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Characterization of edible fig germplasm from Puglia, southeastern Italy: Is the distinction of three fig types (Smyrna, San Pedro and Common) still valid?

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ABSTRACT

Fig. (*Ficus carica* L.) is a gynodioecious species with two major sex types: the caprifig (hermaphroditic), which has male flowers and short-styled female flowers, and the fig (female) with only long-styled female flowers. Many fig varieties require pollen to allow flower fertilization and fruit development in a process known as caprification. Fig varieties produce one or two crops per year; the first is the breba and the second, the main crop. Puglia is characterized by a wide germplasm of both edible (female) figs and (male) caprifigs. Over 100 different fig genotypes, mainly collected in Puglia, are located in the fig repository at the P. Martucci experimental station, University of Bari 'Aldo Moro'. The tendency towards caprification of the three fig types (Common, Smyrna and San Pedro) is still unclear. To investigate the biological behavior of 24 fig genotypes, both caprification trials and microsatellite analysis were used. Fruit-set of brebas was very variable, whereas fruit-set of main crop was medium-high in the Common type varieties and low in the San Pedro types. Almost all genotypes were physiologically biferous. We compared these results with those obtained from molecular and phylogenetic analyses. Out of 49 SSR markers tested, 39 amplified one or two PCR products, and 31 were polymorphic. Phylogenetic analysis of the 24 fig genotypes revealed a clear distinction between Common and San Pedro type figs. Greater understanding of fig biological caprification is important to distinguish fig types into those requiring caprification and those that do not require caprification.

1. Introduction

Figs (*Ficus carica* L.) belong to a genus of the family *Moraceae* in the order *Urticales*. Around 700 of the species in the *Moraceae* belong to the *Ficus* genus (Flaishman et al., 2008; Datwyler and Weiblen 2004), and fig is the most common species among them. Fig is a gynodioecious species, and thus many varieties require pollination to produce fruits, a process known as caprification. Caprification is the pollination of long-styled female flowers of the edible fig by wasps carrying the pollen from the profichi borne on the caprifig (hermaphroditic, with male and female flowers in the profichi fruits). Hand-caprification is a cultural practice very common in the Mediterranean area since ancient times: Aristotle, Theophrastus and Pliny the Elder described it (Vallese 1909; Siniscalchi 1912; Condit, 1955). It consists of placing some crowns of profichi on the shoots of the edible fig to allow pollination by wasps (Fig. 1). Fig-pollinator mutualism is a model system for the study of coevolution (Khadari et al., 2001a) and a feature of particular interest for ecological studies due to the specialized interaction between *Ficus* species and their pollinating wasps (Bandelj et al., 2007). Moreover, the differentiation between hermaphroditic and female strains maintains the close symbiotic relationship between *Ficus* trees and the *Blastphaga* wasps.

Common fig, grape and olive are three classical fruit trees associated with the beginning of Mediterranean horticulture (Giraldo et al., 2008), and consequently, fig is one of the first domesticated fruit tree species (Janick, 2005; Khadari et al., 2005a). The importance of this species since ancient times is widely supported by various citations in both the Holy Bible and the Quran. Fig was very common in the Roman Empire, and various authors (Cato, Pliny the Elder, Columella) described horticultural practices or varieties (African, Winter, Tiburtine, Pompeian, Herculanean, Saguntine, etc.). Puglia was characterized by a wide germplasm of edible figs and caprifigs, probably originating from different Eastern areas of the Empire and thus became a sort of natural fig repository. Caprification commonly occurred where fig trees were cultivated (Vallese, 1909). Fig varieties grow wild in the Mediterranean basin as a consequence of human migration and seed dispersal, but only three types are grown commercially: the Common type, which develops fruits parthenocarpically, either brebas (first crop) or main crop (second crop); the Smyrna type, non-parthenocarpic, which requires pollination with pollen from profichi of the caprifig to develop the main crop; and the San Pedro type, which produces brebas parthenocarpically and the main crop after caprification (Storey, 1976). Thus, every fig variety should fall into one of the four types: caprifig, Smyrna, San Pedro or Common.

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Fig. 1. Profichi to be prepared for the caprification trials (left) and at the end of caprification (right).

Before WWII, the agricultural area dedicated to fig cultivation in Puglia was ~30,000 ha, but today it is less than 500 ha. This dramatic decline in fig cultivation is a consequence of expanded cultivation of other fruit tree species such as table grape, sweet cherry, peach, etc. Although Puglia has favorable pedo-climatic conditions for fig cultivation, this species is considered a low-value crop that attracts very limited interest. A main constraint is the lack of knowledge of the morphological, agronomical, chemical and storage characteristics of both the plant material and the fruits (Ferrara and Papa, 2001). Therefore, synonymies and homonymies, germplasm erosion, the different types (Smyrna, San Pedro or Common), the poor yield of some varieties due to different biological and agronomical factors, as well as caprification, fruit quality and nutrition, and optimum post-harvest treatments are important areas of improvement in order to promote new interest in fig cultivation. Due to this low interest for cultivation, fig has not been subjected to intensive breeding programs like other domesticated crops; thus, many fig populations exhibit a rich genetic diversity that can be exploited to characterize fig germplasm (Perez-Jiménez et al., 2012). Several large-scale comparative studies of gene expression have been conducted to both provide comprehensive data on gene expression and fruit physiology and to elucidate the genetic factors underlying traits involved in type differentiation (Ikegami et al., 2013). For example, a study of gene expression, by performing high-throughput sequencing of cDNA libraries from caprifig and Common type fruits and comparing their transcriptomes using expressed sequence tags, identified several gene complexes involved in fruit physiology and responsible for the mechanisms of phenotypic differentiation among different genotypes (Ikegami et al., 2013).

Despite the progress that has been made using next generation sequencing technologies, molecular markers, and in particular microsatellites, continue to be developed and used (Achtak et al., 2009; Perez-Jiménez et al., 2012). Traditionally, plant germplasm characterization has been carried out using morphological and agronomic traits with fluctuations among years, environments and repetitions, creating difficulties in varietal identification in this species (Giraldo et al., 2010). Currently, simple sequence repeats (SSRs) are the most used molecular markers for breeding programs.

This work describes and characterizes some indigenous fig varieties from Puglia using caprification trials and microsatellite analysis to better understand their biological behavior and genetic relationships, in order to identify uniferous and biferous genotypes and the consequent fig type classification.

2. Materials and methods

2.1. Plant material and location

Twenty-four fig varieties, grown at the fig repository at the 'P. Martucci' experimental station in Valenzano (University of Bari 'Aldo Moro', Italy), were sampled from a collection of over 100 different fig genotypes and used for subsequent analyses (Table 1 and Fig. 2).

2.2. Caprification trials

To investigate the biological behavior of the 24 fig genotypes, fruit-set of both brebas and main crop were evaluated. For this purpose, two trees of each variety and two caprifigs were selected in the fig repository. To investigate brebas production, nine shoots from each tree were chosen during the first half of April and tagged to

Table 1
Twenty-four fig varieties used in this study and their identification number.

ID line	Cultivar
1	Cervone rosso b[a]
2	Dottato Marchese w[a]
3	Dottato Sava w
4	Monaca b
5	Nero Crotone b
6	Regina Gioia b
7	Regina Triggiano b
8	Rosso Comune b
9	Rosso Triggiano b
10	Zingarello b
11	Alba Nera b
12	De Tres Vias w
13	Fasano 1 w
14	Fasano 2 w
15	Melanzana b
16	Nero Sava b
17	Nero Terlizzi b
18	Pasqua w
19	Petrelli w
20	Sant'Antonio w
21	Sant'Elias w
22	Troiano b
23	Indina w
24	Rosso Oria b

[a] b: black skin; w: white skin.

Fig. 2. Fig repository at the P. Martucci experimental station in Valenzano (Bari, Italy) in summer time.

evaluate the presence, number and development of brebas. In the third week of May, fruit-set of brebas was evaluated. For the main crop production, the fruit-set was calculated on six tagged shoots per tree that were left to open pollination.

To verify the caprification requirement of the 24 different varieties, nine fruiting shoots on two trees per variety were selected and figs were counted. Three treatments were tested in order to determine the effect of caprification and consequently the classification type of fig: (i) hand-caprification (bagged shoots with profichi), (ii) no caprification (bagged shoots) and (iii) control (unbagged shoots). Shoots of treatments (i) and (ii) were bagged with a net to prevent the entrance of wasps while preserving air exchange. Hand-caprification was performed in June (first to third week), placing four fruits (profichi) of the caprifig in each bag on June 10th, 15th and 20th. Profichi were taken from two ecotypes of caprifig at early and late ripening, respectively, at the time when the winged, gravid females of *Blasophaga psenes* L. leave the profichi. Bags were removed in the first week of July and figs were counted at the ripening stage of each variety.

2.3. DNA extraction, PCR and electrophoresis

Total genomic DNA was isolated from fresh buds of 24 fig varieties using the DNeasy Plant Mini Kit (Qiagen) following manufacturer's instructions, except for addition of 1% poly-vinylpyrrolidone (PVP 40,000) to the AP1 buffer. gDNA was measured with a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and standardized to 50 ng/μL before amplification.

PCR reactions were performed using BIOTAQ (Bioline) in a 20 μL volume containing 50 ng DNA, 2 μL 10× NH_4 reaction buffer, 0.85 μL 50 mM $MgCl_2$ solution, 0.2 μL 200 μM dNTP mix, 0.6 μL Fam- or Hex-labeled M13 tail, 0.15 μL 1 μM M13 tailed forward primer, 0.6 μL 1 μM reverse primer and 0.07 μL Taq DNA polymerase. PCR was carried out on a BioRad thermal cycler as follows: five min at 95 °C, 20 touchdown cycles of 30 s at 95 °C, 45 s at 60 °C (−0.5 °C each cycle) and 40 s at 72 °C followed by 25 cycles of 30 s at 95 °C, 30 s at 50 °C and 40 s at 72 °C, with a final hold of seven min at 72 °C. An aliquot of 1.6 μL PCR product was mixed with 14 μL formamide and 0.4 μL Rox-500 (Applied Biosystems), the internal molecular weight standard, and denatured at 95 °C for five min. PCR products were then visualized by capillary electrophoresis on a 3500 Genetic Analyzer (Applied Biosystems) and analyzed by Gene Mapper v.5.0 genotyping software as described in Gadaleta et al. (2007,2009).

2.4. Microsatellite analysis and genetic relationship

Forty-nine microsatellite markers were taken from the literature and used to estimate genetic similarity and distances among the 24 fig varieties. Accession number, primer sequences, annealing temperature, and expected allele size are reported on the web site on NCBI (www.ncbi.nlm.nih.gov) and in Table 2. The informativeness of a given DNA marker was measured by the polymorphism information content (PIC). According to Weber (1990) and Anderson et al. (1993) the PIC-value was calculated as follows:

Table 2
Forty-nine microsatellite markers tested in this study, with accession number and expected allelic size for each marker.

Locus	Accession	Expected allelic size (bp)
MFC1	AF333696	192
MFC2	AF333697	172
MFC3	AF333698	136
MFC4	AF333699	218
MCF5	AF333700	140
MCF6	AF333701	313
MCF7	AF333702	150
MCF8	AF333703	179
LMFC11	AY545925	159
LMFC12	AY545926	316
LMFC13	AY545927	289
LMFC14	AY545928	220
LMFC15	AY545929	205
LMFC17	AY545930	192
LMFC18	AY545931	124
LMFC19	AY545932	302
LMFC21	AY545934	143
LMFC22	AY545935	259
LMFC23	AY545936	286
LMFC24	AY545937	132
LMFC25	AY545938	272
LMFC26	AY545939	216
LMFC27	AY545940	232
LMFC28	AY545941	184
LMFC30	AY545942	193
LMFC31	AY545943	253
LMFC32	AY545944	241
LMFC35	AY545946	238
LMFC36	AY545947	252
LMFC37	AY545948	222
LMFC38	AY545949	215
LMFC40	AY545950	216–246
FinsN-1	AM039805	151–160
FinsU-9	AM039811	148–156
Frac110	DQ659282	166–172
FruB38	DQ659291	195–255
Frub61	DQ659291	145–188
Frub93	DQ659292	106–136
Frub391	DQ659294	149–173
Frub416	DQ659289	228–246
Frub415	DQ659297	150–173
Frub422	DQ659298	172–190
Frub436	DQ659299	135–159
FCUP008-2	EF198054	158–184
FCUP013-7	EF198055	196–212
FCUP027-4	EF198058	202–220
FCUP038-6	EF198059	180–193
FCUP044-6	EF198061	208–219
FCUP068-1	EF198066	178–206

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

where k is the total number of alleles detected for a microsatellite and P_i the frequency of the i^{th} i allele in the set of 24 fig genotypes investigated. The null allele was not included to compute PIC value.

A binary matrix containing the SSR scores for each fig accession was constructed and then transformed to a genetic similarity matrix using the Jaccard coefficient in pairwise comparisons. Cluster analysis and building of the dendrogram were performed by using NTSYS pc v.2.1 software, based upon the UPGMA (unweighted pair group method with arithmetical averages) algorithm.

3. Results

3.1. Fruit-set and caprification trials

Our results indicate that ~90% of the 24 varieties examined were biferous and only 10% were strictly uniferous (Table 3). Cervone Rosso and Regina Triggiano did not set brebas at all, thus producing only the main crop. Among the biferous varieties, large differences in fruit-set of brebas were observed, with some varieties setting over 60% brebas (Nero Terlizzi, Petrelli, Sant'Antonio, Rosso Comune, etc.), few with medium percentages and many others (Dottato Marchese, Fasano 1 and 2, Melanzana, Nero Sava, etc.) with poor fruit-set of brebas, generally below 10% (Table 3). Fruit-set of main crop was higher than that of brebas, with high variability among the varieties (ranging from 15 to 94%).

Many varieties showed a high fruit-set when open-pollinated: even for San Pedro type varieties (Petrelli, San Giovanni, etc.), the fruit-set values were not so low. However, the importance of caprification is clear: fruit-set was 76.4% when trees were caprificated and 37.4% when non-caprificated, respectively (Table 4). Several varieties had low fruit-set when not caprificated (Petrelli, Sant'Antonio, San Giovanni, Rosso Comune, etc.), while the others showed high fruit-set as expected from the Common-type figs (Dottato Marchese, Dottato Sava, Nero Crotone, Regina Gioia, etc.).

Table 3
Fruit-set and yield of the fig varieties left to open pollination.

Variety	Breba		Main crop	
	Fruit-set (%)	Yield	Fruit-set (%)	Yield
Alba Nera b[a]	30.0	Low	46.0	Medium
Cervone Rosso b	–	–	88.0	Very high
Des Tres Vias w[a]	35.0	Medium	70.0	High
Dottato Marchese w	5.0	Very low	92.0	Very high
Dottato Sava w	4.0	Very low	94.0	Very high
Fasano 1 w	3.0	Very low	56.0	High
Fasano 2 w	2.0	Very low	65.0	Medium
Indina w	2.0	Very low	89.0	Very high
Melanzana b	4.0	Very low	82.0	Very high
Monaca b	8.0	Very low	71.0	High
Nero Crotone b	5.0	Very low	85.0	Very high
Nero Sava b	5.0	Very low	65.0	High
Nero Terlizzi b	69.0	High	28.0	Low
Pasqua w	2.0	Very low	79.0	Very high
Petrelli w	77.0	High	25.0	Low
Regina Gioia b	30.0	Medium	74.0	High
Regina Triggiano b	–	–	68.0	High
Rosso Comune b	87.0	Very high	15.0	Low
Rosso Oria b	31.0	Medium	57.0	Medium
Rosso Triggiano b	58.0	Medium-high	87.0	Very high
Sant'Antonio w	75.0	High	26.0	Low
Sant'Elias w	72.0	High	24.0	Low
Troiano b	6.0	Very low	66.0	Medium
Zingarello b	32.0	Medium	82.0	Very high

[a] b: black skin; w: white skin.

Table 4
Fruit-set of the main crop of caprificated and non-caprificated fig varieties.

Variety	Main crop (fig)	
	Caprified (%)	Non-caprified (%)
Alba Nera b[a]	76.0	28.0
Cervone Rosso b	83.0	20.0
Des Tres Vias w[a]	73.0	21.0
Dottato Marchese w	89.0	73.0
Dottato Sava w	92.0	83.0
Fasano 1 w	63.0	23.0
Fasano 2 w	69.0	16.0
Indina w	90.0	25.0
Melanzana b	84.0	28.0
Monaca b	77.0	31.0
Nero Crotone b	84.0	59.0
Nero Sava b	62.0	13.0
Nero Terlizzi b	74.0	10.0
Pasqua w	80.0	19.0
Petrelli w	52.0	22.0
Regina Gioia b	87.0	76.0
Regina Triggiano b	86.0	65.0
Rosso Comune b	77.0	10.0
Rosso Oria b	67.0	46.0
Rosso Triggiano b	91.0	78.0
Sant'Antonio w	59.0	20.0
Sant'Elias w	55.0	24.0
Troiano b	78.0	28.0
Zingarello b	85.0	79.0

[a] b: black skin; w: white skin.

3.2. SSR analysis and genetic relationships

Forty-nine fig SSR markers were selected and tested on 24 fig varieties to assess genetic diversity. Thirty-nine SSRs out of 49 (79%) gave at least one allele and were taken into account; the others 10 markers tested produced no product (~21%). Among the 39 amplifying markers, almost all (~95%) gave two or more PCR products, of which 31 (79.5%) amplified multiple discrete PCR products. One hundred ninety-one PCR products were produced and used for phylogenetic analysis (Table 5). One hundred forty-two amplified bands were identified as polymorphic between the 24 fig varieties. The number of alleles per locus ranged from one to nine, while the maximum number of alleles amplified in a single genotype ranged from one to three, with a length of 132–327 bp.

Polymorphism was calculated as the number of primers that gave at least two alleles among the 24 genotypes analyzed, divided by the total number of primers amplifying PCR products. Out of 39 markers examined, 94.9% (31) were polymorphic, of which 20.5% (eight of 31) amplified one discriminating band and were scored as the presence or absence of the PCR product (dominant markers). The remaining 23 primer pairs revealed a co-dominant nature so that polymorphism among fig genotypes referred to the length of the amplified microsatellite bands. The SSR markers were almost uniformly distributed among genotypes; in fact, we were able to identify only five bands that were exclusive of a specific genotype.

The PIC value was calculated for each polymorphic marker and each band in the 24 fig varieties was considered an independent allele (Table 5). The lowest PIC observed was 0.07 for LMFC23 and LMFC27 SSRs and the highest was 0.91 for Frub422 SSR marker. We considered 18 SSR markers as informative (PIC >0.5; see Table 5).

Table 5
Microsatellite (SSR) markers used to assess genetic diversity within 24 fig varieties. For each marker are indicated range of expected product size, total number of PCR product (loci) obtained, number of polymorphic alleles per single locus and PIC value. PIC value has been calculated considering the total number of alleles obtained for each marker tested.

Marker name	Product size range (bp)	Total loci per marker (N)	Polymorphic alleles per locus (N)	Value of PIC
MFC1	191–208	4	4	0.40
MFC2	155–168	4	4	0.37
MFC3	138–151	6	4	0.49
MFC4	213–238	3	3	0.51
MCF5	143–156	5	2	0.16
MCF7	160–173	3	3	0.36
LMFC11	176–178	4	3	0.57
LMFC17	201–214	5	4	0.09
LMFC18	132–138	4	2	0.17
LMFC19	312–327	9	5	0.32
LMFC21	278–284	6	4	0.15
LMFC22	298–300	2	2	0.63
LMFC23	147–150	4	3	0.07
LMFC24	270–275	4	4	0.71
LMFC25	221–251	5	5	0.83
LMFC26	241–251	3	2	0.47
LMFC27	201–212	3	2	0.07
LMFC28	209–220	4	4	0.68
LMFC30	249–277	9	4	0.56
LMFC31	244–258	3	3	0.21
LMFC32	224	1	0	0.2
LMFC35	271–273	2	2	0.80
LMFC36	232–241	2	2	0.28
LMFC37	222–226	2	2	0.72
LMFC38	229–238	6	6	0.62
LMFC40	261–263	2	2	0.75
FinsN-1	144–198	9	9	0.87
FinsU-9	134–244	9	8	0.89
Frub38	220–230	2	2	0.17
Frub391	175–196	5	3	0.53
Frub416	171–174	8	4	0.27
Frub415	237–241	7	2	0.43
Frub422	145–206	9	5	0.91
Frub436	166–176	7	2	0.42
FCUP013-7	213	1	0	0.44
FCUP027-4	201–218	4	4	0.81
FCUP038-6	167–190	9	8	0.76
FCUP044-6	209–218	7	6	0.78
FCUP068-1	176–202	9	8	0.54
Total	132–327	191	142	

A total of 39 microsatellite markers giving 191 loci were used to estimate the genetic diversity among 24 different fig genotypes, and to build a dendrogram showing the genetic distances among them (Fig. 3). The cluster analysis identified two big cluster groups similarly to what determined from caprification trials. Caprification trial combined with SSR markers distinguished the Common and San Pedro type figs.

4. Discussion

Fig caprification is a complex system for fruit production and is a model system for studying the mutualism and coevolution between figs and pollinating wasps (Bandelj et al., 2007; Giraldo et al., 2008). Despite its importance, this mechanism remains unclear, and the classification of fig types on the basis of pollination and fruit production is still ambiguous. In general, all tested varieties responded positively to caprification to a variable degree as also observed elsewhere (Rahami and Jafari, 2008; Zare, 2008). In particular, varieties such as Rosso Comune, Nero Terlizzi, Nero Sava, etc. clearly needed profichi (wasps) to set figs and obtain good yields. Other varieties such as Dottato Marchese, Dottato Sava, Nero Crotone, Regina Gioia, Rosso Triggiano, etc., were not particularly influenced by caprification, showing satisfactory fruit-set and yield with or without caprification. These results indicated that fruit-set was significantly influenced by the presence/absence of profichi in the orchard (i.e., caprifigs), with varieties producing good yield only when caprificated and varieties setting figs parthenocarpically, thus not strictly needing profichi for the pollination. Fruit-set of brebas was highly variable, with some varieties setting insignificant numbers of fruits (Dottato Marchese, Dottato Sava, Monaca, etc.) and others showing potential for brebas production such as Petrelli, Sant'Antonio, Rosso Triggiano, etc.

Most varieties belonging to the San Pedro type were located in the upper branch of the phylogenetic tree, while Common type varieties were positioned in the lower branch, with some exceptions (Fig. 3). The distinction between the two types is not very clear, with some overlapping varieties, indicating some connections between the two types. Both the long-styled pistils and the suppression of the androecium in the edible fig are probably consequences of mutations in the original wild fig (Flaishman et al., 2008; Storey, 1976), with subsequent development of figs that were functionally female instead of hermaphroditic. Common type fig varieties (Brown Turkey, Mission, Dottato, etc.) do not need pollination to set fruits, but are considered persistent rather than parthenocarpic since the fig is not a true fruit but a false fruit called a syconium (Flaishman et al., 2008). Common fig produces one to two crops each year depending on several factors, with a generally small amount of brebas. The other two types of edible figs require pollination by wasps to set the main crop. Botanically, these non-persistent types are classified as caducous and grouped as Smyrna type (e.g. Sarilop, Marabout, etc.) or San Pedro type (e.g. King, San Pedro, Petrelli, etc.) (Flaishman et al., 2008). The San Pedro type is characterized by setting a significant brebas but requiring caprification to set the main crop. This is a unique combination in which persistent and caducous fruits develop on the same branch during the same season.

The effect of caprification is evident in fruit size, flesh thickness and fresh weight (Trad et al., 2013), with caprificated figs heavier and bigger than non-caprificated ones. Flesh thickness increases with caprification and this improves the consistency, taste and flavour of figs when ripe (Flaishman et al., 2008). In fig orchards, caprification should be accomplished two or three times because long-styled flowers become receptive gradually as in other fruit species. So, it is essential to have two or three ecotypes of caprifigs with a different flowering period (Zare, 2008). The ecotypes of caprifig have a significant effect on skin color, ostiole width, total soluble solids, time of ripening, seed germination, size and shape of figs (Rahemi and Jafari, 2008; Gaaliche et al., 2011).

Our results confirmed that when figs were non-caprificated (either manually or naturally), yield was dramatically reduced. Indeed, non-caprificated figs show an internal cavity, dry matter content reduction, a collapse of pedicel development and lack of seeds and the fruit is significantly firmer at harvest (Trad et al., 2013).

Physiologically, caprification acts indirectly as a precursor of several hormone activities. Ethylene, responsible for physiological and biochemical changes in fruit development and maturation, maybe enhanced by pollination. Structural components and secondary metabolites that play a commanding role in determining the final quality of figs are dependent on a good pollination (Trad et al., 2013), although it does not significantly affect aroma (Trad et al., 2012). Our data on caprificated and non-caprificated fruits (Table 4) are quite similar to the percentages reported by others (Rahami and Jafari, 2008; Zare, 2008). This demonstrated that caprification is necessary not only for

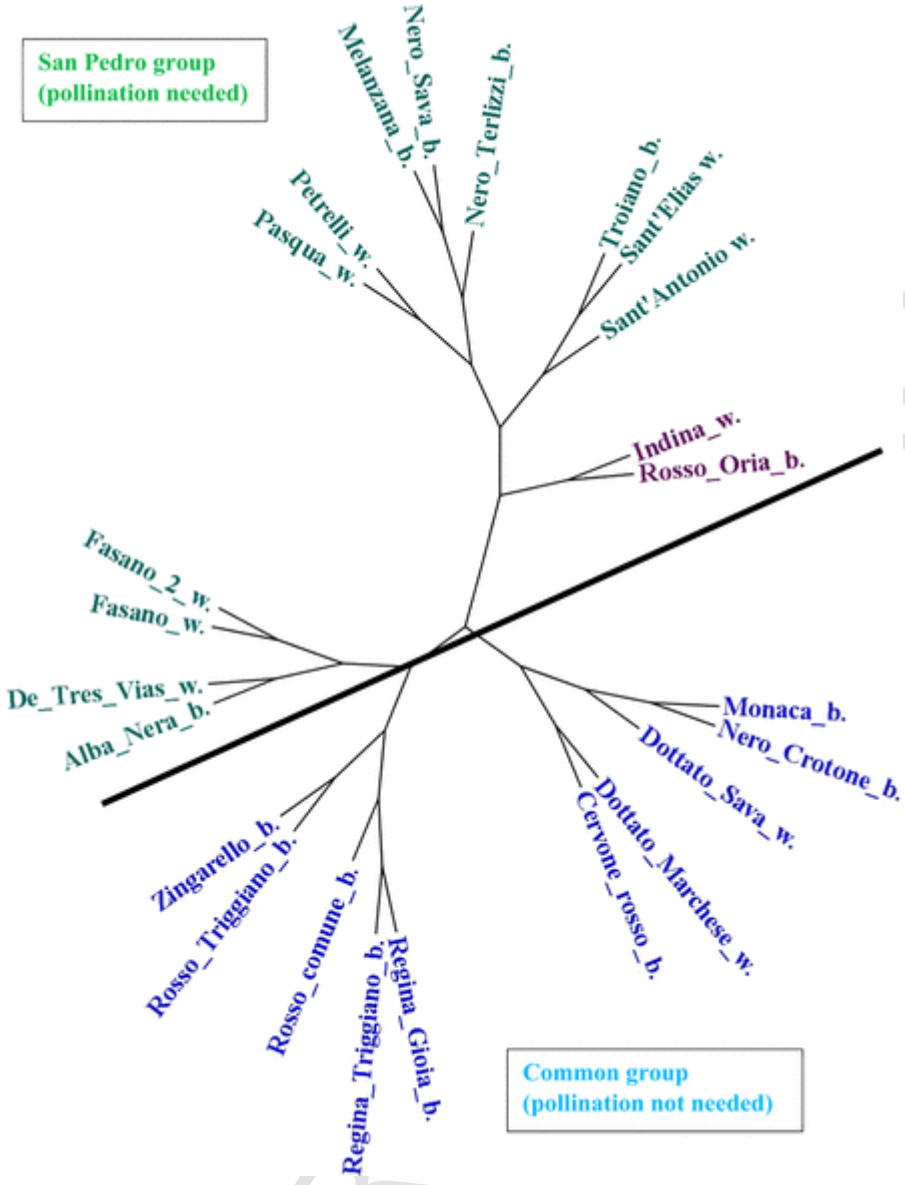

Fig. 3. Evolutionary relationships of taxa. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The upper and the lower part are the two groups determined by caprification trials (San Pedro and Common, respectively).

San Pedro and Smyrna type varieties, but also for Common type varieties. Caprification, whether required or not, can increase fruit-set and improve several organoleptic quality aspects (Ferrara, 1986; Trad et al., 2013). Even though caprification enhances fig production and yield, little information is available on the genetic basis of this complex and interesting system. Large-scale comparative studies of gene expression have provided comprehensive data on its effect on fruit physiology and elucidated genetic factors underlying the traits involved in type differentiation (Ikegami et al., 2013).

In this study, caprification data were coupled with molecular characterization of the same 24 varieties to investigate their genetic diversity and determine whether phylogenetic analysis would reflect the classification obtained via caprification tests. We chose SSR markers because of their abundance and availability in this species (Khadari et al., 2001b; Giraldo et al., 2005; Achtak et al., 2009,2010) and because they have already been used to characterize several fig germplasm collections. We analyzed 49 SSR markers from the literature, including those developed in two neotropical *Ficus* species (Nazareno et al., 2009). SSR markers are widely used to evaluate genetic diversity and characterize fig varieties (Khadariet al. 2005b; Saddoud et al., 2007; Giraldo et al., 2008; Achtak et al., 2009) and have a high intraspecific polymorphism.

The genetic diversity revealed by SSR loci was supported by the observed high PIC values, identifying 18 microsatellites as informative (PIC >0.5), more than those reported in previous studies (Perez-Jiménez et al., 2012; Ahmed et al., 2015). These values indicated allelic richness in the analysed germplasm that could be attributed also to different responses to caprification (Ahmed et al., 2015). In this work, reproduction mode and gene flow explained the majority of the genetic diversity among fig genotypes. Thus, caprification may affect the distribution, diversity and richness of alleles within and among different fig genotypes (Ahmed et al., 2015).

The phylogenetic tree reflected the classification determined in response to caprification. The two main types were Common and San Pedro varieties, with some exceptions in both types, probably caused by mutations in the wild fig, which then evolved into a successive distinction between types, with one having persistent fruits and the other, caducous fruits. In general, figs required pollination, and the appearance of trees that set a crop without pollination was a positive aspect selected by humans and easily propagated through cuttings. We think that figs should be divided into two types: the Common type that does not strictly require pollination (persistent type) and the San Pedro type strictly requiring pollination to set fruit (caducous type). The Smyrna type should be included in the latter category. To support this hypothesis significant difference in nitrate concentration has been detected between persistent and caduceus varieties (Crane, 1986), with an average nitrate concentration in persistent figs three times higher than in caducous figs.

5. Conclusion

The comprehension of the genetic basis of the entire metabolic pathways involved in caprification will be of great importance in order to develop cultivars with higher yield and with longer product shelf-life. The results here reported suggest the importance of considering two approaches, morphological and molecular, to study genetic diversity in fig trees. Molecular analyses in conjunction with morphological and horticultural evaluations are recommended because these provide complementary information, increase the resolving power of genetic diversity analyses, and elucidate the domestication process. Results indicated a possible distinction of the fig types in two groups instead of three. This has important implications for fig management and requires strategies to maintain the longevity and genetic diversity of the species. Knowledge of the genetic diversity present in this germplasm will facilitate its use in breeding programs, improve its management and guarantee this species sustainability in the face of climate change. However, further research is needed to better explain the differences (or similarities?) between brebas and the main crop.

Conflict of interest

The authors declare that they have no conflict of interest.

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