genotypes or loci; Khadari et al. (2004) reported an average of 6.2 alleles per locus and an average H_e of 0.60 with six SSRs and 72 Moroccan fig cultivars. This could reflect a low polymorphism in the accessions studied that could indicate a common origin of cultivated fig in Spain, although further work involving fig cultivars from geographically diverse areas will be needed to address this point. The ease of vegetative propagation in this species and its long history of cultivation, together with the focus on selection of parthenocarpic cultivars

Table 1. Accessions of reference conserved in the Spanish fig germplasm bank and the synonyms found with 20 SSRs.

Accession	Origin	Type	Synonyms in the germplasm bank
Albacor ^z	Balearic Islands	Bifera	Ademuz, Albacor, Alcacer 1, Bacorera, Betera, Breval Málaga, Colar Elche, Cuello de Dama Negra, Del Gra Negre, Goen, Mission, Napolitana Negra Brevera Foyos, Negra, Negra 3, Negra Común R, Negra Málaga, Negra Tocinera, Torre Baja 1, 9602, 9603, 9607, 9616
Albar	Extremadura	Bifera	
Angelina	Balearic Islands	Bifera	Algerina
Arail	Andalusia	Smyrna	
Ayuela	Extremadura	Bifera	
Bec de Perdiu	Catalonia	Bifera	Burriana 1, Napolitana Chelva 1, Napolitana, Napolitana Enguera, Napolitana Negra, Napolitana Negra Foyos, Napolitana Mas Valero, Negra Pozuelo, Torre Baja 3, Napolitana
Bermejí	Extremadura	Bifera	
Bermesca ^y	Balearic Islands	Bifera	Calabacita
Blanca Albondón	Andalusia	Smyrna	
Blanca Betera	Valencia	Bifera	
Blanca Foyos	Valencia	Unifera	
Blanca R	Catalonia	Unifera	
Blava	Balearic Islands	Bifera	
Boja o Farta Belitres	Catalonia	Unifera	
Bota Morada	Castile and Leon	Bifera	Serranilla
Boyuna	Extremadura	Bifera	Porronta
Brocalet ^x	Balearic Islands	Unifera	De Ley 2, Lloral, Martinenca Rimada, Martinenca (Mallorca), Martinenca Mina, Martinenca (Reus), Porquenyà
Brown Turkey	United States	Bifera	Albatera, Desconegut 12
Burjassot Alguaire	Catalonia	Unifera	
Burjassot Blanca	Valencia	Unifera	Albacor, Burjassot V
Burjassot Negre	Catalonia	Unifera	Paratjal, Pota de Cavall
Burreña	Extremadura	Bifera	
Cabatxa	Catalonia	Unifera	Pit de Reina
Calabacita ^y	Extremadura	Bifera	Bermesca
Calderona	Balearic Islands	Unifera	
Casas Bajas	Valencia	Bifera	
Coll de Dama Blanca ^w	Catalonia	Unifera	Breva Q, Coll de Dama Negra, Coll de Dama Rosa, Cuello de Dama Negra, Cuello de Dama Rosa, De Lley 1, Ull de Perdiu Mina, 9603, 9605, 9615
Coll de Dama Negre ^w	Balearic Islands	Unifera	Breva Q, Coll de Dama Blanca, Coll de Dama Rosa, Cuello de Dama Negra, Cuello de Dama Rosa, De Lley 1, Ull de Perdiu Mina, 9603, 9605, 9615
Coll de Dama Rosa ^w	Catalonia	Unifera	Breva Q, Coll de Dama Blanca, Coll de Dama Negra, Cuello de Dama Negra, Cuello de Dama Rosa, De Lley 1, Ull de Perdiu Mina, 9603, 9605, 9615
Conadria	United States	Bifera	
Cordobis	Extremadura	Unifera	
Cornudella	Catalonia	Unifera	
Cucurella	Catalonia	Unifera	De la Gota de Miel, 178, 184
Cuiro de Bou	Balearic Islands	Unifera	
De Baco	Catalonia	Bifera	
De Butxaca	Catalonia	Bifera	
De'n Manel	France	Bifera	Tres voltas L'Any-1
De Rey	Extremadura	Bifera	
Desconegut 85	Catalonia	Bifera	
Doña Maria	Extremadura	Bifera	
Dottato	Balearic Islands	Bifera	Blanca Cabezuela, Cuello de Dama Blanco, Del Guardia, Gota de Miel, Kadota, Napolitana Blanca
Genyiva Mort	Catalonia	Bifera	
Granito	Extremadura	Bifera	
Hoñigal	Extremadura	Unifera	

continued next page

Table 1. Continued.

Accession	Origin	Type	Synonyms in the germplasm bank
Hortella	Balearic Islands	Unifera	
Imperial 76	Catalonia	Unifera	Pell Verd
Jorba	Balearic Islands	Unifera	
La Casta	Extremadura	Unifera	
Lampaga	Andalusia	San Pedro	Ayuela R, Lampa Preta, Pacueca, Tiberio, Villalba, 9809
Mallea ^v	Balearic Islands	Unifera	Alacantina, Albacor, Blanca 1, Bordissot Blanca, Burdissot Verde, Calabacita R, Capoll Llarg, Fraga, Gota de Miel, Maellana Blanca, Napolitana M, Panachée, Sabanita, Verde Pozuelo
Mare de Deu	Balearic Islands	Unifera	Ull de Perdiu Mina (M)
Martina	Balearic Islands	Bifera	
Martinenca ^x	Balearic Islands	Unifera	Brocalet, De Ley 2, Lloral, Martinenca Rimada, Martinenca (Mallorca), Martinenca Mina, Porquenyà
Miralla	Balearic Islands	Unifera	
Mission ^z	United States	Bifera	Ademuz, Albacor, Alcacer 1, Bacorera, Betera, Breval Málaga, Colar Elche, Cuello de Dama Negra, Del Gra Negre, Goen, Napolitana Negra Brevera Foyos, Negra, Negra 3, Negra Común R, Negra Málaga, Negra Tocinera, Torre Baja 1, 9602, 9603, 9607, 9616
Moisoniere	France	Unifera	Becane Noire
Moscatel	Castile-La Mancha	Unifera	
Moscatel Negra	Castile-La Mancha	Unifera	
Napolitana Bacorera	Catalonia	Bifera	
Napolitana Blanca	Catalonia	Unifera	Aldearrubia, Blanca Valenciana, De la Roca, Envernesca-Gota de Miel, Hivernenca M, Llei, Napolitana R
Nazaret	Israel	San Pedro	
Negra Cabezuela	Extremadura	Bifera	
Negra Calabacilla	Andalusia	Unifera	
Negra Común	Andalusia	Bifera	
Panachée ^v	Catalonia	Unifera	Alacantina, Albacor, Blanca 1, Bordissot Blanca, Burdissot Verde, Calabacita R, Capoll Llarg, Fraga, Gota de Miel, Maellana Blanca, Mallea, Napolitana M, Sabanita, Verde Pozuelo
Paratjal	Balearic Islands	Unifera	
Pecho de Reina	Catalonia	San Pedro	Tres Fan Cargas
Pell de Bou	Balearic Islands	Bifera	
Perdigona Negra	Catalonia	Bifera	
Perolaza	Catalonia	Unifera	
Pezonuda	Andalusia	Unifera	
Picholetera	Extremadura	Unifera	
Princesa	Portugal	Bifera	Blanca
Rogisca	Balearic Islands	Unifera	
Roja Almohadin	Castile-La Mancha	Unifera	Franciscana
San Antonio	Extremadura	Bifera	
San Joao Branco	Portugal	Caprifig	
Sang de Rossi	Valencia	Unifera	
Sarrona	Castile-La Mancha	Unifera	
Setjolla Porrera	Catalonia	Unifera	Ull de Perdiu 2
Sitscel	Balearic Islands	Bifera	
Smyrna	Turkey	Smyrna	
Tocal	Andalusia	Caprifig	
Torrebaia 2	Balearic Islands	Bifera	
Tres Collitas	Catalonia	Bifera	
Tres Voltas L'Any-2	Catalonia	Bifera	
Ull de Perdiu 1	Catalonia	Unifera	Setjola
Verdal	Balearic Islands	Unifera	Verdal M, Verdal Mina, Verdal R, 9612
Verdaleta	Valencia	Bifera	
Verdejo	Extremadura	Bifera	
Verdejuela	Extremadura	Bifera	

continued next page

Table 1. Continued.

Accession	Origin	Type	Synonyms in the germplasm bank
White Genoa	United States	Unifera	
Zuguele	Extremadura	Bifera	
120	France	Bifera	
152	Catalonia	Bifera	
176	Catalonia	Unifera	
179	Catalonia	Unifera	181
183	Catalonia	Unifera	
185	Catalonia	Unifera	
218	Catalonia	Unifera	
9606	Aragon	Unifera	
9608	Aragon	Unifera	
9611	Aragon	Bifera	
9614	Aragon	Unifera	

^{z,y,x,w,v} Genotypes of reference with the same SSR profile but that show phenotypic differences among them.

Table 2. Locus name, sequence, repeat motif, GenBank accession number, and literature reference for the 20 polymorphic microsatellites used in the identification of fig cultivars.

SSR	Sequence (5'–3')	Motif	GenBank accession no.	Reference
LMFC12	F: TTAAACCTACTTCAACAAT R: GTAATCCCCGAGATATAGT	(CT) ₅₅	AY545926	Giraldo et al., 2005
LMFC13	F: CCTCTTCTCTCTCTTAATTTT R: TTTATCAAACCCACTGATTC	(GA) ₂₈	AY545927	Giraldo et al., 2005
LMFC14	F: CAAAACTCACACCAATAATC R: TAATCTGCAAAAAGATGACTA	(GA) ₁₆	AY545928	Giraldo et al., 2005
LMFC15	F: CGGAGAAAAGATTTAGAATTTG R: ATTCCAGAGACGAAAAGGTCT	(TC) ₂₂	AY545929	Giraldo et al., 2005
LMFC17	F: TTAAGAATACGTCCTTGGTAT R: GAGATTTGTTGACTTCATT	(GA) ₁₆	AY545930	Giraldo et al., 2005
LMFC18	F: CACATCCACACACCAAAGAG R: TACCACAGACTCACCCAATTAT	(GA) ₉	AY545931	Giraldo et al., 2005
LMFC19	F: CTTATGAAAACCTCGGTAGAAG R: AATGAATGGAAATGATCTTG	(AT) ₁₁ (AG) ₁₂	AY545932	Giraldo et al., 2005
LMFC21	F: ATGTCAAAACACCAGCTCTA R: AAGAATAGAAAACCTGAAAAAG	(TC) ₉	AY545934	Giraldo et al., 2005
LMFC23	F: TTTCTGTCTAACGATCAAAAA R: CTCCCATCTCCAACCTCCATC	(AG) ₂₀	AY545936	Giraldo et al., 2005
LMFC24	F: ACTTCTTCATATTTGGTATAGG R: TTCATAAACTGGTCTAAAAGA	(CT) ₁₀	AY545937	Giraldo et al., 2005
LMFC26	F: ATGTTATAGTTGAGTGAGGATAA R: AAATAGTGGATCTTGCATGT	(GA) ₁₅	AY545939	Giraldo et al., 2005
LMFC27	F: ATTTCTTCAACTTTTGTAAATGA R: CCTTTTGTCTACATATACCTTT	(TG) ₁₇ (AG) ₆	AY545940	Giraldo et al., 2005
LMFC28	F: TGATTCTTTTACTTGTAGATT R: AAGACATTGAGACATACCAG	(CT) ₁₄	AY545941	Giraldo et al., 2005
LMFC30	F: TTGTCCGTTTCTTATACAAT R: TCTTTTATAGGCAGATGTTAG	(CT) ₁₈ (CA) ₆	AY545942	Giraldo et al., 2005
LMFC31	F: GTAAAATGAAAATTGGAGTATT R: TTGAAGATATTGTTGTATGCT	(GA) ₁₅	AY545943	Giraldo et al., 2005
LMFC32	F: GAAAGAAAGTCGAATAATGTA R: TATAAAGAGGGTGGTCTTAGT	(GA) ₂₃	AY545944	Giraldo et al., 2005
MFC1	F: ACTAGACTGAAAAAACATTGC R: TGAGATTGAAAGGAAACGAG	(CT) ₁₃	AF333696	Khadari et al., 2001
MFC2	F: GCTTCCGATGCTGCTCTTA R: TCGGAGACTTTTGTTCAT	(AC) ₁₈ (AT) ₇	AF333697	Khadari et al., 2001
MFC4	F: CCAAACCTTTTAGATACAACCTT R: TTTCTCAACATATTAACAGG	(AT) ₄ (AC) ₁₁	AF333699	Khadari et al., 2001
MFC5	F: ACCAATCCAAATAATAATCC R: ACACGCTTACTAGAATTACC	(GA) ₁₃	AF333700	Khadari et al., 2001

Table 3. Locus name, range size of the amplified fragments, number of alleles (A), observed (Ho) and expected (He) heterozygosities, probability of identity (PI), and Wright's fixation index (F) calculated for 20 SSR markers in 209 fig cultivars.

SSR	Size (bp)	A	Ho	He	PI	F
LMFC12 ^z	350–376	3				
LMFC13 ^z	270–298	4				
LMFC14	213–215	3	0.38	0.36	0.51	–0.06
LMFC15	183–207	3	0.20	0.21	0.66	0.05
LMFC17 ^z	188–203	4				
LMFC18	120–126	2	0.41	0.37	0.60	–0.11
LMFC19 ^z	299–334	5				
LMFC21	265–271	3	0.12	0.12	0.78	0.00
LMFC23	132–134	2	0.10	0.09	0.84	–0.11
LMFC24	242–278	4	0.58	0.47	0.57	–0.23
LMFC26	224–237	3	0.36	0.31	0.56	–0.16
LMFC27	186–196	2	0.59	0.47	0.61	–0.26
LMFC28 ^z	183–203	6				
LMFC30 ^z	231–262	9				
LMFC31	228–242	2	0.61	0.49	0.62	–0.24
LMFC32 ^z	199–223	7				
MFC1 ^z	160–192	4				
MFC2	157–170	5	0.80	0.74	0.20	–0.08
MFC4 ^z	198–221	3				
MFC5	128–142	4	0.36	0.31	0.60	–0.16
Mean		3.9	0.41	0.36	0.60	–0.12

^zIndicates amplification of more than two fragments in some genotypes.

(common type), have allowed the widespread transfer of selected plant stocks among different regions. This could explain the high number of synonyms found in the accessions studied [the 78 polymorphic amplification fragments detected with the 20 SSRs studied allowed the unambiguous identification of only 98 genotypes (46.86%) of all accessions preserved in the bank] due probably to local naming of clones propagated vegetatively that, in some cases, may show some phenotypic differences due to different local environmental factors. In any case, a low level of polymorphism has been reported in other works in fig with different molecular markers and cultivars even from different countries (Galderisi et al., 1999; Giraldo et al., 2005; Khadari et al., 1995; Papadopoulou et al., 2002).

SELECTION OF REFERENCE GENOTYPES. Due to the high number of synonyms and homonyms detected in the germplasm bank, a reference cultivar name was chosen within each genetic profile except for five of the 98 groups, where two or three reference cultivars have been selected because clear morphological differences were found; those differences include flesh color, as with 'Albacor' and 'Mission' or 'Coll de Dama Blanca', 'Coll de Dama Negre', and 'Coll de Dama Rosa'; fruit size and shape, as with 'Bermesca' and 'Calabacita', and 'Brocalet' and 'Martinenca'; or fruit color, as with 'Mallea' and 'Panachée'. The agronomic and economic importance of the cultivar in the consulted bibliography was the main criterion for selection of the reference cultivars. Table 1 shows the synonyms found in the germplasm bank and the reference cultivars selected. Establishment of the database with molecular profiles of reference genotypes will assist in management of the germplasm bank and aid in the screening of new accessions before their incorporation into the collection.

Examples for the most well-known cultivars are described below.

'COLL DE DAMA'. Risso (1826) described a cultivar known as Figue des Dames, and Simonet et al. (1945) used a similar name. Later, this cultivar was known as 'Col de Dame.' According to Condit (1955), Mazieres (1920) mentioned that this cultivar was distributed over southern France and Spain, where it originally came from. This would explain the high number of synonyms in this group among the fig genotypes prospected in Spain. However, the presence of different SSR profiles under the same denomination of 'Col de Dame' indicates also de presence of homonyms. According to Condit (1955), 'Col de Dame' is synonymous with 'Cuello de Dama Blanca', although in this study these names were associated with different genotypes (similarity of 0.73). Moreover, we have found some phenotypic differences among some of the cultivars that present the same SSR profile as 'Col de Dame' ('Coll de Dama Blanco', 'Coll de Dama Negre', and 'Coll de Dama Rosa'), due probably to microenvironmental factors or somatic mutations which probably have taken place throughout the history of fig cultivation, resulting in phenotypic intra-cultivar variability (Galderisi et al., 1999).

'LAMPAGA'. Both molecular results and phenotypic evaluation show that 'Lampaga' and 'Lampa Preta' are synonyms in our collection. According to Condit (1955), the Portuguese cultivar Lampo Preto, described by Bobone (1932), could be the same cultivar as Lampeira or Lampapas. The accession known in our collection as 'Pacueca' shows the same genetic profile as 'Lampaga' and 'Lampa Preta' based on SSRs, and 'Pacueca' is listed as a synonym for Lampaga within the Spanish parthenocarpic violet cultivars (Flores 1990). Because we do not have 'Lampeira' in our collection, we have preferred to maintain the name Lampaga for the selected cultivar of this group.

'PANACHÉE'. The accession known in our collection as 'Calabacita R' is morphologically different from 'Calabacita', and this is corroborated by molecular analysis in this work. Morphological comparison (J.P. Roger, personal communication) indicates that Calabacita R is likely the cultivar known in France as Panachée, a very distinct cultivar with yellow- and green-striped fruit. Barron (1869) described it as an improved cultivar of Col de Signora Bianca, a synonym for Fraga according to Condit (1955). In fact, 'Fraga' presents the same genetic profile than 'Mallea' and 'Panachée' ('Calabacita R'). Then, 'Panachée' could have been derived from 'Fraga', probably by mutation, because they are clearly different at the morphological level but indistinguishable at the molecular level with the SSRs used in this work.

However, in other cases, the bibliographic data do not agree with the obtained results. Some examples are described below.

The unifera cultivars Jorba and Alacantina have been reported as synonyms (Estelrich, 1910). However, the results obtained show that 'Jorba' and 'Alacantina' present a similarity value of 0.72. After comparing the morphological descriptions established by the different authors with the phenotype observed in the germplasm bank, we can conclude that the cultivar Alacantina in our collection is probably mislabeled.

Eisen (1901) identified 'Mission' as the Spanish 'Franciscana'. Condit (1955) described 'Franciscana' as a black fig cultivated in Malaga, Spain. It has been suggested (Condit, 1955) that the cultivars known as Negra and Breval introduced

in California originated in Malaga and were identical to Franciscana. The SSR analysis of this study indicates that 'Mission' presents the same genetic profile as 'Negra', 'Negra Malaga', and 'Breval Malaga'. However, the genetic distance with 'Franciscana' (given the reference name of 'Roja Almohadin' in our collection) is very high because both accessions show a similarity index of 0.62. Therefore, the cultivar Franciscana in our germ-plasm bank is probably not the same as the one identified by Eisen. This homonym could be due to an error in the labeling of the bank. To solve this controversy it would be necessary to find new specimens under this denomination and carry out new analyses.

Similarly, with the SSR markers used in this work it has not been possible to distinguish genotypes putatively derived from mutations from the original ones, such as 'Panachée' and 'Mallea'; 'Mission' and 'Albacor Negro'; 'Coll de Dama Negra', 'Coll de Dama Rosa', and 'Coll de Dama Blanca'; 'Brocalet' and 'Martinenca'; or 'Bermesca' and 'Calabacita', that can be easily distinguished phenotypically.

GENETIC RELATIONSHIPS BETWEEN FIG CULTIVARS. Among all possible dendrograms, the dendrogram with the highest cophenetic correlation coefficient (0.63) was chosen. Figure 1 represents the final dendrogram with the 104 reference genotypes after selecting one cultivar for each one of the 98 groups with the same genetic profile and six additional genotypes from five groups where two or three cultivars were selected because the morphological differences were clear (Table 1). The cultivars can be put into three main groups (A, B, and C) and five additional unique profiles (D). Accessions from the different geographic areas prospected are present in the three main groups, and, therefore, geographic origin is not the main criterion for the classification obtained. This could be explained due to the easy vegetative propagation of this species and its low edaphic and climatic requirements, which allow easy exchange of plant material among different geographical regions. However, certain subgroup classifications can be related to the production type, and some are supported by high bootstrap values. Examples include the unifera groups 'Hoñigal' and 9606 (bootstrap of 100%); 'Panachée', 'Mallea', 'Blanca Foyos', 'Blanca R', 'Burjassot Blanca', and 'Boja o

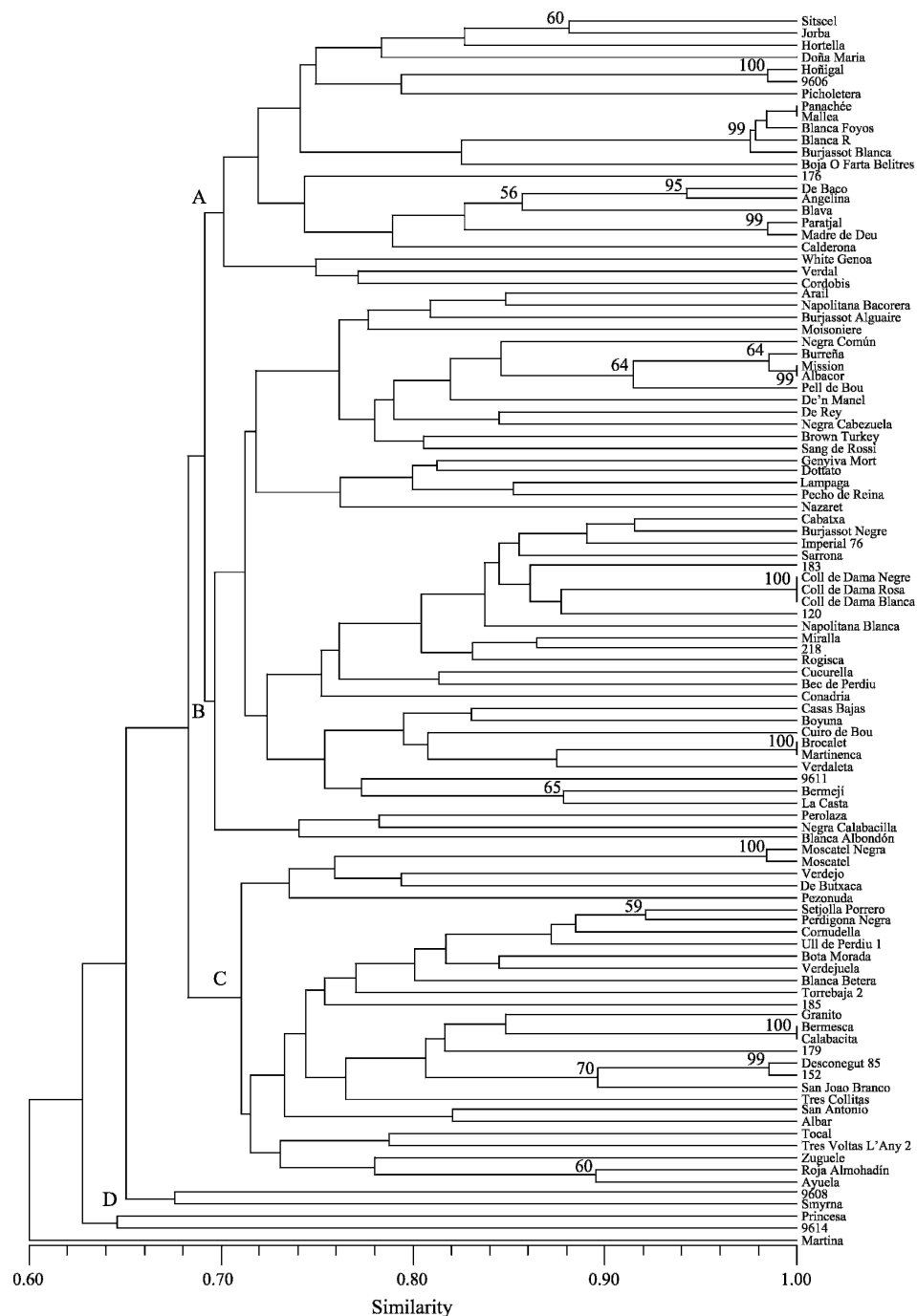


Fig. 1. Dendrogram of the 104 fig reference genotypes based on UPGMA analysis using the similarity matrix generated by the Nei and Li (1979) coefficient after amplification with 20 pairs of SSR primers. Bootstrap values out of 2000 replicates are shown if 50% or higher.

Farta Belitres' (bootstrap of 99%); 'Paratjal' and 'Mare de Deu' (bootstrap of 99%); and the bifera groups 'De Baco', 'Angelina', and 'Blava' (bootstrap of 56%) and 'Burreña', 'Mission', and 'Albacor' (bootstrap of 64%). Moreover, the presence of the two caprifig cultivars (Tocal and San Joao Branco) clustered with parthenocarpic accessions could indicate a common origin derived from crosses between caprifigs and parthenocarpic accessions because in the presence of caprifigs, the second crop of common fig can be the result of pollination producing seeded figs.

In addition to the main groups obtained in the dendrogram, five genetic profiles are different from the rest: 'Princesa', 'Martina', 'Smyrna', 9608, and 9614. The geographical origin of these genotypes could explain their differentiation from the rest of the accession conserved in the germplasm bank. 'Princesa' from Portugal is synonymous with 'Blanca', from Huelva in southwestern Spain. According to Gallesio (1817), the cultivar Blanca was present in Italy but not in Spain or France. In fact, this cultivar was not included in old publications of Spanish fig cultivars (Priego Jaramillo and Sanchiz, 1934). Nevertheless, in more recent Spanish publications (Flores, 1990), it appears as a good cultivar for dried figs, with good size fruit. The fact that the similarity value between this profile and the rest of the cultivars is low could be explained by accepting the hypothesis that this genotype comes from the Italian cultivar not introduced in Spain until the 20th century. Regarding 'Martina', this accession originated from a prospecting trip carried out in 1993 in the Balearic Islands. Studies based on the genetic diversity of wild fig tree populations in those islands show the low diversity and the strong differentiation of wild fig tree populations. This differentiation could be due to a possible establishment of Balearic fig populations before fig domestication (Khadari et al., 2005). Thus, some Balearic domesticated figs, such as 'Martina', could be derived from those ancient populations. Further studies with additional cultivars and wild material from the Balearic islands are needed to address this point. 'Smyrna' is a cultivar of Turkish origin, and this could explain its separation from the cultivars analyzed in this work which are mainly from Spain. Regarding 9608 and 9614, they correspond to recent introductions in the germplasm bank from collections in Aragon (northern Spain).

The results obtained in this work indicate that SSRs are excellent codominant markers to optimize fig germplasm management, allowing distinction of synonyms and homonyms and studies of conserved diversity. This resulted in a reduction of the initial number of 209 accessions to 104 referenced cultivars. Further work with other fig germplasm collections is required to establish common criteria for cultivar naming and optimizing fig germplasm conservation worldwide.

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