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ANALYSIS OF GENETIC DIVERSITY OF *Ficus carica* L. (Moraceae) COLLECTION USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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ABSTRACT

Modern technologies and accurate information on genetic diversity and structure are contributing to improve the plant breeding, in particular for all the minor species with a lack of data. Genetic diversity of 139 different *Ficus carica* L. genotypes collected from Italy and Croatia, and divided into two subgroups: uniferous (only main crop) and biferous (breba and main crop), was investigated using 49 microsatellite markers. A total of 70 alleles were generated, of which 64 (91.4%) showed a polymorphic pattern indicating high level of genetic diversity within the studied collection. The mean heterozygosity over the 64 single locus microsatellites was 0.33 and the expected and observed averaged variance were 16.50 and 184.08, respectively. The 139 fig genotypes formed two clusters in the PCoA analysis, suggesting a division between Italian and Croatian genotypes. Moreover, the fig accessions could be divided into two main clusters based on the STRUCTURE analysis according to the biological type, uniferous or biferous, with partly overlapping varieties. In conclusion, our results demonstrated that molecular markers were able to discriminate among genotypes and useful for the authentication of fig tree varieties (homonymies and synonymies).

Key words: fig, breba, SSRs, genetic diversity, population structure

INTRODUCTION

Ficus carica L., the common fig (2n = 2x = 26), an ancient species from the eastern portion of Mediterranean basin, including Turkey and Iran [Ikegami et al. 2009], belongs to *Ficus*, a genus including 600 to 1,900 species [Datwyler and Weiblen 2004, Flaishman et al. 2008].

Large number of the existing fig varieties may be the result of selection focused on agronomic characteristics and/or selection and transportation to distant regions by growers and breeders [Condit 1955]. The identification of commonly used varieties depends on phenotypic traits, which is not completely correct as same varieties may have different names depending on the location (synonyms) or different varieties may have the same name (homonyms). Fig varieties grow wild throughout the Mediterranean basin, but only three types (parthenocarpic and/or non-parthenocarpic) are grown commercially: 1) the Common-type,

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with parthenocarpic fruits, either breba (first crop) or main crop (second crop); 2) the Smyrna-type, with non-parthenocarpic fruits (main crop); and 3) the San Pedro-type, with parthenocarpic fruits (breba) and non-parthenocarpic (main crop) [Storey 1976, Ferrara et al. 2016]. However, this division into three groups could be questionable if we rely only on the pollination requirement (caprification or not), thus dividing varieties only into two groups [Ferrara et al. 2016].

The world fresh fig production was estimated to be 1,050,459 tons per year [FAO 2016], of which its production in Turkey was estimated to be 305,450 tons (29% of the world fig production) [FAO 2016].

Italy ranks 16th in world fig cultivation with 11,297 tons per year (1.1% of the worldwide production) and Croatia 38th (1,165 tons) amongst the fig producing countries [FAO 2016].

Traditionally, plant germplasm characterization has been carried out using morphological and agronomical traits with fluctuation among years, environments and repetitions [Giraldo et al. 2010] and consequently difficulties have arisen in identifying the varieties. Because plant phenotype is unstable due to genotype-environment interactions, a genetic analysis is crucial for the assessment and accurate characterization of fig genetic resources.

Despite the progresses that have been made with the next generation sequencing technologies, in recent years, molecular markers, and in particular microsatellites (SSR), continue to be developed and used [Achtak et al. 2009, Perez-Jiménez et al. 2012].

The present work aimed to describe and characterize a collection of 139 different fig (*F. carica* L.) genotypes through microsatellite marker analysis to better understand their core biological behavior and their genetic relationships in order to create a molecular markers database for fig breeding.

As part of an ongoing germplasm characterization effort, the presented fig collection, including both Italian and Croatian genotypes, was analyzed with SSR markers. Since these two countries are on two faces of the Adriatic Sea, an exchange of plant material has been carried on since ancient times (exchange of fig varieties in the Roman Empire and even earlier) and cases of synonyms and homonyms are found to be present and widespread within these regions [Prgomet and Bohac 2003]. This study attempts to assess genetic diversity and differentiation within the collection following both the geographical origin/site of cultivation and the number of crops per year.

MATERIAL AND METHODS

Plant material

Genetic analysis was carried out on a set of 139 different *F. carica* L. genotypes originating from Italy and Croatia (Tab. 1). Italian genotypes were collected from small private orchards in the Puglia region, in commercial farms or at the fig repository located at the 'P. Martucci' experimental station in Valenzano (University of Bari 'Aldo Moro', DiSSPA, Fruit Tree Unit, Italy). The Croatian accessions were kindly provided by Skink Ltd from Rovinj (Istria county) from their fig collection orchard populated with figs from different parts of Croatia, Slovenian Istria and part of Bosnia and Herzegovina, as well as from Italy (Tuscany region). Fig varieties that were purchased in Tuscany can be found in Croatian orchards, as it is not rare for Croatian fig growers to buy plant material in Italy.

Molecular characterization

Total genomic DNA was isolated from fresh leaves of 139 different fig genotypes using the DNeasy Plant Mini Kit (Qiagen) according to manufacturer's instruction, except for the addition of 1% of Poly-vinylpyrrolidone (PVP 40,000) to the buffer AP1. Genomic DNA concentration was measured using the Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and standardized to 50 ng/µl prior to amplification.

PCR reactions were performed using BIOTAQ™ (Bioline) in a 20 µl volume containing: 150 ng of DNA, 2 µl of 10× NH_4 reaction buffer, 0.85 µl of 50 mM $MgCl_2$ solution, 0.2 µl of 200 µM dNTP mix, 0.6 µl of Fam- or Hex-labeled M13 tail, 0.15 µl of 1 µM of M13 tailed forward primer, 0.6 µl of 1 µM of reverse primer and 0.07 µl of Taq DNA polymerase.

PCR was carried out on a BioRad thermal cycler as follow: 5 min at 95°C, and 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 7 min at 72°C. Aliquot of 1.6 µl of PCR product was mixed with 14 µl of formamide and 0.4 µl of Rox-500

Table 1. List of the 139 analyzed *Ficus carica* L. genotypes, their name, geographical origin, biological type and the site (country/region) of cultivation/collection

ID line	Genotype	Origin	Type	Site
1	2	3	4	5
1	22 T	Italy	Biferous	Italy
2	23 T	Italy	Biferous	Italy
3	24 T	Italy	Biferous	Italy
4	35 T	Italy	Biferous	Italy
5	Cammartone Bifera N.	Italy	Biferous	Italy
6	Verdone	Italy	Biferous	Italy (Puglia)
7	Fiorone precocissimo	Italy	Biferous	Italy (Sardinia)
8	Fico di Atessa	Italy	Biferous	Italy (Sardinia)
9	21 T	Italy	Biferous	Italy
10	20 T	Italy	Biferous	Italy
11	19 T	Italy	Biferous	Italy
12	17 T	Italy	Biferous	Italy
13	Caprifico	Italy	Biferous	Italy
14	15 T	Italy	Biferous	Italy
15	14 T	Italy	Biferous	Italy
16	10 T	Italy	Biferous	Italy
17	9 T	Italy	Biferous	Italy
18	8 C	Italy	Uniferous	Italy
19	8 T	Italy	Biferous	Italy
20	7 T	Italy	Biferous	Italy
21	7 C	Italy	Uniferous	Italy
22	6 T	Italy	Biferous	Italy
23	5 C	Italy	Uniferous	Italy
24	4 C	Italy	Uniferous	Italy
25	Fico Melanzana Verde	Italy	Biferous	Italy (Puglia)
26	Fico Nero_Sava	Italy	Biferous	Italy (Puglia)
27	Fico Dell'Abate	Italy	Biferous	Italy (Puglia)
28	Fico Faraone	Italy	Biferous	Italy (Puglia)
29	Fico Vernea	Italy	Uniferous	Italy (Puglia)
30	Fico Nero_Crotone	Italy	Biferous	Italy (Calabria)
31	Fico Troia	Italy	Biferous	Italy
32	1 C	Italy	Uniferous	Italy
33	1 a C	Italy	Biferous	Italy
34	2 T	Italy	Biferous	Italy
35	3 C	Italy	Uniferous	Italy
36	3 T	Italy	Biferous	Italy
37	Fico Polvere	Italy	Biferous	Italy (Puglia)
38	17 M	Italy	Biferous	Italy
39	18 M	Italy	Biferous	Italy
40	6 A/M	Italy	Biferous	Italy
41	28 M	Italy	Biferous	Italy
42	4 A/M	Italy	Biferous	Italy
43	9 M	Italy	Biferous	Italy
44	3 A/M	Italy	Biferous	Italy

Table 1 cont.

1	2	3	4	5
45	37 M	Italy	Biferous	Italy
46	15 M	Italy	Biferous	Italy
47	1 A/M	Italy	Biferous	Italy
48	43 M	Italy	Biferous	Italy
49	5 M	Italy	Biferous	Italy
50	23 M	Italy	Biferous	Italy
51	42 M	Italy	Biferous	Italy
52	4 M	Italy	Biferous	Italy
53	10 A/M	Italy	Biferous	Italy
54	Fico Fracazzano Oria	Italy	Biferous	Italy (Puglia)
55	Petrelli Bianco Oria	Italy	Biferous	Italy (Puglia)
56	Fiorone di S.Giovanni	Italy	Biferous	Italy (Puglia)
57	Fiorone B Oria	Italy	Biferous	Italy (Puglia)
58	Stafiero AZ Facoltà	Italy	Biferous	Italy (Puglia)
59	Caprifico Cep 2 Bari	Italy	Biferous	Italy (Puglia)
60	22 M	Italy	Biferous	Italy
61	41 M	Italy	Biferous	Italy
62	24 M	Italy	Biferous	Italy
63	20 M	Italy	Biferous	Italy
64	Fico verdone Sava	Italy	Biferous	Italy (Puglia)
65	Fiorone della Regina	Italy	Biferous	Italy (Puglia)
66	Fiorone bianco	Italy	Biferous	Italy (Puglia)
67	Fico A Bianco Oria	Italy	Biferous	Italy (Puglia)
68	Fiorone Testa di Gatto	Italy	Biferous	Italy (Puglia)
69	Fico 3 volte Gioia	Italy	Biferous	Italy (Puglia)
70	Caprifico Cep 1 Bari	Italy	Biferous	Italy (Puglia)
71	Troiano Nero Crotone	Italy	Biferous	Italy (Calabria)
72	33 M	Italy	Biferous	Italy
73	44 M	Italy	Biferous	Italy
74	19 M	Italy	Biferous	Italy
75	35 M	Italy	Biferous	Italy
76	34 M	Italy	Biferous	Italy
77	45 M	Italy	Biferous	Italy
78	13 M	Italy	Biferous	Italy
79	Vera Bianca Crotone	Italy	Biferous	Italy (Calabria)
80	7 M	Italy	Biferous	Italy
81	1 M	Italy	Biferous	Italy
82	26 M	Italy	Biferous	Italy
83	Nerello Marchese	Italy	Uniferous	Italy
84	Fico Nataline Crotone	Italy	Biferous	Italy (Calabria)
85	D	Italy	Biferous	Italy
86	G	Italy	Biferous	Italy
87	F	Italy	Biferous	Italy
88	6 M	Italy	Biferous	Italy
89	2 M	Italy	Biferous	Italy
90	29 M	Italy	Biferous	Italy
91	M	Italy	Biferous	Italy
92	21 M	Italy	Biferous	Italy
93	14 M	Italy	Biferous	Italy

Table 1 cont.

1	2	3	4	5
94	Columbro B Crotone	Italy	Biferous	Italy
95	7 M	Italy	Biferous	Italy (Calabria)
96	10 M	Italy	Biferous	Italy
97	27 M	Italy	Biferous	Italy
98	40 M	Italy	Biferous	Italy
99	Bružetka bijela	Croatia	Uniferous	Croatia
100	Bružetka crna	Croatia	Uniferous	Croatia (Medulin)
101	Miljska figa	Slovenia	Uniferous	Croatia (Medulin)
102	Zamorčica	Croatia	Uniferous	Croatia (Seca)
103	Zimica	Croatia	Uniferous	Croatia/Bosnia
104	Petrovača bijela	Croatia	Biferous	Croatia (Medulin)
105	Poli pistoia	Italy	Biferous	Croatia
106	Šaraguja	Croatia	Uniferous	Croatia
107	Bjelica	Croatia	Biferous	Croatia (Medulin)
108	Bottaccio	Italy	Biferous	Croatia (Plavje)
109	Rezavica	Croatia	Uniferous	-
110	Fico della Madonna	Croatia/Bosnia	Biferous	Croatia (Medulin)
111	Crnica	Croatia	Uniferous	Croatia (Medulin)
112	Rosso di Trani	Italy	Biferous	Croatia (Medulin)
113	Verdino	Italy	Uniferous	Croatia
114	Columbro bianco_B	Italy	Biferous	Croatia
115	Divlja (Wild)	Croatia	Biferous	Croatia
116	Fiorone	Italy	Biferous	Croatia
117	Split	Croatia	Uniferous	Croatia
118	Cavalierino	Italy	Uniferous	Croatia
119	S.Martino	Italy	Biferous	Croatia
120	Faraone	Italy	Biferous	Croatia
121	Sardo bianco	Italy	Biferous	Croatia
122	Piombionese	Italy	Biferous	Croatia
123	Melanzana Bianca	Italy	Biferous	Croatia
124	Corvo siculo	Italy	Uniferous	Croatia
125	New Bianco Nic.	Italy	Uniferous	Croatia
126	Montalcino rosa	Italy	Uniferous	Croatia
127	Bianco di Carmingano	Italy	Uniferous	Croatia
128	Bamborino	Italy	Uniferous	Croatia
129	Raffaone	Italy	Biferous	Croatia
130	Francuska crna	Croatia	Uniferous	Croatia
131	Caietti nero	Italy	Biferous	Croatia
132	Bianchetto	Italy	Uniferous	Croatia
133	Dattero	Italy	Uniferous	Croatia
134	Ficazzano	Italy	Biferous	Croatia
135	Columbro bianco_U	Italy	Uniferous	Croatia
136	Troiano	Italy	Uniferous	Croatia
137	Zuchetto	Italy	Biferous	Croatia
138	Momjan	Croatia	Uniferous	Italy
139	Brogiotto Bi. Bocci	Italy	Uniferous	Croatia

Table 2. Allelic frequencies reported for all the alleles of each microsatellite tested in this study

Locus	Allele	Allelic frequency
1	2	3
MFC1	190	0.635
	203	0.530
	208	0.525
MFC3	138	0.326
	151	0.532
	140	0.408
	143	0.319
MFC4	213	0.446
	233	0.408
	237	0.424
LMFC17	203	0.218
	207	0.845
	214	0.077
LMFC18	131	0.763
	138	0.481
LMFC19	312	0.338
	316	0.868
	320	0.221
LMFC21	280	0.750
	284	0.229
LMFC22	297	0.474
	299	0.755
LMFC25	228	0.204
	232	0.092
	236	0.199
LMFC26	241	0.087
	251	0.423
LMFC27	202	0.332
	213	0.347
LMFC28	209	0.071
	214	0.255
	216	0.332
	220	0.071
LMFC30	249	0.071
	258	0.342
	270	0.265
	277	0.071
LMFC31	244	0.296
	258	0.403
LMFC36	241	0.469
	243	0.097
LMFC37	222	0.378
	227	0.270
LMFC38	231	0.316
	237	0.393
	220	0.841
	233	0.536
Frub391	175	0.500
	179	0.191
	182	0.562
	196	0.041
Frub422	185	0.224
	195	0.102
	208	0.240
	221	0.077
Frub436	167	0.554
	172	0.154
FCUP027-4	202	0.300
	206	0.333
	218	0.357
FCUP038-6	170	0.165
	175	0.456
	188	0.210
	192	0.278

(Applied Biosystems), the internal molecular weight standard, and denatured at 95°C for 5 min. PCR products were then visualized by capillary electrophoresis on 3500 Genetic Analyzer (Applied Biosystems) and analyzed by Gene Mapper v.5.0 genotyping software.

Microsatellite analysis and genetic relationship

A number of 49 microsatellite primer pairs taken from the literature and available on NCBI [www.ncbi.nlm.nih.gov] was tested to estimate genetic similarity and distances among the 139 fig genotypes. The selection of the fig SSRs was based on their high polymorphism information content.

Genetic data were converted into a pairwise by individual genetic distance matrix using the haploid SSR markers distance matrix. Once a genetic distance matrix was calculated, a principal coordinate analysis (PCoA) was carried out using GenALEx software (v. 6.5) [Peakall et al. 2006, 2012] and phylogenetic tree was performed by NTSYS pc v. 2.1 software based upon the UPGMA (Unweighted Pair Group Method with Arithmetical averages) method. The Bayesian

clustering program STRUCTURE (version 2.3.4) was used selecting an admixture model with correlated allele frequencies. The number of sub-groups (K) was estimated by 20 independent runs for each K (from 1 to 10) applying the admixture model, with allele frequencies uncorrelated for SSR markers, 100,000 Markov Chain Monte Carlo (MCMC) repetitions, and a 100,000 burning period. Means of the log-likelihood estimates for each K were calculated. The true K was determined using both an estimate of the posterior probability of the data for a given K [Pritchard et al. 2000], and the Evanno ÄK [Evanno et al. 2005]. A genotype was considered to belong to a group if its membership coefficient was ≥0.50.

Statistical analysis

Genetic diversity parameters of the fig collection were reported. Percentage of polymorphic loci (P%), number of different alleles (N_a), number of alleles with a frequency greater than 5% (N_a F ≥ 5%), number of effective alleles (N_e), Shannon's information index (I), number of private alleles (NPA, equivalent to the number of alleles unique to a single genotype in the data set), heterozygosity (H), expected variance (V_e), observed variance (V_o), and disequilibrium index (V_o/V_e), were calculated by GenALEx software (v. 6.5) [Peakall et al. 2006, 2012]. The same software was also used to perform the Analysis of MOlecular VAriance (AMOVA), within and among geographical groups (Italy and Croatia). Heterozygosity was calculated following the formula ($1 - Óp_i^2$), where p_i^2 is the frequency of allele i averaged over the subpopulations.

RESULTS

Molecular analysis of fig genotypes

The collection included a total of 139 genotypes, of which 98 from Italy and 41 from Croatia was divided into two subgroups, biferous and uniferous, according to the number of crops per year, thus obtaining 107 and 32 genotypes for each group, respectively (Tab. 3).

DNA polymorphisms were scored across the fig collection and out of 49 tested primer pairs, 24 (48%) gave clear and reliable amplification products and thus could be used for molecular characterization of the collection. PCR amplification produced a total of 70 microsatellite alleles: 6 (8.6%) monomorphic.

Table 3. Genetic diversity statistics estimated for all the analysed microsatellite loci in the *Ficus carica* L. collection

Population	P	N_a	N_a F	N_e	I	H	V_e	V_o	V_o/V_e
Overall	82.1	69.5	2.34	1.65	0.54	0.33	16.50	184.08	11.15
Italy	100	2.69	2.30	1.79	0.67	0.41	15.45	76.45	4.92
Croatia	64.3	2.00	1.74	1.51	0.41	0.26	9.58	64.31	6.71

P = percentage of polymorphic loci; N_a = number of different alleles; N_a F = number of alleles with frequency greater than 5%; N_e = number of effective alleles = $1/(\Sigma p_i^2)$; I = Shannon's information index = $-\Sigma p_i^2 * \text{Ln}\ (p_i)$; H = heterozygosity ($1 - \Sigma p_i^2$); V_e = expected variance; V_o = observed variance; V_o/V_e = disequilibrium index
p_i is the frequency of the i^{th} allele for the population and Σp_i^2 is the sum of the squared population allele frequencies

Table 4. Analysis of molecular variance (AMOVA) within and among *Ficus carica* L. accessions from two different countries (Italy and Croatia)

Source	df*	Sum of square	Estimated variance	Percentage of variation
Among groups	1	9272.944	159.466	75%
Within groups	137	7362.739	53.743	25%
Total	138	16635.683	213.209	100%

* degree of freedom
Fixation index (Fst) = 0.748

Whereas, the remaining 64 alleles (91.4%), produced by 22 primer pairs, showed a polymorphic pattern, and thus they were suitable to evaluate the genetic diversity and estimate genetic distances in the collection. The number of amplified alleles per locus ranged between 2–4, with a length of the amplified alleles ranged between 131–320 bp (Tab. 4).

The estimation of genetic diversity in the fig collection is summarized in Table 1. The overall number of different alleles, alleles with frequency greater than 5% and effective ones were 69.5, 2.34 and 1.65, respectively.

Shannon's index of the whole collection (0.54) and heterozygosity (0.33) were used as two intra-region gene diversity. The disequilibrium index (V_o/V_e) was 11.15 for the whole collection with a value of 4.92 for the Italian group and 6.71 for the Croatian one. The lowest number of polymorphic loci within geographical groups was detected for Croatia (64.3), while Italy showed 100% polymorphism.

Heterozygosity and Shannon's information index, as two useful intra-region gene diversity indices, were 0.41 and 0.67 for Italy, and 0.26 and 0.41 for Croatia, respectively.

In order to discriminate between different groups in the fig collection, analysis of molecular variance (AMOVA) was also performed and the percentage of intra and inter-region genetic variation was estimated (Tab. 2). The fixation index (F_{st}) of the whole collection was 0.748 ($P < 0.001$), as reported in Table 2, meaning that 75% of the total variation occurred among different groups, while 25% was found within individual groups. The results of AMOVA for this germplasm collection indicated that, at a molecular level, fig accessions coming from the same geographical areas were similar compared to accessions of different origin.

Collection structure

All the 70 polymorphic loci were used to estimate genetic diversity among the 139 fig genotypes and to determine the structure of the collection. Principal coordinate analysis (PCoA) (Fig. 1) was carried out to identify genetic structure within the data set. The first three coordinates explained 63.40%, 9.95% and 7.40% of genetic variation, respectively, accounting for 80.75% in total. The PCoA was reported in two different ways identifying the geographical origin/location (Italy and Croatia) (Fig. 1a), and the number of crops (breba and/or main crop) per year of each accession (uniferous and biferous) (Fig. 1b). As shown in the first plot (Fig. 1a), genotypes were split by the first axis in two distinct groups according to the geographical site.

In order to elucidate genetic relationships among the 139 fig genotypes, 70 polymorphic microsatellite loci were used to build a phylogenetic tree based on the UPGMA algorithm using Jaccard's similarity coefficient matrix. The highly dissected nature of the tree suggested that accessions were distinct from each other and most variation was confined to within clusters. Interestingly, cluster analysis split different genotypes in two groups based on their molecular similarity matching the geographical site (Fig. 2). Group I included the highest number of genotypes (89), of which the majority Italian (84 genotypes) and only 5 Croatians.

PCoA analysis and phylogenetic tree showed that group I included biferous genotypes, whereas group II included both uniferous and biferous types without distinction.

To assign the individuals into subpopulations based on genetic similarity, we used a Bayesian approach implemented in STRUCTURE. Following the methodology described in Evanno et al. [2005], the ΔK were plotted against the K numbers of the subgroups, indicating the most likely number of subpopulations was 2 (Fig. 3a). Therefore, considering $K = 2$, the collection was split in two sub-groups (group 1, group 2) containing 55 and 84 accessions each (Fig. 3b). In particular, all of the Croatian and some Italian genotypes were assigned to group 1, with a Q1 mean of 0.86, while the Italian genotypes clustered to group 2, with a Q2 mean membership of 0.87.

Looking at the number of crops per year, group 1 contained 45% of uniferous accessions and 55% of biferous, whereas group 2 was composed by all biferous genotypes, except for the Italian lines 4 C, 7 C, 3 C, 1 C, 5 C and Fico Vernea, and the Croatian line Crnica (Tab. 5). The reason why some uniferous accession clustered with the biferous in the group 2 could be due to a possible common origin of these varieties.

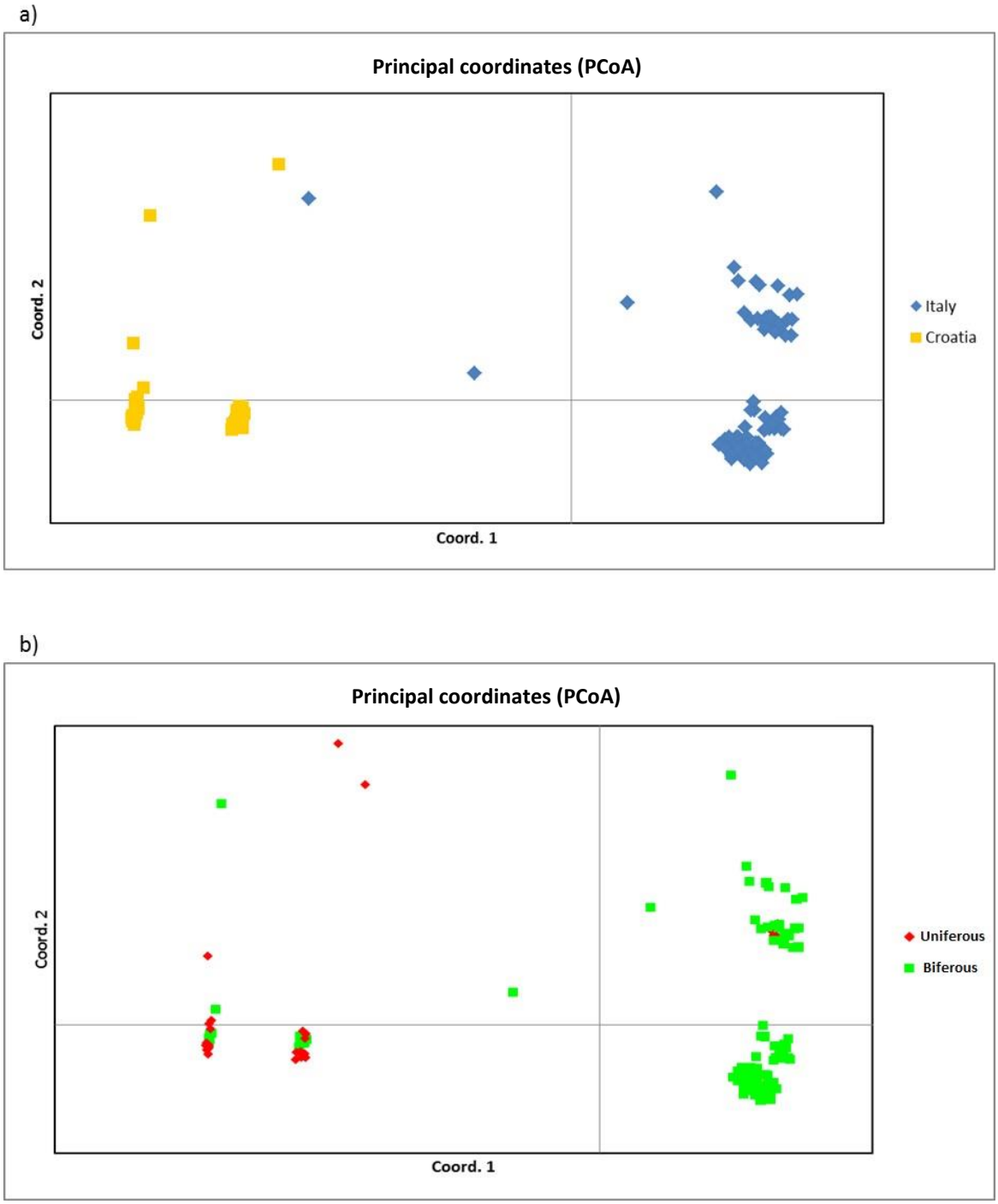

Fig. 1. Principal coordinates analysis (PCoA) plot of the first two components obtained from 70 SSRs for 139 fig accessions. The first three axis explained the 63.40%, 9.95% and 7.40% of genetic variation, respectively for a total of 80.75%. The graph reported the subdivision following a) the geographical origin/location of the accessions and b) the number of crops per year (uniferous or biferous)

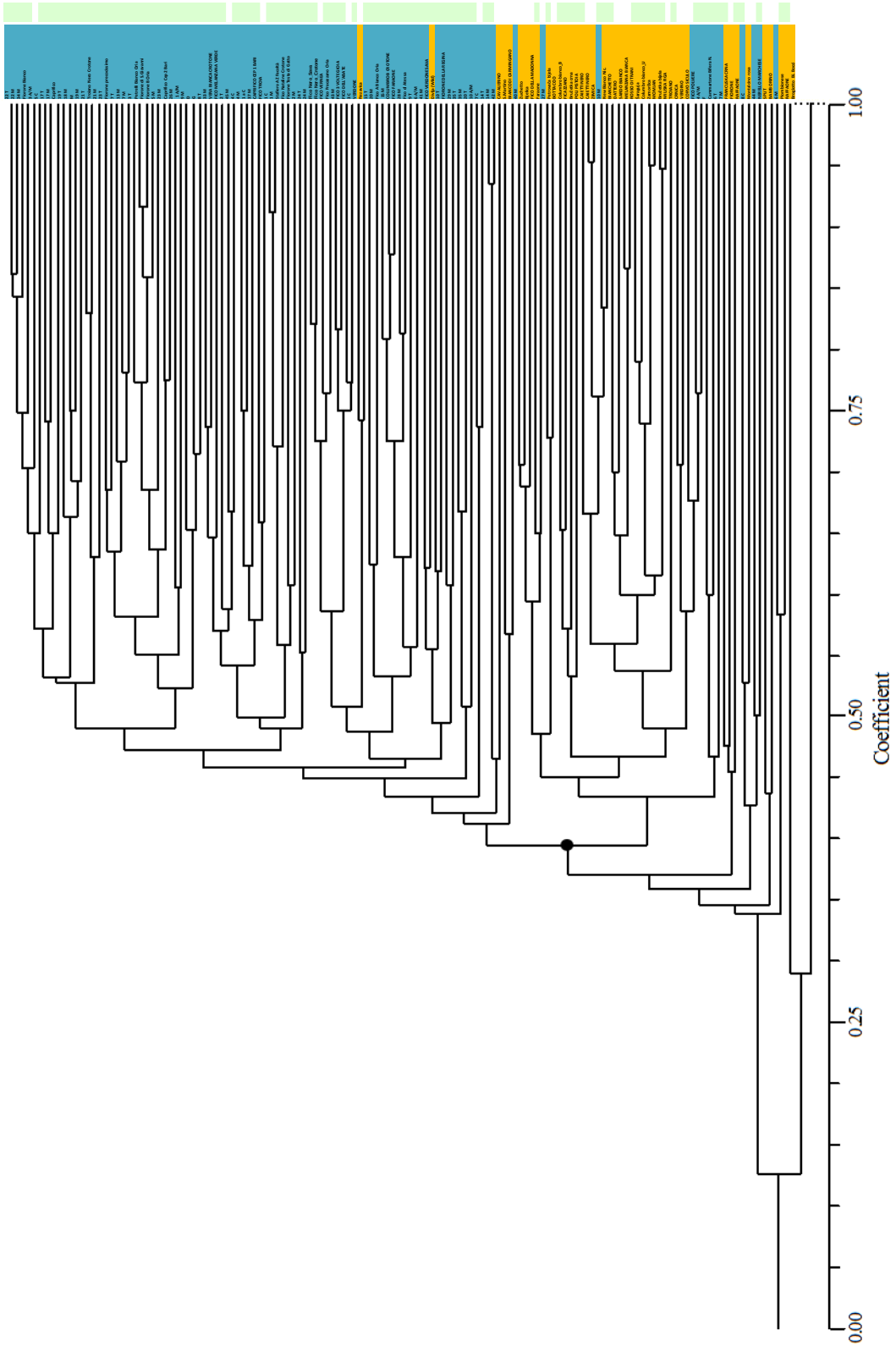

Fig. 2. Phylogenetic tree showing genetic distances within 139 fig genotypes resulting from SSR markers analysis. Font colors represent the two geographical origin/location of the accessions: blue for the Italian genotypes and yellow for the Croatian ones. Light green (biferous) and no color (uniferous) at the right of the tree indicate the number of crops per year. Cluster analysis and phylogenetic tree construction were performed by the NTSYS pc v. 2.1 software, based upon the UPGMA (unweighted pair group method with arithmetical averages) method

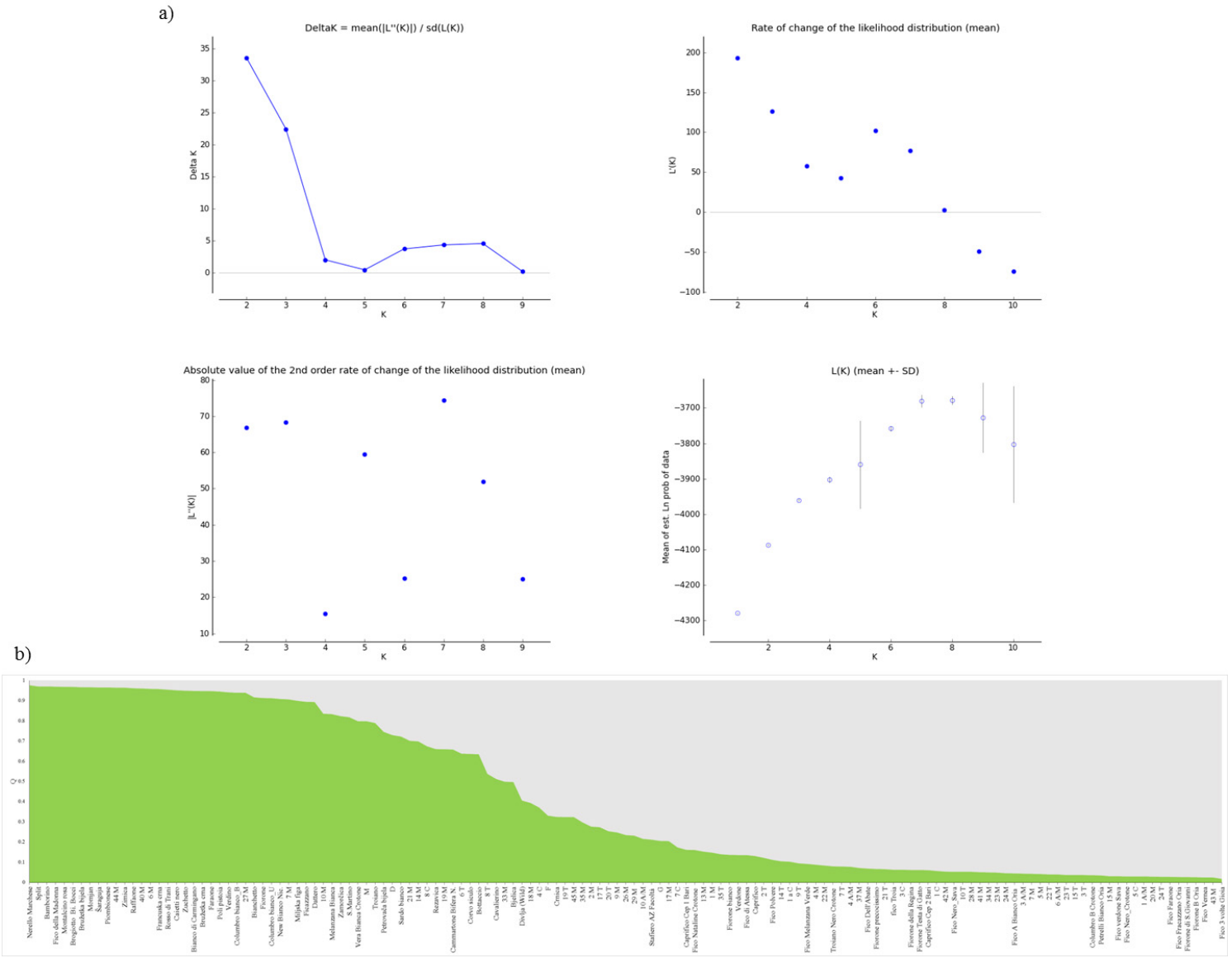

Fig. 3. Figure 3a) Results of ΔK computation [Evanno et al. 2005] and mean log likelihood of the data [L(K)] for the fig collection for each scenario tested (K = 1−10) showing support for K = 2. Figure 3b) Population structure of the 139 fig accessions using microsatellites markers. The graph shows the STRUCTURE bar plot for K = 2 based on 70 SSR markers data and the Q values representing the proportion of ancestry to a given subpopulation. All parameters were calculated by STRUCTURE software (version 2.3.4)

Table 5. List of the 139 *Ficus carica* L. genotypes with the site of origin/collection and the number of crops per year (uniferous or biferous) with the Q values derived from the STRUCTURE analysis at K = 2

Genotype	Site	Type	Q value
1	2	3	4
Nerello Marchese	Italy	Uniferous	0.977
Split	Croatia	Uniferous	0.971
Bamborino	Italy/Croatia	Uniferous	0.971
Fico della Madonna	Croatia	Biferous	0.970
Montalcino rosa	Italy/Croatia	Uniferous	0.969
Brogiotto Bi. Bocci	Italy/Croatia	Uniferous	0.969
Bružetka bijela	Croatia	Uniferous	0.967
Momjan	Croatia	Uniferous	0.967
Šaraguja	Croatia	Uniferous	0.966
Piombionese	Italy/Croatia	Biferous	0.966
44 M	Italy	Biferous	0.965
Zimica	Croatia	Uniferous	0.965
Raffaone	Italy/Croatia	Biferous	0.962
40 M	Italy	Biferous	0.961
6 M	Italy	Biferous	0.959
Francuska crna	Croatia	Uniferous	0.958
Rosso di Trani	Italy/Croatia	Biferous	0.955
Caietti nero	Italy/Croatia	Biferous	0.952
Zuchetto	Italy/Croatia	Biferous	0.950
Bianco di Carmingano	Italy/Croatia	Uniferous	0.949
Bružetka crna	Croatia	Uniferous	0.948
Faraone	Italy/Croatia	Biferous	0.948
Poli pistoia	Italy/Croatia	Biferous	0.946
Verdino	Italy/Croatia	Uniferous	0.942
Columbro bianco_B	Italy/Croatia	Biferous	0.940
27 M	Italy	Biferous	0.940
Bianchetto	Italy/Croatia	Uniferous	0.917
Fiorone	Italy/Croatia	Biferous	0.914
Columbro bianco_U	Italy/Croatia	Uniferous	0.913
New Bianco Nic.	Italy/Croatia	Uniferous	0.909
7 M	Italy	Biferous	0.907
Miljska figa	Slovenia/Croatia	Uniferous	0.900
Ficazzano	Italy/Croatia	Biferous	0.895
Dattero	Italy/Croatia	Uniferous	0.894
10 M	Italy	Biferous	0.836
Melanzana Bianca	Italy/Croatia	Biferous	0.834
Zamorčica	Croatia	Uniferous	0.824
S. Martino	Italy/Croatia	Biferous	0.819
Vera Bianca Crotone	Italy	Biferous	0.799
M	Italy	Biferous	0.799
Troiano	Italy/Croatia	Uniferous	0.790
Petrovača bijela	Croatia	Biferous	0.746
D	Italy	Biferous	0.730
Sardo bianco	Italy/Croatia	Biferous	0.723
21 M	Italy	Biferous	0.702

Table 5 cont.

1	2	3	4
14 M	Italy	Biferous	0.699
8 C	Italy	Uniferous	0.675
Rezavica	Croatia	Uniferous	0.661
19 M	Italy	Biferous	0.660
Cammartone Bifera N.	Italy	Biferous	0.659
6 T	Italy	Biferous	0.638
Corvo siculo	Italy/Croatia	Uniferous	0.637
Bottaccio	Italy/Croatia	Biferous	0.635
8 T	Italy	Biferous	0.538
Cavalierino	Italy/Croatia	Uniferous	0.513
33 M	Italy	Biferous	0.499
Bjelica	Croatia	Biferous	0.498
Divlja (Wild)	Croatia	Biferous	0.406
18 M	Italy	Biferous	0.394
4 C	Italy	Uniferous	0.371
F	Italy	Biferous	0.331
Crnica	Croatia	Uniferous	0.325
19 T	Italy	Biferous	0.324
45 M	Italy	Biferous	0.324
35 M	Italy	Biferous	0.298
2 M	Italy	Biferous	0.277
17 T	Italy	Biferous	0.274
20 T	Italy	Biferous	0.254
9 M	Italy	Biferous	0.248
26 M	Italy	Biferous	0.235
29 M	Italy	Biferous	0.232
10 A/M	Italy	Biferous	0.216
Stafiero AZ Facoltà	Italy	Biferous	0.212
G	Italy	Biferous	0.206
17 M	Italy	Biferous	0.205
7 C	Italy	Uniferous	0.174
Caprifico Cep 1 Bari	Italy	Biferous	0.162
Fico Nataline Crotone	Italy	Biferous	0.161
13 M	Italy	Biferous	0.153
1 M	Italy	Biferous	0.148
35 T	Italy	Biferous	0.140
Fiorone bianco	Italy	Biferous	0.137
Verdone	Italy	Biferous	0.136
Fico di Atessa	Italy	Biferous	0.136
Caprifico	Italy	Biferous	0.131
2 T	Italy	Biferous	0.122
Fico Polvere	Italy	Biferous	0.112
14 T	Italy	Biferous	0.105
1 a C	Italy	Biferous	0.103
9 T	Italy	Biferous	0.095
Fico Melanzana Verde	Italy	Biferous	0.092
4 M	Italy	Biferous	0.088
22 M	Italy	Biferous	0.084

Table 5 cont.

1	2	3	4
Troiano Nero Crotone	Italy	Biferous	0.080
7 T	Italy	Biferous	0.079
4 A/M	Italy	Biferous	0.078
37 M	Italy	Biferous	0.072
Fico Dell'Abate	Italy	Biferous	0.069
Fiorone precocissimo	Italy	Biferous	0.067
21 T	Italy	Biferous	0.066
Fico Troia	Italy	Biferous	0.063
3 C	Italy	Uniferous	0.063
Fiorone della Regina	Italy	Biferous	0.062
Fiorone Testa di Gatto	Italy	Biferous	0.062
Caprifico Cep 2 Bari	Italy	Biferous	0.061
1 C	Italy	Uniferous	0.057
42 M	Italy	Biferous	0.054
Fico Nero Sava	Italy	Biferous	0.053
10 T	Italy	Biferous	0.053
28 M	Italy	Biferous	0.053
41 M	Italy	Biferous	0.051
34 M	Italy	Biferous	0.050
23 M	Italy	Biferous	0.049
24 M	Italy	Biferous	0.046
Fico A Bianco Oria	Italy	Biferous	0.045
3 A/M	Italy	Biferous	0.044
7 M	Italy	Biferous	0.043
5 M	Italy	Biferous	0.042
22 T	Italy	Biferous	0.040
6 A/M	Italy	Biferous	0.040
23 T	Italy	Biferous	0.037
15 T	Italy	Biferous	0.037
3 T	Italy	Biferous	0.037
Columbro B Crotone	Italy	Biferous	0.036
Petrelli Bianco Oria	Italy	Biferous	0.035
15 M	Italy	Biferous	0.031
Fico verdone Sava	Italy	Biferous	0.031
Fico Nero Crotone	Italy	Biferous	0.030
5 C	Italy	Uniferous	0.030
1 A/M	Italy	Biferous	0.030
20 M	Italy	Biferous	0.029
24 T	Italy	Biferous	0.028
Fico Faraone	Italy	Biferous	0.028
Fico Fracazzano Oria	Italy	Biferous	0.027
Fiorone di S.Giovanni	Italy	Biferous	0.027
Fiorone B Oria	Italy	Biferous	0.026
Fico Vernea	Italy	Uniferous	0.025
43 M	Italy	Biferous	0.025
Fico 3 volte Gioia	Italy	Biferous	0.018

DISCUSSION

Germplasm characterization of plant accessions has been restricted, thus limiting the breeding programs. Fig is one of the ancient species from the Mediterranean basin adapted to a wide range of climate conditions characterized by numerous ecotypes and landraces selected and cultivated in different agricultural areas, with a growth of homonyms and synonyms, causing problems for genotype categorization [Galet 1990, Lebot and Aradhya 1991, Aradhya et al. 1995]. More than 700 varieties were listed by Condit [1955] in the monograph on fig varieties and many of them have large numbers of synonyms. The lack of information and the occurrence of extensive synonyms complicated the deciphering of genetic identity and relationships among the genotypes. Moreover, genetic and site of origin of most of the fig genotypes is unknown and all data concerning the genotypes are incomplete, inaccurate, or missing in most germplasm collections.

In this study, 70 microsatellite loci were used to investigate the genetic relationships among 139 fig genotypes collected in two different Mediterranean countries (Italy and Croatia) in order to describe the relationship among the collection and try to characterize uniferous and biferous genotypes.

In the present study, the average expected heterozygosity (H) of 0.335 was similar or slightly lower to previously published works, 0.482 in 194 worldwide fig tree accessions [Aradhya et al. 2010], 0.44 in 19 European and Asian fig tree varieties [Ikegami et al. 2009] and 0.53 in 57 Spanish fig tree accessions [Perez-Jimenez et al. 2012]. However, this value was lower compared to 0.678 reported in 76 Turkish fig varieties [Caliskan et al. 2012].

In addition, AMOVA analysis indicated that only 25% of the total genetic diversity is distributed within groups, whereas 75% of the diversity is attributed to differences between regions. This high variability between regions indicated that, at a molecular level, fig accessions coming from different geographical areas were diverse with respect to accessions with the same area of origin. Although Croatia and Puglia are on two sides of the Adriatic Sea and exchange of plant material have occurred since ancient times (Roman, Byzantines, Venetians, Turkish, etc.) and Croatian have carried fig varieties from Italy, the fig varieties diversified their biological features in the two different countries. Our data differ from a previous report on the analysis of genetic diversity among European and Asian fig varieties [Ikegami et al. 2009], which explained the low divergence between collections/groups with the occurrence of gene flow or common origin of the populations [Salhi-Hannachi et al. 2005, Ikegami et al. 2009], probably because they analyzed only 19 fig genotypes.

Our data were confirmed by PCoA, phylogenetic tree and the structure analysis. These results indicated that the examined fig genotypes clustered in two distinct groups according to their geographical location. Moreover, Bayesian analysis showed that at K = 2, without prior population information, the simulation attained the highest likelihood value and had the higher clusterness, confirming the previous work on genetic diversity and structure of Mediterranean basin *Ficus carica* genotypes [Ganopoulos et al. 2015]. Herein, we supposed that the fig collection was characterized by a typical continuous genetic diversity, supported by two independent clusters obtained in relation to their origin/location. Maybe figs in Italy and Croatia had common ancestors in ancient times and diversified their features in the successive centuries as a consequence of different criteria: a different selection of growers (types of crops), other introduced materials, use for fresh or dried consumption, etc. Furthermore, as reported in literature, many uniferous and biferous varieties clustered together in the same group indicating a possible common origin of these varieties [Salhi Hannachi et al. 2006, Chatti et al. 2007, Baraket et al. 2009, 2011, Ganopoulos at al. 2015], due to the monoecious origin of *Ficus* that has evolved into two gynodioecious forms as suggested by Machado et al. [2001]. Probably all the fig varieties were 'biferous', at least physiologically, and can be considered as 'commercially uniferous' since the breba crop is absent or in traces in many 'uniferous' varieties. However, the buds of the breba (fruit buds) are present also in the uniferous varieties thus suggesting the fig to be at least physiologically biferous for all the varieties (with some triferous varieties). The distinction on the PCoA of two not well defined groups for uniferous and biferous could confirm this idea indicating that the difference should be

only related to varieties requiring pollination or not as previously reported [Ferrara et al. 2016].

CONCLUSION

Data presented in the current study on a collection of fig genotypes from Italy and Croatia could be a useful tool towards understanding the fig biology and breeding programs. We demonstrated that (1) genetic diversity of this fig collection was higher compared to the other previous studies, and (2) SSR markers successfully contributed to the estimation of the relatedness of fig at the varietal level.

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