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RESEARCH ARTICLE

Identification and characterization of Italian common figs (*Ficus carica*) using nuclear microsatellite markers

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Abstract Common fig (*Ficus carica* L.) is one of the most ancient domesticated species, originated, supposedly in Arabia, from where it diffused to the Middle East and Asia, and to the Mediterranean basin, where it greatly diffused. More than 600 fig varieties have been described, but it is conceivable that this number is underestimated. Along all the Italian territory, there is a rich germplasm of fig composed of a large number of varieties (approximately 300) of very not well defined origin. Effectively, during several centuries of cultivation and propagation by seed, a large number of genotypes appeared and were selected, leading to the generation of an uncountable number of genotypes, different in numerous traits, particularly in those related to leaves and fruits features. Unfortunately, the extensive existing fig genetic patrimony is facing genetic erosion; for this reason, it is extremely important to study and valorised it, in order to preserve the remaining biodiversity. The purpose of this study was to genetically characterize, with nSSR markers, 79 fig accessions, collected in several areas in Italy. The set of chosen markers resulted highly polymorphic, and allowed the characterization of all the studied accessions. Data were analysed by cluster analysis, and the results demonstrated a great genetic variability within the population. The nSSR used, moreover, allowed us to identify all accessions and to recognised possible homonyms and synonyms, and cases of intravarietal clones.

Keywords nSSR · *Ficus carica* L. · Biodiversity · Germplasm · Cultivar identification · Genetic variability

Introduction

Common fig (*Ficus carica* L.), with olive tree, grape and palm, is one of the most ancient domesticated species (Mawa et al. 2013). Common fig has originated, supposedly, in Arabia (Storey 1976), from where it diffused to the Middle East and Asia, and to the Mediterranean basin, where it greatly diffused. Therefore, many authors consider the East Mediterranean region the area of the common fig domestication (Ercisli et al. 2012), and from there the cultivation spread to the West Mediterranean area, where fig

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populations were already present in natural habitats before domestication (Veberic and Mikulic-Petkovsek 2016). The process of domestication resulted in sweeter and bigger fruits (Falistocco 2009). Approximately, worldwide, more than 600 fig varieties have been described (Condit 1955), but it is conceivable that this number is underestimated. Unfortunately, changes in alimentary habits, the lack of intensive system of fig cultivation and the short fig shelf life, caused the downfall of fig cultivation. In the Mediterranean basin, fig biodiversity has been preserved, because its cultivation is merely based on the use of autochthonous genotypes. In Italy, fig germplasm consists of a large number of varieties (approximately 300) of not well defined origin (Grassi 1998; Barberis et al. 2001; Chessa et al. 2001; Minonne et al. 2001), diffused, primarily, in four regions of southern Italy (Campania, Calabria, Puglia and Sicily), and in Tuscany. Particularly, archeobotanical researches, combined with historical sources, date the presence of the fig tree from the Neolithic, through the Roman Empire to the Middle Ages (Turfa 2012; Mariotti Lippi et al. 2009; Rattighieri et al. 2013; Buonincontri et al. 2014; Mariotti Lippi et al. 2015) and further on; as a matter of fact, historical references, found in XIII–XVI century documents, report the names of some varieties of fig, Dottato, Verdino, Brogiotto Nero and Brogiotto Bianco, cultivated in Tuscany (Baldini 1953). Even though, in Italy, fig cultivation has a long tradition and its germplasm is still wide, with more than 90 genotypes (Baldini 1953; Basso 1960a, b), information on their genetic identity are scarce and dubious, making their cataloguing very difficult. Therefore, the establishment strategies for the preservation of the local fig germplasm is a necessity. For this reason, for example, in Tuscany, "Slow food", a global organization to prevent the disappearance of local food cultures and traditions, individuated the "dry fig of Carmignano" as a typical regional production to be valorized and preserved. The scientific community, instead, is committed to characterize the existing fig germplasm. To assess the diversity among fig cultivars, different methods have been utilized, from morphological to biochemical and genetic characterizations. Morphological descriptors were used to evaluate and characterize fig cultivars in the Mediterranean area (Papadopoulou et al. 2002; Gaaliche et al. 2012; Çalişkan and Polat 2012; Giraldo et al. 2010) to determine diversity in different accessions of *F. carica* L.

Biochemical markers, such as isozymes (Cabrita et al. 2001), and DNA markers, such as mitochondrial DNA-RFLP (Khadari et al. 2005), AFLP (Laddomada et al. 2008; Aradhya et al. 2010), and RAPD (Khadari et al. 1995; Chessa et al. 2001; De Masi et al. 2003; Ciarmiello et al. 2015) have been used to characterize fig varieties, revealing high polymorphism among fig accessions from different Mediterranean areas. Genomic microsatellite markers have been developed for common fig tree in recent years (Khadari et al. 2003; Giraldo et al. 2005, 2008; Achtak et al. 2009; Ferrara et al. 2016). Khadari et al. (2001), identified 8 SSR primers on different varieties of *F. carica* L. and other plants of the genus Ficus, showing the efficiency of the primers on fig varieties, but a partial transferability on other plants of the genus Ficus. The same primers were then utilized on 30 fig varieties from France and Morocco (Khadari et al. 2003), and 5 of the 8 primers were selected for the characterization of the entire population, considering their highest discrimination power. Giraldo et al. (2005), identified 26 new SSR primers and tested them on 15 fig cultivars from France and Spain; the primers were able to discriminate 9 different genotypes in the 15 accessions. Achtak et al. (2009) individuated, trough selection and validation, 6 SSR primers characterized by high discriminant capacity, useful for the identification of all the genotypes present in a Morocco's fig collection. In a study of Abou-Ellail et al. (2014), seven fig cultivars were characterized using biochemical and microsatellite markers; the firsts showed different relation among the cultivars, but they were considered not very efficient, because their low polymorphism and because, in some cases, they were influenced by environmental factors; instead, the use of SSR allowed the discrimination of all the 7 cultivars, proving a higher efficiency. Recently, the analysis of simple sequence repeats (SSR or microsatellites) showed good efficiency in the identification and characterization of fig accessions of different origins (Baraket et al. 2011; Khadari 2012; Essid et al. 2015). Knap et al. (2016), developed 16 new SSR markers and evaluated their transferability on other species, with successful results.

The objective of the present work was to describe the genetic variability of common fig germplasm still present in Italy by identifying any genetic similarities,

synonyms and homonyms, with the aid of a set of nuclear SSR (nSSR) markers. The study of genetic variability within the germplasm is the best condition for a correct preservation of genetic resources, and for the identification and valorisation of the most suitable genotypes, in view of a recovery of the fig industry, not only in Italy, but all over the world too.

Materials and methods

Plant material

The plant material used in this study was collected in ex situ fig germplasm private collection, located in Pescia (Pistoia, Tuscany, IT) (43°53′13″N, 10°41′18″E, 42 m a.s.l.). The plant collection, propagated by cuttings, is composed by 79 parthenocarpic fig accessions (Table 1). For each accession, leaves from healthy and actively growing shoots, were collected in August, and randomly sampled from different parts of each tree. Samples were placed in airtight plastic bags, immersed in liquid nitrogen, and frozen at − 80 °C, prior to subsequent analysis.

DNA extraction and molecular characterization

Genomic DNA was extracted following the CTAB (Cetyl Trimethylammonium Bromide) procedure (Doyle and Doyle 1987). After the spectrophotometer quantification, sample were analysed with nSSR markers.

For DNA amplification, 7 couples of nSSR dinucleotide primers (MFC and LMFC series), set already for fig characterization for their high level of polymorphism (Khadari et al. 2001; Giraldo et al. 2005), were used (Table 2).

PCR amplification was performed in a final volume of 20 µL containing: 1 × Reaction Buffer (International PBI, Milano, Italy), 0.2 mM dNTPs (Amersham Biosciences, Piscataway, USA), 2 mM $MgCl_2$ (International PBI, Milano, Italy), 0.2 µM primer (MWG Biotech, Ebersberg), 20 ng genomic DNA and 1 U di Taq polymerase (Fisher Molecular Biology, Trevose, USA).

PCR amplification reaction was optimized in thermal cycler MJ PCT 100 Research (Watertown, Mass.), programming a first passage at 94 °C for 1 min followed by 35 cycles of 30 s at 94 °C, 30 s at the specific annealing temperature for each couple of primers (Table 2), and 1 min at 72 °C, for denaturation, annealing and primer extension, respectively; at the end of the cycles were allowed 8 min of incubation at 72 °C.

One of the two PCR primers in each reaction was end-labelled with a fluorescent dye (CY5, MWG Biotech, Ebersberg, Germany). The amplification products were separated with a CEQ 2000 Genetic Analysis System (Beckman Coulter, Inc.) sequencer on acrylamide gel CEQ Separation Gel LPA-1 (Beckman Coulter, Inc.). A marker CEQ DNA Size Standard kit 400 (Beckman Coulter, Inc.) was used to estimate the approximate molecular weight of the amplified products. Two reference samples were used in all runs.

Statistical analysis

Fragments were sized using a conservative binning approach (Kirby 1990), through the statistical R software (R Development Core Team 2005), which takes into account the type of replicate and compensates for the limits of fragment resolution. Genotypes showing a single allele in a given locus were indicated as homozygote.

All analyses were developed after removing duplicates, previously identified by using pair-wise comparisons among all genotypes, based on their multilocus nSSR profile, using an Excel spreadsheet (© Microsoft Corporation).

Data were processed using the software Identity 1.0 (Wagner and Sefc 1999), available at the web site http://www.boku.ac.at/zag/forsch/identity.htm. Identity 1.0 was used for the calculation of the number of alleles per locus (*N*), allele frequency, observed (*Ho*) and expected (*He*) heterozygosity, frequency of null alleles $r = (He - Ho)/(1 + He)$ (Brookfield 1996), probability of genetic identity (*PI*) (Paetkau et al. 1995).

The number of effective alleles ($Ne = 1/(1 - He)$, Brown and Weir 1983) was calculated using Microsoft Excel spreadsheet (© Microsoft Corporation).

The level of similarity/dissimilarity among examined accessions was obtained through the genetic similarity matrix utilizing Manhattan distance. Cluster analysis and construction of the dendrogram relative to genetic distances were obtained by using the unweighted pair-group method with arithmetic mean

Table 1 Fig cultivars included in this study, and their origin, number of leaf lobes (NLL), external fruit colour (EFC), internal fruit colour (IFC) and fruit shape (FS)

Cultivar	Origin	NLL*	EFC*	IFC*	FS*	Cultivar	Origin	NLL*	EFC*	IFC*	FS*
"Albo"	Prato	3	Green yellow	Pink	Spherical	"Fiorone bianco" 2	Prato	3	Green	Pink	Pyriform
"Bianco"	Pisa	3	Green	Brown yellow	Pyriform	"Fiorone della Garfagnana"	Lucca	5	Green	Pink	Turbinate
"Bianco di Carmignano"	Prato	3	Green	Pink	Spherical	"Gentile bianco"	Lucca	5	Green	Brown yellow	Spherical
"Bianco gigante"	Prato	3	Green	Pink	Pyriform	"Luzzano"	Potenza	5	Yellow	Light brown	Pyriform
"Bianco della Tunisia"	Tunisia	5	Green	Light red	Pyriform	"Martigiano"	Prato	5	Green	Red	Pyriform
"Bianchetto"	Pistoia	5	Green yellow	Pink	Pyriform	"Melanzana"	Cosenza	5	Black	Dark red	Pyriform
"Bottaccio"	Pistoia	5	Green	Red	Spherical	"Montalcino rosa"	Siena	5	Green	Light red	Pyriform
"Buti"	Pisa	3	Green	Dark pink	Pyriform	"Montecarlo"	Pistoia	5	Green	Dark red	Spherical
"Brogiotto bianco" 1	Lucca	5	Green	Red	Spherical	Montesano"	Salerno	5	Purple	Light red	Spherical
"Brogiotto bianco" 2	Florence	5	Green	Red	Spherical	"Monaco"	Prato	3	Green	Red	Pyriform
"Brogiotto bianco" 3	Pistoia	5	Green	Red	Spherical	"Natalegna"	Naples	3	Green	Pink	Spherical
"Brogiotto nero" 1	Pistoia	5	Purple	Red	Spherical	"Nero gigante"	Prato	5	Brown	Dark pink	Turbinate
"Brogiotto vero"	Pistoia	5	Purple	Red	Spherical	"Paradiso" 1	Pisa	5	Green yellow	Red	Turbinate
"Brogiotto nero" 2	Pistoia	5	Purple	Red	Spherical	"Paradiso" 2	Pisa	5	Green yellow	Red	Turbinate
"Brogiotto nero" 3	Prato	5	Purple	Red	Spherical	"Pecciolo nero" 1	Pistoia	5	Black	Red	Pyriform
"Brogiotto"	Cosenza	5	Purple	Red	Spherical	"Pecciolo nero"2	Prato	5	Black	Red	Pyriform
"Brogiotto nero" 4	Pisa	5	Purple	Red	Spherical	"Perticone"	Prato	5	Dark green	Pink	Pyriform
"Castagnolo"	Lucca	5	Brown	Light red	Spherical	"Petrelli"	Bari	3	Green	Red	Pyriform
"Cavaliere"	Lucca	3	Purple	Red	Pyriform	"Portogallo"	Pistoia	5	Brown	Brown	
"Cavallierino"	Pistoia	5	Purple	Red	Spherical	"Rocco nero"	Pistoia	5	Black	Dark pink	Spherical
"Cerreto"	Ascoli Piceno	3	Purple-green	Light red	Turbinate	"Rosso Trani"	Trani	5	Purple	Dark pink	Pyriform
"Columbro nero"	Cosenza	5	Purple	Pink	Spherical	"Salato"	Pistoia	5	Black	Red	Ovoidal
"Corvo"	Pistoia	5	Black	Dark red	Pyriform	"Seccareccio"	Lucca	5	Green	Pink	Pyriform
"Datterino"	Pisa	5	Brown	Light red	Ovoidal	"Sementino"	Pistoia	5	Black	Red	Spherical
"Dattero"	Pistoia	5	Green-brown	Dark red	Turbinate	"San Piero"1	Prato	3	Purple	Red	Turbinate

Table 1 continued

Cultivar	Origin	NLL*	EFC*	IFC*	FS*	Cultivar	Origin	NLL*	EFC*	IFC*	FS*
"Datto"	Prato	5	Green	Red	Pyriform	"San Piero" 2	Naples	3	Purple	Red	Turbinate
"Di tre volte"	Florence	3	Green yellow	Red	Spherical	"San Piero" 3	Grosseto	3	Purple	Red	Turbinate
"D'oro"	Lucca	5	Green	Pink	Pyriform	"Siro"	Prato	3	Green	Dark red	Spherical
"D"oro di Capezzana"	Prato	5	Green	Pink	Pyriform	"Sanguinella"	Pistoia	5	Purple	Dark red	Pyriform
"Dottato" 3	Pistoia	3	Green	Pink	Pyriform	"Trapani"	Trapani	3	Green	Pink	Pyriform
"Dottato" 4	Pisa	3	Green	Pink	Pyriform	"Troiano"	Benevento	3	Green	Red	Pyriform
"Dottato" 5	Pisa	3	Green	Pink	Pyriform	"Verdino"	Pistoia	5	Green	Pink	Pyriform
"Dottato" 6	Pisa	3	Green	Pink	Pyriform	"Verdino bianco"	Pistoia	5	Green	Pink	Pyriform
"Dottato" 7	Spain	3	Green	Pink	Pyriform	"Viola della Turchia"	Turkey	5	Purple	Red	Pyriform
"Dottato" 1	Prato	3	Green	Pink	Pyriform	"Zucchetto"	Pistoia	3	Green	Red	Pyriform
"Dottato" 2	Prato	3	Green	Pink	Pyriform	"Pippo"	Prato	5	Green	Red	Pyriform
"Dottato" 8	Arezzo	3	Green	Pink	Pyriform	"Buti Verdino"	Pisa	5	Green	Pink	Pyriform
"Faraone"	Pistoia	5	Brown	Pink	Cucurbiform	"Unknown" 2	Syracuse	5	Green	Light red	Pyriform
"Fico della signora"	Pistoia	3	Green	Light brown	Pyriform	"Fiorone bianco"3	Prato	3	Green	Pink	Pyriform
"Fiorone Bianco" 1	Lucca	3	Green Yellow	Pink	Pyriform						

*UPOV 2010

Table 2 List of nSSR loci, primer sequence, fragment size and annealing temperature (T) of each primer used (*Khadari et al. 2001; **Giraldo et al. 2005)

Primer	For 5′ → 3′	Rev 5′ → 3′	Size	T (°C) (annealing)
*MFC2	GCTTCCGATGCTGCTCTTA	TCGGAGACTTTTGTTCAAT	172	55
*MFC3	GATATTTTCATGTTTAGTTTG	GAGGATAGACCAACAACAAC	136	55
*MFC4	CCAAACTTTTAGATACAACTT	TTTCTCAACATATTAACAGG	218	55
**LMFC12	TTAAACCCTACTTTCAACAAT	GTAATCCCCCGAGATATAGT	376	55
**LMFC24	ACTTCTTCATATTTGGTATAGG	TTCATAAACTGGTCTAAAAGA	272	55
**LMFC30	TTGTCCGTTTCTTATACAAT	TCTTTTTAGGCAGATGTTAG	253	55
**LMFC31	GTAAAATGAAAATTGGAGTATT	TTGAAGATATTGTTGTATGCT	241	55

(UPGMA) algorithm, with XLSTAT 2009 software (Addinsoft™ 1995–2009).

Results

SSR markers polymorphism

The molecular analysis on 79 accessions was performed using seven SSR markers, belong to the series MFC and LMFC. For a better comprehension of markers characteristics, the number of amplified alleles per locus, frequency, heterozygosity, discrimination power and null allele frequency were observed (Table 3). From the 79 analysed accessions, SSR markers identified 43 alleles (Table 3). Allelic frequencies (f) varied from 0.006 and 0.651; the average expected heterozygosity (He) and observed heterozygosity (Ho) value were 0.664 and 0.670 respectively. The highest expected heterozygosity was reach by locus LMFC30 with 0.810, instead the higher observed heterozygosity was reach by locus LMFC4 with 0.798. The less observed and expected heterozygosity reached the values respectively of 0.456 and 0.520, both for the locus LMFC24.

The number of alleles (N_o) amplified, ranged from 3 alleles for the primer MFC4, to 9 alleles for LMFC30. The most frequent alleles in the examined population were alleles 278, for the locus LMFC24, with a frequency of 0.652 and 353 for the locus LMFC12, with a frequency of 0.513; the alleles less frequent were alleles 270 for the locus LMFC24 and allele 244 for LMFC30, with a frequency of 0.006.

Microsatellite LMFC30 showed the highest PIC (= 0.778) values (Table 3), reflecting the large number of alleles observed and similar allele frequency distribution in the population when compared to the other microsatellites (Table 3). The lowest PIC was found in microsatellite LMFC24 (= 0.466).

Identification of the examined population and internal relationship

The utilization of the seven SSR markers, on 79 accessions allowed the discrimination and identification of 56 genotypes, of which 12 (named "Group") consisted of a number of accessions from 2 to 6 (Fig. 1). Genetic profiles of fig trees are reported in Supplementary Data (Table S1). The relationship among all accessions was analysed and observed by cluster analysis (UPGMA) at Euclidean distances, by which dissimilarities between the studied figs are highlighted (Fig. 1). The dendrogram (Fig. 1) includes all the accessions and shows 56 different genotypes and differences can be observed between the accession with a dissimilarity index between 0 and 40,83. The examined population is divided in two main clusters (I and II); the firs cluster includes 1/3 of the identified genotypes, and contains two subgroups, I.1 and I.2. Subgroup I.1 is divided in two sets (I.1.A and I.1.B): set I.1.A is composed by five dark figs ("Pecciolo nero 1", "Datterino", "Sanguinella", "Datto", "Viola della Turchia"), and three light figs ("Perticone", "Faraone", "Cavallierino"); set.I.1.B, is mainly composed by dark figs ("Rocco nero", "Dattero", "Pecciolo nero 2", "Cavaliere", "Brogiotto nero 2") and "Brogiotto nero 3"). In subgroup I.2, only the genotype "Corvo" is present.

Cluster II is divided in two subgroups, in which the majority of the studied accessions are included.

Table 3 Allele sizes (base pairs) and allele frequency (f), observed (N_o) and effective (N_e) number of alleles, observed (H_o) and expected heterozygosity (H_e), probability of null alleles (r), Polymorphism Information Content (PIC) at seven nSSR loci in the total fig genotypes

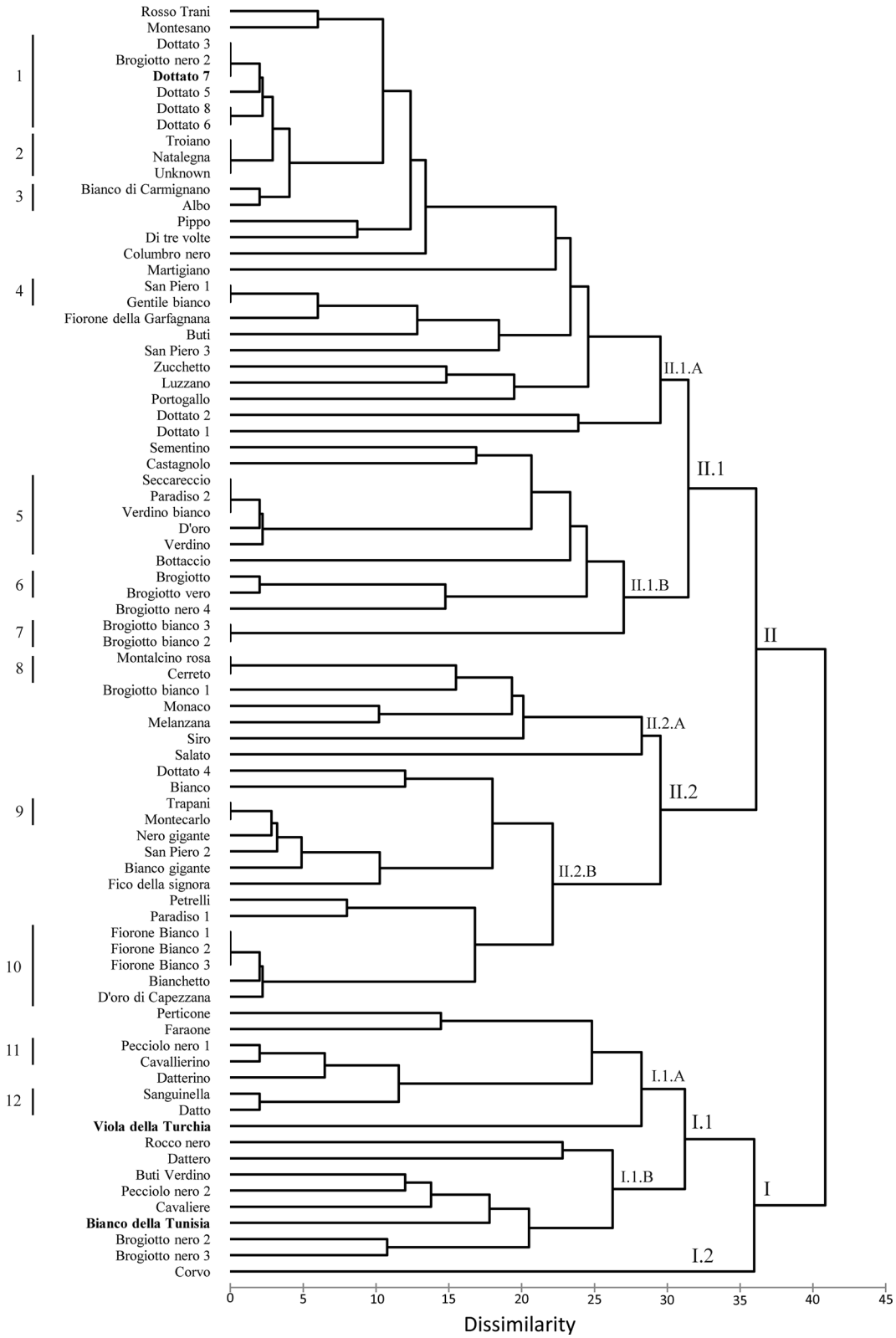
	Locus		Locus		Locus		Locus		Locus		Locus		Locus	
	MFC2	f	MFC3	f	MFC4	f	LMFC12	f	LMFC24	f	LMFC30	f	LMFC31	f
a	159	0.335	124	0.032	202	0.329	353	0.513	270	0.006	236	0.038	229	0.095
b	161	0.089	126	0.184	222	0.392	355	0.013	276	0.222	244	0.006	231	0.386
c	167	0.038	128	0.076	226	0.279	372	0.095	278	0.652	246	0.247	243	0.076
d	169	0.032	130	0.190			375	0.006	280	0.095	248	0.013	245	0.443
e	171	0.013	132	0.063			381	0.367	284	0.006	252	0.051		
f	173	0.481	136	0.019			401	0.006	295	0.019	258	0.291		
g	179	0.013	138	0.392							260	0.146		
h			144	0.044							262	0.139		
i											266	0.070		
No	7		8		3		6		6		9		4	
Ne	2.854		4.390		2.978		2.481		2.082		5.258		2.809	
H_O		0.747		0.688		0.798		0.544		0.456		0.696		0.683
H_E		0.650		0.772		0.664		0.597		0.520		0.810		0.644
r		− 0.082		− 0.014		− 0.092		0.033		0.082		0.078		− 0.032
PIC		0.586		0.733		0.586		0.515		0.466		0.778		0.571

Subgroup II.1 is composed by the sets II.1.A and II.1.B. Set II.1.A is split in two sub sets; in one sub set, there are two accessions of the genotype "Dottato" (2 and 1), that are positioned near each other (Table S1). In the other sub set (above), it is possible to observe the similarity and genetic nearness between "Dottato" accessions (3, 5, 6, 7, 8) (Group 1) (Fig. 1 and Table S1). Other interesting observations are synonymies, that are accessions named differently, but sharing the same alleles amplicons (Table S1), for example, "Troiano", "Natalegna" and unknown (Group 2), and the accessions "San Pietro 1" and "Gentile Bianco" (Group 4).

In set II.1.B, it is possible to observe similarities between two accessions named "Brogiotto bianco" (2 and 3), that have different geographic origins, and low genetic differences between "Brogiotto", and "Brogiotto vero", considered the same genotypes (Group 6). Moreover, three genotypes with different names ("Seccareccio", "Paradiso 2", "Verdino bianco") share the same microsatellite amplicon (Table S1) and have genetic similarities with "Doro" and "Verdino" (Group 5). Other synonymies are observed in the accessions "Montalcino rosa" and "Cerreto" and "Trapani" and "Montecarlo" (Group 8 and 9) (Fig. 1 and Table S1).

Discussions

The seven primer pairs used in this study (Table 2) were selected from a set of 8 microsatellites identified and developed by Khadari et al. (2001) and from a set of 26 primers developed by Giraldo et al. (2005). For the selection of primers, the number of alleles amplified by the markers, the polymorphism and the results of amplification in other studies were considered (Saddoud et al. 2007; Giraldo and López-Corrales 2008; Aradhya et al. 2010; Saddoud et al. 2011; Abou-Ellail et al. 2014; Ferrara et al. 2016). Molecular analysis enables the identification of the genetic distances in the Tuscany fig population, allowing the observation of a rich biodiversity. SSR markers, together with other molecular methods, were previously used in the study of fig biodiversity in France (Khadari et al. 2001; Khadari 2012), Spain (Giraldo et al. 2005), Tunisia (Saddoud et al. 2007), in Brazil


Rosso Trani
Montesano
Dottato 3
Brogiotto nero 2
Dottato 7
Dottato 5
Dottato 8
Dottato 6
Troiano
Natalegna
Unknown
Bianco di Carmignano
Albo
Pippo
Di tre volte
Columbro nero
Martigiano
San Piero 1
Gentile bianco
Fiorone della Garfagnana
Buti
San Piero 3
Zucchetto
Luzzano
Portogallo
Dottato 2
Dottato 1
Sementino
Castagnolo
Seccareccio
Paradiso 2
Verdino bianco
D'oro
Verdino
Bottaccio
Brogiotto
Brogiotto vero
Brogiotto nero 4
Brogiotto bianco 3
Brogiotto bianco 2
Montalcino rosa
Cerreto
Brogiotto bianco 1
Monaco
Melanzana
Siro
Salato
Dottato 4
Bianco
Trapani
Montecarlo
Nero gigante
San Piero 2
Bianco gigante
Fico della signora
Petrelli
Paradiso 1
Fiorone Bianco 1
Fiorone Bianco 2
Fiorone Bianco 3
Bianchetto
D'oro di Capezzana
Perticone
Faraone
Pecciolo nero 1
Cavallierino
Datterino
Sanguinella
Datto
Viola della Turchia
Rocco nero
Dattero
Buti Verdino
Pecciolo nero 2
Cavaliere
Bianco della Tunisia
Brogiotto nero 2
Brogiotto nero 3
Corvo
1
2
3
4
5
6
7
8
9
10
11
12
II.1.A
II.1
II.1.B
II
II.2.A
II.2
II.2.B
I.1.A
I.1
I.1.B
I
I.2
0
5
10
15
20
25
30
35
40
45
Dissimilarity

◀**Fig. 1** UPGMA dendrogram based on Euclidean distance of accessions of *Ficus carica* L. The bars marked with numbers (1–12) indicate the genotype groups with high degree of similarity index

(do Val et al. 2013) and in different Mediterranean countries (Khadari 2012).

Giraldo et al. (2005) analysed 15 fig cultivars from different geographical areas, using 26 SSR markers, and obtained low value of observed heterozygosity (0.47); the data was endorsed by the easiness in agamic propagation of fig, by the narrow genetic base and by the small genetic variability in the studied population. However, results reported in this study on Italian fig biodiversity show a good level of polymorphism and genetic variability, with a higher average heterozygosity (0.67) (Table 3). The rescored value is, instead, in accord with a prior study of 72 Tunisian fig genotypes (Saddoud et al. 2007), where the observed heterozygosity reached the value of 0.7. The lower value of observed heterozygosity in the population studied in this research, compared to expected heterozygosity in four loci, is in accord with the study of Saddoud et al. (2007, 2011). In the set of primers used in this study, primers LMFC30 and MFC3 showed the best results in terms of polymorphism, amplifying 9 and 8 alleles respectively. These data are in accord with prior studies of Achtak et al. (2009) and Aradhya et al. (2010), in which the amplification of the same number of alleles for the considered primers is shown. As concern observed heterozygosity (Ho), the highest level was reached by the locus MFC4 with 0.798, instead the major expected heterozygosity (He) was found in locus LMFC30, with a value of 0.810 (data in partial accord with the study of Giraldo et al. 2005). Prior study (Khadari et al. 2003) showed low value of heterozygosity for the locus MFC4 (0.375 in 14 accessions analysed from Conservatoire Botanique National Méditerranéen de Porquerolles collection, and 0.560 in 16 Moroccan genotypes analysed); instead, locus MFC4, showed high level of heterozygosity in the study of Aradhya et al. (2010) on 194 fig genotypes, reaching the value of 0.818; the higher value registered in this study, was probably due to the wide number of fig samples analysed and to the worldwide origin of the accessions.

In this study, PIC value was higher than 0.5 for all loci analysed (data in accord with Ferrara et al. 2016), except for the locus LMFC24, that turned to be the less informative; instead, the locus LMFC30, with 0.7783 achieved the highest value. From the allelic analysis it is possible to observe the presence of unique alleles ($f = 0.0063$), thus typical of single genotypes, like allele 270 and 284 (locus LMFC24), specific for the genotype "Paradiso 1" and "Dottato 2" respectively; allele 401 (locus LMFC12) specific for "Corvo"; allele 244 (LMFC30), for "Siro" genotype; the presence of unique allele is not shown for the locus of the series MFC. The existence in the considered population of unique alleles could be a starting point for the univocal discrimination of genotypes (Jakše et al. 2004), originated in a restricted area of Italy.

Dendrogram observations, based on the differences in their allelic profile, allowed the separation of the analysed accessions in different groups (Fig. 1). Genetic structure does not show particular grouping, probably due to narrow genetic base from which the fig were domesticated. From the analysis of the population, it was not possible to discriminate between common fig (partenocarpic) and "San Pedro" fig type, as was already partially observed by Ferrara et al. (2016). Instead, our results are in accord with other studies (Saddoud et al. 2007; Baraket et al. 2011) in which correlation was not highlighted. Nevertheless, it is possible to observe in the I cluster, the presence almost exclusive of dark figs, thus showing not only phenotypic but also genetic similarity, even if some other dark varieties are distributed along the dendrogram.

The observation of the allelic profile and the dendrogram, allowed the identification of cases of homonymy, synonymy and possible denomination mistakes that caused varietal confusion (Giraldo and López-Corrales 2008). Of the four accessions named "Brogiotto nero", only two have relevant similarities ("Brogiotto nero" 2 and 3) (cluster I dendrogram), instead, the other two accessions possess different allelic profiles and are clustered in genetically distant groups; for this reason, this can be considered wrong denomination. The same result is shown for the two "Paradiso" accessions analysed: "Paradiso 2" showed the same allelic profile of the accession "Seccareccio" (case of synonymy) (Table S1), instead, "Paradiso 1" is situated in a different cluster in the dendrogram, showing important genetic differences if compared with "Paradiso 2" (Table S1). Another particular case concerns the 7 analysed accessions of "Dottato"

cultivar (Fig. 1), coming from different cultivated areas: five of them showed genetic proximity (II.1.A in dendrogram, Group 1), instead, the other two accessions ("Dottato" 1 and 2), are positioned in another subgroup, showing an additional case of wrong nomination. Case of synonymy is shown for the cultivars "San Pietro 1" and "Gentile bianco" (Group 4), and homonymy is shown between San Piero 1 and "San Piero 2" (Fig. 1 and Table S1). The unknown cultivar was probably recognized: it possess the same allelic profile of the two synonymy "Natalegna" and "Troiano" (Group 2). The reason of the varietal confusion and the presence of homonymies and synonymies is probably due to the ancient origin of fig cultivars and migrations, so that, during century figs cultivar were renamed differently (Aradhya et al. 2010); nevertheless, wrong naming by growers, mistake during propagation or errors in labelling, are other possible hypothesis. Denomination errors and varietal confusion were yet observed in fig population by Aradhya et al. (2010) and Giraldo and López-Corrales (2008), where fig coming from germplasm banks were studied and characterized. Moreover it is known that in fig, as in other ancient species, the lack of varietal standards has led, with the time, to the formation of a heterogeneous population of clones, that increased the confusion in the classification within the species (Condit 1955). In our study, some accessions showed a less than 10% genetic dissimilarity (group 1, 5, 10, 11 and 12), due to minor genetic differences between the allelic profiles; in accord to several Authors (Cipriani et al. 2002; Hocquigny et al. 2004; Beghé et al. 2013), such small genetic discrepancies might have been originated by somatic mutations, which frequently occur in long vegetatively propagated species. Accessions with such characteristics, therefore, might be considered as heterogeneous clones of the same cultivar (polyclonal cultivar).

Conclusion

Seventy-nine Italian accessions, collected in a collection field in Tuscany, were studied, using SSR markers, allowing the individuation of 56 different genotypes. Moreover, thanking the allelic analysis, homonymies and synonymies were individuated, making a step forward in the characterization of the germplasm collection. In the present study, it was possible to identify a cluster, in which the majority of dark figs are contained, but the primers set utilized does not recognized all dark figs accessions; however, phenotypic characters, such as peel pigmentation, are probably controlled by simple Mendelian gene and may be unrelated to molecular markers.

The present study permit the detection of identity errors in figs denomination and allowed the creation of a databank in which, Tuscany and Italian fig genotypes are included. This result will help a univocal varietal identification and the evaluation of the real fig biodiversity. In addition to the genetic analyses, to better characterize and valorise Italian fig germplasm, morphological and agronomical analyses could be integrate in further studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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