Spanish Journal of Agricultural Research
16 (3), e0703, 12 pages (2018)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2018163-11662
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Genetic diversity and population structure in caprifigs (*Ficus carica* var. *caprificus*) using SSR markers

Oguzhan Caliskan[1], Safder Bayazit[1], Muruvvet Ilgin[2], Nesrin Karatas[3] and Ali Ergul[4]

[1]Hatay Mustafa Kemal University, Fac. Agriculture, Dept. Horticulture, Hatay, Turkey. [2]Kahramanmaras Sutcu Imam University, Fac. Agriculture, Dept. Horticulture, Kahramanmaras, Turkey. [3]Alata Horticultural Research Institute, Mersin, Turkey. [4]Ankara University, Biotechnology Institute, Ankara, Turkey.

Abstract

Abundant wild and cultivated fig germplasm can be found in Turkey, a center of diversity for figs; however, many of these valuable genetic resources have not yet been identified or characterized using molecular markers. In the present study, microsatellite markers were used to characterize a set of 96 caprifig (*Ficus carica* var. *caprificus*) accessions from Turkey. The caprifig accessions showed considerable polymorphism with an average of 8.3 alleles per locus. The number of alleles per locus varied from three for the loci LMFC18 and LMFC23, to 14 for the loci FCUPO38-6 and FCUPO08. Genetic distance values and cluster analyses revealed high genetic similarities, except for the reference group, among the caprifig groups. Factorial correspondence analysis also separated the caprifig groups, suggesting that caprifig populations from Turkey were unmixed, probably because of low gene flow, likely because germplasm has not yet been moved among geographical areas and because many caprifig populations arose from propagation by seed. In our population structure analysis, the caprifig accessions could be grouped according to the regions from where they were sampled. Our molecular data revealed great genetic diversity within this caprifig germplasm. This genetically rich caprifig germplasm resource will be useful for both fig breeding programs and analysis of the complex genetic structure of figs that reproduce using various pollination strategies.

Additional keywords: genetic resources; microsatellite markers; genetic differentiation analysis.

Abbreviations used: BAPS (Bayesian analysis of population structure); FCA (factorial correspondence analysis); F_{st} (fixation index); H_e (expected heterozygosity); H_o (observed heterozygosity); HW (Hardy-Weinberg equilibrium); LD (linkage disequilibrium); NYSYSpc (numerical taxonomy and multiware analysis system software); PCR (polymerase chain reaction); PI (probability of identity); SSR (simple sequence repeat); UPGMA (unweighted pair-group with arithmetic mean).

Authors' contributions: Designed the experiments, coordinated the works, wrote and edited the manuscript: OC and SB. Sampled the plant material: MI and NK. Performed the microsatellite analysis: AE. All authors read and approved the final manuscript.

Citation: Caliskan, O.; Bayazit, S.; Ilgin, M.; Karatas, N.; Ergul, A. (2018). Genetic diversity and population structure in caprifigs (*Ficus carica* var. *caprificus*) using SSR markers. Spanish Journal of Agricultural Research, Volume 16, Issue 3, e0703. https://doi.org/10.5424/sjar/2018163-11662

Received: 05 May 2017. **Accepted:** 27 Sep 2018.

Copyright © 2018 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Funding: Scientific and Technical Research Council of Turkey (TÜBİTAK-114O034).

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Oguzhan Caliskan: caliskanoguzhan@gmail.com or ocaliskan@mku.edu.tr

Introduction

The fig is one of the primary horticultural plants cultivated by humans in the Lower Jordan Valley, about 11,400 to 11,200 years ago (Kislev *et al.*, 2006). Anatolia and Syria, as natural habitats of figs, were also centers of origin from where they were transferred to other regions (Condit, 1947). The natural fig populations in the region represent a rich genetic resource for fig breeding.

Fig is a functionally gynodioecious genus that includes the monoecious caprifig and the edible fig (Beck & Lord, 1988). Fig cultivars are grouped into four types, based on their pollination requirement and cropping stages (Flaishman *et al.*, 2008). The first type, the caprifig, is not edible. The caprifig crops are valuable as a pollen source both to promote fruit set in the edible figs and as germplasm in fig breeding programs. Thus, caprifigs are used for breeding parthenocarphic cultivars and high-quality edible figs (Stover & Aradhya, 2008; Flaishman *et al.*, 2015). The second and third types comprise the two groups of edible figs, *Smyrna* and *San Pedro*, require caprification, or the pollination of edible figs with pollen carried from caprifig fruits by *Blastophaga psenes* wasps (Galil & Neeman, 1977), to set their main crops of fruit. The fourth type, the

common figs (*Ficus carica*), are called 'persistent' figs because they can bear one or two crops per season with or without caprification.

Turkey is the world's leading fig-producing country and is part of the center of diversity of figs, where numerous cultivated and wild forms of fig, including caprifigs, with great diversities of color, shape and ripening periods are grown (Caliskan *et al.*, 2016). However, vulnerability to biotic and abiotic stresses and loss of agricultural land to intensive urbanization has adversely affected fig production in Turkey (Caliskan *et al.*, 2012). The main fig cultivars such as 'Sarılop' and 'Bursa Siyahı', and most local cultivars in Turkey, require caprification for fruit set. Thus, it has become essential to establish a germplasm evaluation and preservation program for caprifigs.

In previous studies, morphological parameters (Giraldo *et al.*, 2010; Podgornik *et al.*, 2010; Caliskan & Polat, 2012) and molecular markers (Giraldo *et al.*, 2005; Ikegami *et al.*, 2009; Aradhya *et al.*, 2010; Caliskan *et al.*, 2012) have been used to demonstrate the significant phenotypic and genetic variability in edible fig germplasm. In particular, molecular markers have been used to genetically distinguish fig genetic resources for which only phenotypic data were previously available. The genome of fig is relatively small, at about 356 Mb (Mori *et al.*, 2017). However, little is currently known about the level of genetic diversity in caprifig germplasm (Dalkilic *et al.*, 2011; Essid *et al.*, 2015).

This is the first study using microsatellite markers to evaluate the genetic diversity and population structure of caprifig accessions. These results will improve our understanding of the level of diversity of caprifig germplasm in Turkey and will help to devise an effective strategy for the conservation, management and use of these genetic resources in breeding programs for edible fig.

Material and methods

Plant materials

The present study was carried out using 90 caprifig accessions selected from the Eastern Mediterranean region of Turkey (Table 1) and six caprifig cultivars as reference ('Ak İlek', 'Armut İlek', 'Elma İlek', 'Hamza', 'Küçük Konkur' and 'Taşlık'). The caprifig accessions were sampled from native populations in the Eastern Mediterranean region of Turkey, and the reference cultivars are used here for analysis of the evolution of caprifig in the Aegean region of Turkey. The caprifig accessions, but not the reference cultivars, were grouped and coded as follows according to the region from which they were sampled: A (Adana), H (Hatay), K (Kahramanmaraş, abbreviated hereafter as K'maraş), M (Mersin), and O (Osmaniye). Morphological characteristics of these caprifig accessions are listed in Table 1. *Profichi* (early) fruits were used for evaluation of some fruit parameters (Caliskan *et al.*, 2016) and the numbers of leaf lobes were also evaluated in 2014 and 2015 years (IPGRI & CIHEAM, 2003). For each caprifig accession, 30 profichi fruits and 30 leaves were used for morphological parameters. Fruit size was evaluated on a scale ranging from very small (<30 mm) to very large (>59 mm); the number of gall flowers per fruit was evaluated on a scale ranging from low (<250) to very high (>750) and the number of male flowers per fruit was evaluated on a scale ranging from very low (<75) to very high (>150). 'Persistent' caprifig accessions 'Mersin06' and 'Osmaniye02' have parthenocarphic fruit set, and other accessions that we used were 'cauducous' (non-parthenocarpic) caprifigs.

SSR genotyping

DNA was extracted using the procedure described by Lefort *et al.* (1998). The concentration and purity of the extracted DNA were analyzed using a NanoDrop® ND-1000 spectrophotometer.

Microsatellite polymorphisms were identified using 15 SSR (simple seaquence repeat) markers previously characterized in fig, namely LMFC18, LMFC23, LMFC24, LMFC25, LMFC27 and LMFC30 (Giraldo *et al.*, 2005); FCUPO08-2, FCUPO38-6, FCUPO044, FCUPO68-1 and FCUPO70 (Bandelj *et al.*, 2007); and MFC1, MFC2, MFC4 and MFC8 (Khadari *et al.*, 2001). These 15 fig SSRs were chosen based on their high polymorphism information content (PIC).

SSR-PCR amplifications were carried out in 11.1-µL reactions containing 0.5 units (0.07 µL) of GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA), 15 ng (in 6 µL) of template DNA, 0.5 pmol of each forward and reverse primer, 0.5 mM of each dNTP (1 µL of each primer), 25 mM $MgCl_2$ (1 µL) and 5X PCR buffer (2 µL). The temperature cycling conditions for DNA amplification were 94 °C for 3 min; followed by 35 cycles of 1 min at 94 °C, 1 min at 50–60 °C, 2 min at 72 °C and a final extension at 72 °C for 10 min (Caliskan *et al.*, 2012). Forward primers for each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France) to allow these amplicons to be distinguished by their fluorescent dye tags when PCR products are separated in the same capillary. PCR products were diluted in sample loading solution (20 µL SLS) and standards from the GenomeLab™ DNA Standard-400

Table 1. Fruit and leaf characteristics of the caprifig accessions analyzed in the present study.

Accession	Fruit size	AMF[a]	AGF[b]	NLL[c]	Accession	Fruit size	AMF[a]	AGF[b]	NLL[c]
Adana01	Medium	High	Medium	7	K'maraş01	Small	Very low	Medium	3
Adana02	Medium	Very high	Very High	5	K'maraş02	Small	Very low	Medium	5
Adana03	Medium	Very high	Very High	5	K'maraş03	Small	Very low	Medium	3
Adana04	Medium	Low	Low	3	K'maraş04	Small	Very low	High	5
Adana05	Small	High	High	3	K'maraş05	Small	Medium	Medium	5
Adana06	Small	Medium	Medium	5	K'maraş06	Medium	High	Very low	4
Adana07	Medium	Very low	Low	3	K'maraş07	Small	Low	Medium	4
Adana08	Small	Very high	High	5	K'maraş08	Small	High	Very low	4
Adana09	Medium	High	Medium	5	K'maraş09	Medium	Medium	High	4
Adana10	Medium	Very high	High	3	K'maraş10	Small	Low	High	3
Adana11	Small	Medium	Medium	5	K'maraş12	Medium	Medium	Medium	3
Adana12	Medium	Very high	Medium	3	K'maraş14	Small	Very low	Very low	3
Hatay01	Medium	Medium	High	7	K'maraş15	Medium	Medium	Medium	3
Hatay02	Very large	Low	High	7	K'maraş16	Large	Low	Medium	5
Hatay03	Small	Medium	Medium	7	K'maraş17	Small	Medium	Medium	4
Hatay04	Medium	High	High	5	K'maraş18	Medium	Low	Very low	3
Hatay05	Medium	Medium	Very high	7	Mersin01	Medium	Low	High	3
Hatay06	Medium	Very high	High	3	Mersin02	Medium	Very low	Medium	1
Hatay08	Medium	High	High	5	Mersin03	Medium	High	High	7
Hatay09	Small	Medium	High	3	Mersin04	Medium	Very high	Medium	1
Hatay11	Small	Low	Medium	3	Mersin05	Medium	High	High	4
Hatay13	Medium	Very high	Very high	5	Mersin06	Medium	High	Very low	5
Hatay14	Small	High	Medium	3	Mersin07	Small	High	Medium	4
Hatay15	Medium	Medium	High	3	Mersin08	Medium	High	Medium	5
Hatay16	Medium	Very high	Medium	5	Mersin09	Medium	High	Medium	5
Hatay17	Small	Very high	High	7	Mersin10	Small	Low	Very low	4
Hatay18	Medium	Very high	Low	5	Mersin11	Medium	Very high	Medium	3
Hatay19	Medium	Very high	High	5	Mersin12	Medium	Very high	Medium	5
Hatay20	Large	Very high	Very high	5	Mersin13	Large	Very high	High	3
Hatay21	Medium	High	High	7	Mersin15	Very small	Very low	Very low	5
Hatay22	Medium	High	Medium	7	Mersin16	Small	Very low	Very low	5
Hatay23	Large	High	Medium	5	Mersin17	Small	Medium	Medium	3
Hatay24	Medium	Very high	High	7	Mersin18	Medium	High	High	3
Hatay25	Medium	Low	Medium	3	Mersin19	Very small	Low	Medium	3
Hatay26	Large	Medium	Medium	5	Mersin20	Small	Low	Medium	4
Hatay28	Medium	Medium	High	3	Mersin21	Small	Medium	High	4
Hatay29	Large	Low	High	3	Mersin22	Small	Medium	High	4
Hatay30	Medium	Low	Medium	3	Osmaniye01	Very small	Very high	High	5
Hatay32	Small	Very high	High	5	Osmaniye02	Very small	High	High	5
Hatay33	Medium	Very high	Medium	5	Osmaniye03	Very small	Very low	Very low	3
Hatay34	Medium	Medium	Medium	3	Osmaniye06	Small	High	Medium	3
Hatay35	Medium	High	Medium	4	Osmaniye08	Medium	Very high	Medium	3
Hatay36	Medium	Low	High	5	Osmaniye09	Medium	Medium	Very high	4
Hatay37	Medium	Very high	High	5	Osmaniye10	Small	Low	High	5
Hatay38	Small	Medium	High	5	Osmaniye11	Small	Medium	High	1

Table 1. Continued.

Accession	Fruit size	AMF[a]	AGF[b]	NLL[c]	Accession	Fruit size	AMF[a]	AGF[b]	NLL[c]
Küçük Konkur	Medium	Medium	Very high	5	Elma İlek	Large	High	Medium	5
Taşlık	Medium	High	High	5	Armut	Medium	High	High	5
Hamza	Medium	Medium	High	5	Ak İlek	Medium	High	High	5

[a] AMF: number of male flower per *profichi* fruit. [b] AGF: number of gall flower per *profichi* fruit. [c] NLL: number of leaf lobes.

(0.5 µL) were included. The amplified fragments were analyzed at least twice using a CEQ 8800XL Capillary DNA Analysis System (Beckman Coulter, Fullerton, CA, USA) to confirm reproducibility. Allele sizes were determined for each SSR locus using Beckman CEQ DNA Analysis Software (Version 8.0).

Molecular diversity analysis

The number of alleles (n), allele frequency, expected (H_e) and observed (H_o) heterozygosity, estimated frequency of null alleles (r), probability of identity (PI) and presence of identical genotypes were evaluated for each locus using IDENTITY version 1.0 software (Paetkau *et al.*, 1995). The fixation index (Fst) is equal to $(H_{exp}-H_{obs})/H_{exp}$, where H_e and H_o indicate expected and observed heterozygosity (Wright, 1965). The PI was calculated as $PI = \Sigma (p_i)^4 - \Sigma\Sigma (2p_ip_j)^2$, where p_i is the frequency of the ith allele.

Genetic similarity and cluster analysis

Microsat version 1.5 was used to calculate the proportion of shared alleles using the ps option (option 1-(ps)) to assess genetic distances between individuals, as described by Minch *et al.* (1995). Data were then converted into a similarity matrix, and a dendrogram was constructed using the unweighted pair-group with arithmetic mean (UPGMA) method (Sneath & Sokal, 1973) using the Numerical Taxonomy and Multiware Analysis System (NTSYSpc) (Rohlf, 2004).

Population genetic structure and genetic differentiation analysis

Population genetic parameters of the regional groups of caprifig accessions were investigated using Arlequin vers 3.5 software. In addition, Hardy-Weinberg (HW) equilibrium and linkage disequilibrium (LD) were analyzed between each pair of loci (Excoffier & Lischer, 2010). A neighbor-joining tree was designed using Nei's genetic distances in NTSYSpc (Rohlf, 2004). Gene flows (N_m) among accession groups were evaluated using Genetix 4.05 (Belkhir *et al.*, 2004). The population structures of the whole set of accessions and of each regional group of caprifigs were analyzed using the Bayesian Analysis of Population Structure (BAPS) vers 6.0 software (Corander *et al.*, 2008). The most likely number of clusters was predicted according to the procedure described by Evanno *et al.* (2005) using the ΔK statistic based on the rate of change with respect to K in the log probability of data.

Results

Caprifig morphological characteristics and SSR analysis

The preponderance of fruit sizes in the caprifig *profichi* crops were medium (52 accessions) or small (31 accessions). Fruit size was characterized as large for seven accessions, very small for five accessions and very large for one accession. The number of male flowers per profichi fruit was medium or higher in 70 of the accessions. The accessions were grouped into the following classes according to the number of gall flowers per fruit: medium (39 accessions), high (38 accessions), very low (9 accessions), very high (7 accessions) or low (3 accessions). Most accessions (39) had leaves with five lobes, while 31 accessions had leaves with three lobes and three accessions had unlobed leaves (Table 1).

As shown in Table 2, 15 microsatellite markers from 96 caprifig accessions grown and sampled in Turkey were analyzed, and a total of 124 polymorphic alleles were detected. The number of alleles per locus varied from 3 for LMFC18 and LMFC23 to 14 for FCUPO38-6 and FCUPO08, with an average allele number of 8.3. Mean H_e and H_o were 0.594 and 0.449, respectively. The H_o values for MFC1 and MFC4 were higher than those for other markers, and their r values (frequencies of null alleles) were also negative. Wright's Fst values show whether there was a deficiency or excess of heterozygosity, related to expected values. An excess of heterozygotes (negative Fst) was found for three markers and a deficiency of heterozygotes was found for 12 markers. The PI values for the most informative loci were 0.940 in LMFC23 with three alleles, 0.730 in LMFC24 with four alleles, and 0.682 in LMFC30 with 11 alleles. The least informative loci were found

Table 2. Locus names, allele size ranges (A) in bp, number of alleles (n), expected heterozygosity (H_e), observed heterozygosity (H_o), fixation index (F_{st}), frequency of null alleles (r), and probability of identity (PI) for polymorphic SSR loci in caprifigs.

Locus	A	n	H_e	H_o	F_{st}	r	PI
MFC1	159–193	6	0.622	0.854	-0.373	-0.142	0.307
MFC2	156–190	8	0.789	0.656	0.169	0.074	0.138
MFC4	197–221	4	0.649	0.718	-0.106	-0.041	0.323
MFC8	166–180	6	0.459	0.041	0.911	0.286	0.430
LMFC18	118–130	3	0.405	0.031	0.923	0.266	0.576
LMFC23	128–144	3	0.030	0.031	-0.033	-0.000	0.940
LMFC24	221–277	4	0.156	0.010	0.936	0.126	0.730
LMFC25	210–224	8	0.547	0.541	0.011	0.003	0.338
LMFC27	175–209	10	0.386	0.354	0.083	0.023	0.443
LMFC30	231–263	11	0.841	0.500	0.405	0.185	0.682
FCUPO038-6	142–178	14	0.856	0.729	0.148	0.068	0.065
FCUPO044	190–206	8	0.714	0.197	0.724	0.301	0.191
FCUP068-1	143–185	13	0.799	0.677	0.153	0.067	0.089
FCUPO08	142–178	14	0.822	0.593	0.279	0.125	0.092
FCUP070	150–174	12	0.842	0.802	0.048	0.022	0.075
Total	118–277	124					
Mean		8.3	0.594	0.449			0.361

in FCUPO038-6 (PI = 0.065), FCUPO70 (PI = 0.075), FCUPO68-1 (PI = 0.089) and FCUPO08 (PI = 0.092).

The non-random association of alleles at different loci (LD), namely between a marker locus and a phenotypic trait locus, is the starting point for association mapping studies. Levels of LD base on the amount and distribution of the genetic diversity, the mating system, selection regimes and recombination events in the ancestry of the genotypes (Song *et al.*, 2009; Rodriguez *et al.*, 2012). Some significant (p<0.05) LD was identified among the 15 loci analyzed (data not shown). Groups of regional accessions varied in the numbers of loci pairs that exhibited significant LD. Accessions sampled in Hatay had the most pairs of loci in LD (17), whereas those accessions sampled in Mersin or Osmaniye had the fewest pairs of loci in LD (2). In some caprifig groups, several loci diverged from HW expectations. Accessions included in the Hatay group had the most pairs of loci that diverged from HW equilibrium (12 of 15 loci), whereas the reference group had the fewest pairs loci that diverged from HW equilibrium had (3 of 15 loci).

Genetic relationships among caprifig accessions

The largest similarity index values were observed between the accessions Hatay19 and Hatay22 (0.97), Hatay28 and Hatay29 (0.90) and Hatay19 and Hatay37 (0.90) (data not shown). To explain the genetic relationships among these caprifig accessions, a dendrogram was generated using UPGMA hierarchical clustering of pairwise genetic distances over 15 SSR loci. The genetic relationships among these caprifig accessions are shown in Figure 1. These accessions grouped predominantly into Group III, which is further comprised of different subgroups, whereas the other accessions clustered into Groups I, II, IV, V, VI and VII. Putatively synonymous accessions were not found within clusters among the caprifigs.

The accessions Mersin01, Mersin04 and Mersin05, which have medium-sized fruit, were included in Group I together with Hatay09, which had small fruit. Six accessions were included in Group II. The accession Mersin06, which had persistent fruit set, clustered together with Mersin08. These accessions also had similar fruit size, numbers of male flowers per profichi fruit and leaf lobe numbers. The majority of these caprifig accessions (75) clustered within Group III displayed a diverse set of morphological characteristics, including fruit size, the number of male flowers per *profichi* fruit, the number of gall flowers per profichi fruit and the number of leaf lobes. In most cases, the cluster positions of accessions were not related to their morphological characteristics or area of origin. Accessions from the Hatay province were grouped together more often than accessions from other provinces. The accessions Hatay22 and Hatay19 were very closely genetically related, but differed in some

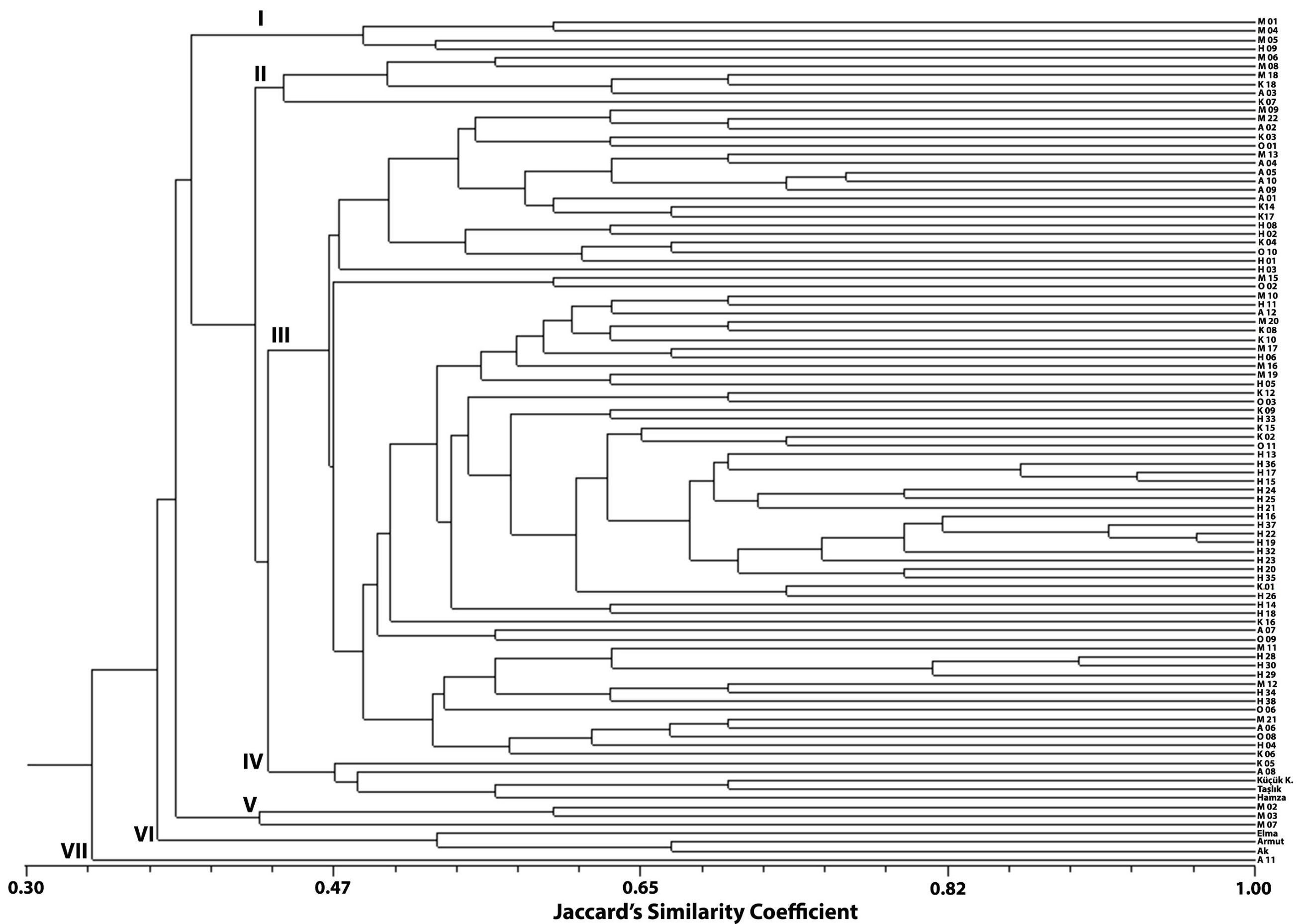

Figure 1. Dendrogram of genetic similarity of 96 caprifig accessions analyzed using 15 SSR markers.

morphological characteristics, such as the number of gall flowers per *profichi* fruit and the number of leaf lobes. Two accessions with persistent fruit set, Osmaniye02 and Mersin15, were grouped together, and separate from all other accessions. These two accessions also shared fruit size (small) and leaf morphology (five lobes).

The reference cultivars 'Küçük Konkur', 'Taşlık' and 'Hamza' were closely genetically related and clustered together in Group IV, which also included the accessions Adana08 and K'maraş08. 'Küçük Konkur', 'Taşlık' and 'Hamza' have medium-sized fruit and leaves with five lobes, whereas the accessions Adana08 and K'maraş08 have small fruits. In Group V, the accessions Mersin02, Mersin03 and Mersin 07 have distinct fruit sizes, numbers of male and gall flowers, and numbers of leaf lobes, but were nonetheless found to have close genetic relationships. Group VI comprised three cultivars, including 'Armut İlek', 'Ak İlek' and 'Elma İlek'. The 'Armut İlek' and 'Ak İlek' cultivars have medium-sized fruit, high numbers of male and gall flowers, and leaves with five lobes. The Adana011 accession, which has small fruit and an intermediate number of male and gall flowers, was distinct from all the other accessions and cultivars in Group VII.

Genetic relationships and population structure in caprifig groups

Some genetic variables such as H_e and H_o and the number of alleles per locus were investigated for the five regional caprifig groups and the reference group shown in Table 3. Mean H_o values were lower than H_e values except in the Osmaniye caprifig group. The proportion of polymorphic loci ranged between 0.867 and 0.933. The regional caprifig groups varied significantly in allele frequencies and profiles at the loci analyzed. Each regional caprifig group had three or more high-frequency alleles. The mean number of alleles per locus varied from 3.20 for the reference group to 5.60 for the Mersin group. Genetic differentiation (F_{st}) values (Table 4) ranged from 0.007 between the Osmaniye and K'maraş groups to 0.182 between the reference and Hatay groups. These genetic parameters showed that some caprifig groups differed genetically from the others, but all were more similar among them than compared with the reference group. In addition, there was significant gene flow between some caprifig regional groups such as Osmaniye and K'maraş or Osmaniye and Mersin (Table 5). Genetic similarity among the caprifig groups was evaluated using Nei's

Table 3. Expected and observed heterozygosities of caprifig groups.

Group	Heterozygosity		Polymorphic locus[c]		Mean of alleles/locus
	He[a]	Ho[b]	*p* (0.95)	*p* (1.00)	
Mersin	0.615±0.232	0.451±0.328	0.933	0.933	5.60
Adana	0.547±0.257	0.500±0.369	0.933	0.933	4.80
K'maraş	0.552±0.281	0.488±0.332	0.933	0.933	5.33
Osmaniye	0.542±0.273	0.458±0.339	0.867	0.867	4.07
Hatay	0.527±0.260	0.438±0.347	0.867	0.933	5.00
Reference	0.453±0.263	0.289±0.298	0.867	0.867	3.20

[a] H_e: expected heterozygosity. [b] H_o: observed heterozygosity. [c] A locus is considered polymorphic if the frequency of the common allele does not exceed 0.95.

Table 4. Pairwise F_{st} values among caprifig groups.

Group	Mersin	Adana	K'maraş	Osmaniye	Hatay	Reference
Mersin	-					
Adana	0.030*	-				
K'maraş	0.041***	0.050***	-			
Osmaniye	0.016ns	0.018ns	0.007ns	-		
Hatay	0.057***	0.097***	0.050***	0.041*	-	
Reference	0.135***	0.134**	0.118***	0.120**	0.182***	-

**p* <0.05, ***p* <0.01, ****p* <0.001. ns: not significant.

Table 5. Gene flow (N_m) among caprifig groups.

Group	Mersin	Adana	K'maraş	Osmaniye	Hatay	Reference
Mersin	-					
Adana	11.12	-				
K'maraş	6.92	5.36	-			
Osmaniye	58.02	26.22	59.99	-		
Hatay	4.48	2.44	5.20	7.08	-	
Reference	1.83	1.82	2.09	2.30	1.19	-

standard coefficient of genetic distance, and clustering was performed using the genetic distance data (Fig. 2). Genetic distance values and cluster analyses revealed high genetic similarities between caprifig groups, except for the reference group. The Hatay caprifig accessions demonstrated the lowest similarity (67.1%) to the reference caprifigs. K'maraş caprifigs showed high similarity to the geographically close Osmaniye caprifig group, with a high gene flow value (N_m = 59.99) between these two caprifig groups (Tables 5 and 6).

Factorial correspondence analysis (FCA) revealed little substructure within caprifig groups (Fig. 3). The first axis represented 40.61%, the second 26.40% and the third 20.69% of the overall variability between caprifig individuals. The most genetically distinct caprifig accessions were found in the Hatay and Adana groups. Osmaniye caprifigs showed relatively little overlap with the Mersin and K'maraş caprifig groups. In addition, the Adana caprifig group overlapped only slightly with the Mersin group. The unremarkable overlaps among these caprifig groups indicated the low level of genetic similarity among these caprifig accessions (Tables 5 and 6).

Population structure analyses using k-means hierarchical clustering revealed six caprifig clusters, with k = 5 best modeling the population structure of these caprifig accessions (Fig. 4). Genetic distance values were high between the Hatay and reference caprifig groups, but each had homogenous within-group population structure. Slight overlap was detected between Osmaniye and Mersin or between Osmaniye and K'maraş caprifigs, but the FCA analysis showed homogenous within-group population structure.

Discussion

SSR genotyping

Phenotypic variation in some plant species, especially clonally propagated fruit species such as

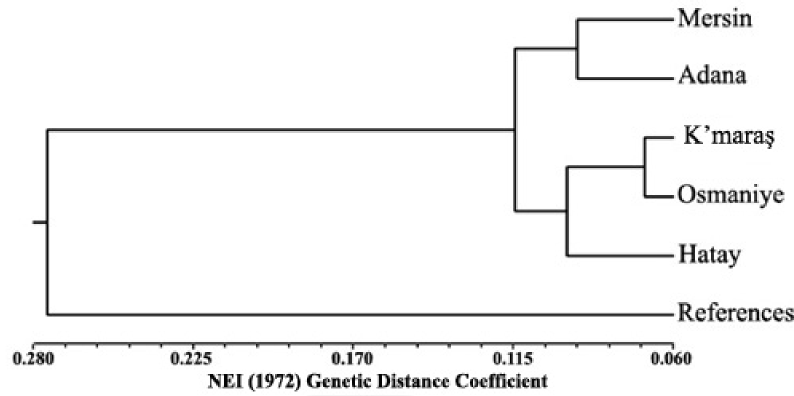

Figure 2. Genetic relationships among the caprifig regional groups based on Nei's (1972) genetic distance.

fig, can depend on plant age, cultural management and genotype-by-environment interactions. Therefore, morphological parameters are often not entirely dependable for classifying fig germplasm. However, molecular markers are useful for both identifying and classifying individual accessions and for eliminating similar, synonymous and homonymous accessions from a germplasm collection. Our results showed that SSR markers were useful for caprifig germplasm characterization, in agreement with previous results for edible figs (Giraldo *et al.*, 2005; Ikegami *et al.*, 2009; Aradhya *et al.*, 2010).

Figs have become adapted to the Mediterranean region after a long history of domestication and cultivation in the region. Currently, Turkey represents one center of diversity for figs, with various fig subspecies including *F. carica* var. *caprificus* (caprifigs), *F. carica* var. *domestica* (edible figs) and *F. carica* var. *rupestris* (Davis, 1978) growing throughout the country. Archaeobotanical studies have also shown that early fig culture in Anatolia corresponds to the current fig-growing areas of the Eastern Mediterranean region of Turkey (Ulas & Fiorentino, 2010). Further, Caliskan & Polat (2012) reported that the most important cultivar, 'Sarılop', which is grown in the Aegean region for dried figs, is genetically very close to the 'Sultani', 'Meryemi' and 'Armut Sapı' local cultivars grown in Hatay in the eastern Mediterranean region of Turkey. Similarly, the caprifig cultivars 'Küçük Konkur', 'Taşlık' and 'Hamza' are closely genetically related to the Adana08 and K'maraş08 accessions.

Estimates of allelic richness can be affected by sample size, plant species and marker systems (Bashalkhanov *et al.*, 2009; Landguth *et al.*, 2012). The numbers of alleles at MFC1, MFC2 and MFC8 (Giraldo *et al.*, 2008; Aradhya *et al.*, 2010; Caliskan *et al.*, 2012), and at LMFC27 and LMFC30 (Ikegami *et al.*, 2009), were much lower in fig germplasm collections comprised of numerous fig accessions compared to the number of alleles at those markers in this set of caprifigs from Turkey. Essid *et al.* (2015) revealed 6 alleles for LMFC30, 3 for MFC1 and 3 for MFC2 in Tunisian caprifigs. Our data indicate a high level of allelic richness in caprifigs grown in Turkey, perhaps because these accessions came from geographically diverse areas near the origin of fig culture. The allelic diversity of these caprifigs could also be due to outcrossing mediated through pollination by *Blastophaga* wasps.

The H_e and H_o values for various loci showed that the gene and genotype frequencies in caprifig varied from Hardy-Weinberg expectations. However, mean H_e was higher than H_o. The heterogeneity among loci for

Table 6. Nei's (1972) genetic distance between caprifig groups[a].

Group	Mersin	Adana	K'maraş	Osmaniye	Hatay	References
Mersin	1.000					
Adana	0.093 (90.7)	1.000				
K'maraş	0.105 (89.5)	0.119 (88.1)	1.000			
Osmaniye	0.088 (91.2)	0.093 (90.7)	0.070 (93.0)	1.000		
Hatay	0.107 (89.3)	0.174 (82.6)	0.093 (90.7)	0.100 (90.0)	1.000	
Reference	0.299 (70.1)	0.265 (73.5)	0.233 (76.7)	0.251 (74.9)	0.329 (67.1)	1.000

[a] Values represent N_m, and each value in parentheses indicates percentage of genetic similarity between that pair of groups.

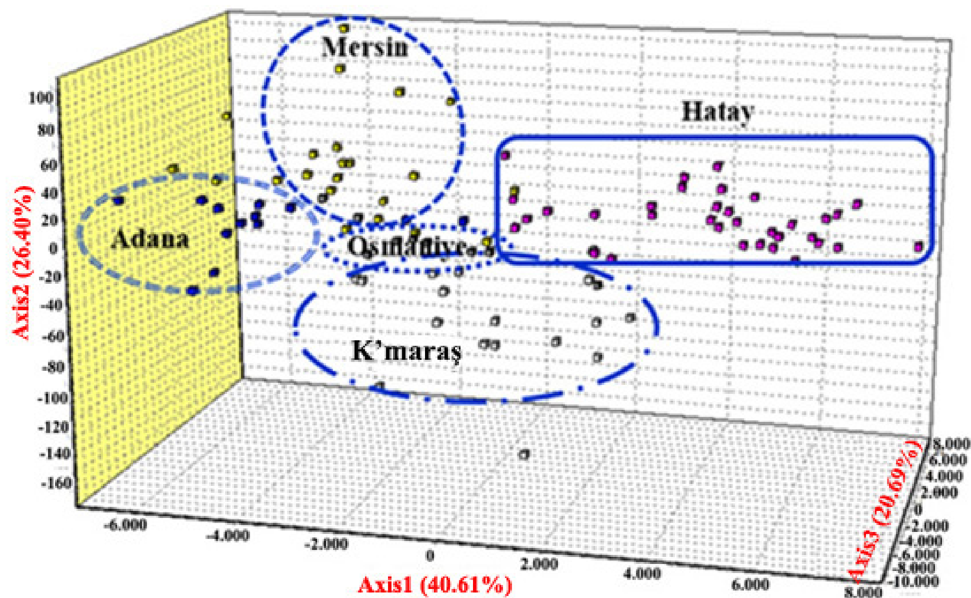

Figure 3. Factorial correspondence analysis of five caprifig groups.

both heterozygosity and F_{st} values reflect a complex breeding system and panmixis in the history of caprifig germplasm. In addition, genetic diversity within the caprifigs, revealed as sharply divided clusters, shows that much of the variation in caprifig is confined to the level of individuals as multilocus heterozygotes. Thus, our data agree with those of Aradhya *et al.* (2010), who reported that most genetic variation within figs is locked up at the level of individuals as polymorphic.

The clusters identified here did correspond to the geographic origin of the caprifig accessions. Especially, Osmaniye caprifigs, which showed the lowest F_{st} and genetic distance, and highest gene flow were closely related to other caprifigs. The result was consistent with its central geographic location among the five provinces. In addition, the caprifig accessions grown in Hatay did cluster together by geographic origin, although some of them clustered together with the caprifigs grown in Kahramanmaraş (K'maraş). We know that growers of edible figs in the Kahramanmaraş area come to Hatay to obtain caprifig profichi fruits, because caprifigs in their region mature too late to use for early caprification in Kahramanmaraş. Thus, some caprifigs grown in Kahramanmaraş can be expected to be closely related genetically to those from Hatay. However, previous studies also indicated some limited clustering of fig genotypes according to geographic region (Salhi-Hannachi *et al.*, 2006; Dalkilic *et al.*, 2011; Essid *et al.*, 2015).

Traditionally, fig cultivars are classified according to skin and flesh color, floral characteristics and pollination requirement or parthenocarpy. However, classifications made according to these characteristics can differ from those based on genetic markers (Giraldo *et al.*, 2008; Aradhya *et al.*, 2010). The caprifig classifications based on molecular markers here were consistent with those based on fruit size, floral characteristics or the number of leaf lobes. However, persistent accessions clustered together with cauducous accessions in marker-based classifications. Adana11 was not genetically similar to the other caprifigs accessions, which suggests that Adana11 is a wild caprifig. Congruently, Storey & Condit (1969) had previously indicated that wild Mediterranean figs exhibit diverse morphological characteristics and ecological adaptations. Thus, such a result is not unexpected.

Genetic relationships among caprifig groups

Our analysis could clearly distinguish six caprifig groups. The Hatay and reference caprifig groups were distinct from all of the other groups, and also had the lowest genetic similarity to and gene flow with each other. The caprifig accessions in the Osmaniye group overlapped only slightly with those in the Mersin and K'maraş caprifig groups according to our FCA analysis. The genetic relationship between the Osmaniye and K'maraş caprifig groups was also supported by Neighbor Joining analysis and they exhibited relatively homogenous within-group genetic structure. This result could be due to gene flow between these caprifig groups or to transfer of individual plants (human-mediated migration) between these regions. Our data suggest that there was some mechanism for exchange of caprifig genetic material between these groups.

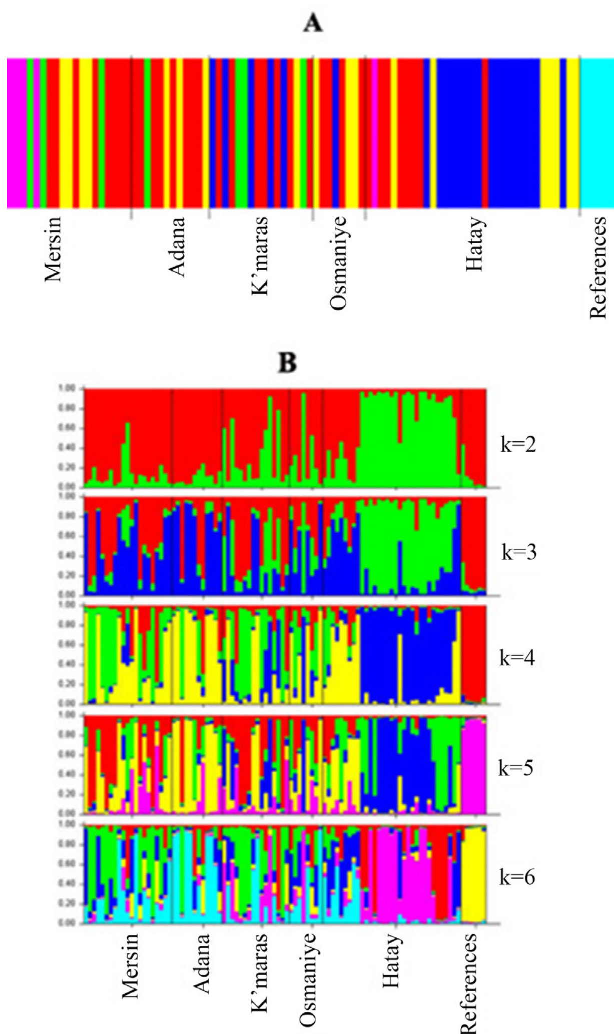

Figure 4. Population STRUCTURE analysis of all caprifig accessions organized by regional group (A). The population structure for k = 2, k = 3, k = 4, k = 5 and k = 6 as the number of clusters represented in these 96 caprifig accessions (B). Each individual accession is symbolized by a slim band divided into k colored sections representing k clusters.

As in the FCA, the population structure analysis showed that the Hatay and reference caprifig groups were more genetically uniform than other caprifig groups. The data showed that there was little gene flow between these groups and the other regional caprifig groups. The F_{st} values reflecting genetic diversity showed that the reference and Hatay caprifig groups were the most genetically distant (F_{st} = 182), whereas the Osmaniye and K'maraş caprifig groups were the least genetically distant (F_{st} = 0.007). This analysis identified differential gene flow between specific caprifig groups. Geographical distance could have had a negative effect on genetic exchange between some sampling areas. Thus, the high inter-group F_{st} values between the reference and Hatay caprifig groups are consistent with the relatively distant locations from which these accessions were sampled in Turkey. In contrast, the low F_{st} value between the Osmaniye and K'maraş groups revealed high gene flow between these groups, which is not surprising due the close proximity of the areas from which accessions in these groups were sampled. The data were also similar to the gene flow and genetic distance values (Tables 5 and 6).

Population structure analysis revealed six caprifigs groups that reflect the areas in Turkey from which the accessions were sampled. Cluster analysis (CA) revealed different clusters than those identified using genetic distance-based analysis. Aradhya *et al.* (2010) indicated that differences in results of population structure analysis when using CA or Bayesian approaches are likely due to differences between distance- and model-based hypotheses. In addition, our genetic data suggested that our caprifig accessions included groups with moderate population substructure that were comprised mostly of segregating individuals.

Some significant LD was apparent between pairs of the 15 microsatellite loci analyzed here. LD was strongest in the Hatay and reference groups (between 17 and 10 gene pairs, respectively). However, the Mersin, Osmaniye, Adana and K'maraş groups showed low LD values (between 2 to 8 pairs of loci). Akcay *et al.* (2014) indicated that high LD in pears could be due to gene flow between groups of accessions. Campoy *et al.* (2016) showed that LD could be important in sweet cherry because vegetative propagation of this horticultural species results in relatively fewer recombination events. However, LD decay can occur more quickly in cross-pollinated species compared to self-pollinated plant species due to lower heterozygosity in the latter (Gaut & Long, 2003), as well as in small populations (Dunning *et al.*, 2000).

Our analysis of the molecular genetics and population genetic structure of caprifig accessions from the center of origin of figs in Turkey revealed great genetic diversity and intensive differentiation of caprifigs. This rich genetic variation could have been due to establishment of caprifig populations propagated by seed. Our data for caprifigs reaches conclusions opposite to those of some previous studies on edible figs (Khadari *et al.*, 2001; Giraldo *et al.*, 2005; Aradhya *et al.*, 2010; Caliskan *et al.*, 2012), which hypothesized a narrow genetic basis for edible figs due to their long history of domestication and cultivation of relatively few major cultivars.

In summary, this microsatellite-based analysis represents a first step towards a database for marker-assisted classification and analysis of the genetic structure of caprifig genetic resources that grow in the center of fig diversity around the Mediterranean. The data

presented here support the efficiency of microsatellite markers for both the description of genetic diversity and management of caprifig germplasm. These results revealed the great genetic variation available in caprifig germplasm resources from Turkey. The present study provides essential information to design a caprifig germplasm collection without duplication of plant material, to sustainably manage fig breeding programs and to establish strategies for conserving caprifig genetic resources.

References

Akcay ME, Burak M, Kazan K, Yuksel C, Mutaf F, Makir M, Ayanoglu H, Ergul A, 2014. Genetic analysis of Anatolian pear germplasm by simple sequence repeats. Ann Appl Biol 164: 441-452. https://doi.org/10.1111/aab.12113

Aradhya M, Stover E, Velasco D, Koehmstedt A, 2010. Genetic structure and differentiation in cultivated fig (*Ficus carica* L.). Genetica 138: 681-694. https://doi.org/10.1007/s10709-010-9442-3

Bandelj D, Javornik B, Jakse J, 2007. Development of microsatellite markers in the common fig, *Ficus carica* L. Molec Ecol Notes 7: 1311-1314. https://doi.org/10.1111/j.1471-8286.2007.01866.x

Bashalkhanov S, Pandey M, Rajora OP, 2009. A simple method for estimation genetic diversity in large populations from finite sample sizes. BMC Genetics 84: 1-10.

Beck NG, Lord EM, 1988. Breeding system in *Ficus carica*, the common fig. I. Floral diversity. Amer J Bot 75: 1904-1912. https://doi.org/10.1002/j.1537-2197.1988.tb11271.x

Belkhir K Goudet J, Chikhi L, Bonhomme F, 2004. Genetix, logiciel sous WindowsTM pour la génétique des populations, vers 4.05. 10 January 2017. http://www.genetix.univ-montp2.fr/genetix/genetix.htm.

Caliskan O, Polat AA, 2012. Morphological diversity among fig (*Ficus carica* L.) accessions sampled from the eastern Mediterranean region of Turkey. Turk J Agric For 36: 179-193.

Caliskan O, Polat AA, Celikkol P, Bakir M, 2012. Molecular characterization of autochthonous Turkish fig accessions. Span J Agric Res 10: 130-140. https://doi.org/10.5424/sjar/2012101-094-11

Caliskan O, Bayazit S, Ilgın M, Karatas N, Kocatas H, 2016. Effects of some applications on in vitro pollen germination of caprifig genotypes (*Ficus carica* var. *caprificus*). VII Int Sci Agric Symp, Jahorina (Bosnia & Herzegovina), 6-9 Oct, pp: 238-244.

Campoy JA, Lerigoleur-Balsemin E, Christmann H, Beauvieux R, Girollet N, Quero-Garcia J, Dirlewanger E, Barreneche T, 2016. Genetic diversity, linkage disequilibrium, population structure and construction of a core collection of *Prunus avium* L. landraces and bred cultivars. BMC Plant Biol 16: 49. https://doi.org/10.1186/s12870-016-0712-9

Condit IJ, 1947. The fig. Chronica Botanica, Waltham, MA, USA.

Corander J, Siren J, Arjas E, 2008. Bayesian spatial modeling of genetic population structure. Comp Stat 23:111-129. https://doi.org/10.1007/s00180-007-0072-x

Dalkilic Z, Mestav HO, Gunver-Dalkilic G, Kocatas H, 2011. Genetic diversity of male fig (*Ficus carica caprificus* L.) genotypes with random amplified polymorphic DNA (RAPD) markers. Afr J Biotec 10: 519-526.

Davis PH, 1978. Flora of Turkey and the East Aegean Islands. Edinburgh Univ Press, Edinburgh.

Dunning AM, Durocher F, Healey CS, Teare MD, McBride SE, Carlomagno F, Xu CF, Dawson E, Rhodes S, Ueda S, *et al.*, 2000. The extent of linkage disequilibrium in for populations with distinct demographic histories. Amer J Human Genet 67: 544-554. https://doi.org/10.1086/316906

Essid A, Aljane F, Ferchichi A, Hormoza JI, 2015. Analysis of genetic diversity of Tunusian caprifig (*Ficus carca* L.) accessions using simple seaquence repeat (SSR) markers. Hereditas 152: 1-7. https://doi.org/10.1186/s41065-015-0002-9

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14: 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier L, Lischer HEL, 2010. Arlequin suite vers 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Flaishman MA, Rodov V, Stover E, 2008. The fig: Botany, horticulture, and breeding. Hortic Rev 34: 113-197.

Flaishman MA, Zohar RP, Freiman ZE, Yablovitz Z, 2015. Conventional and molecular breeding systems in fig (*Ficus carica* L.). V. Int Symp on Fig, Napoli (Italy), Aug 31-Sept 3, Abstract Book, p. 8.

Galil J, Neeman G, 1977. Pollen transfer and pollination in the common fig (*Ficus carica* L.). New Phytol 79: 163-171. https://doi.org/10.1111/j.1469-8137.1977.tb02192.x

Gaut BS, Long AD, 2003. The lowdown on linkage disequilibrium. Plant Cell 15: 1502-1508. https://doi.org/10.1105/tpc.150730

Giraldo E, Viruel MA, Lopez-Corrales M, Hormaza JI, 2005. Characterisation and cross-species transferability of microsatellites in common fig (*Ficus carica* L.). J Hortic Sci Biotec 80: 217-224.

Giraldo E, Lopez-Corrales M, Hormaza JI, 2008. Optimization of the management of and ex-situ germplasm bank in common fig with SSRs. J Amer Soc Hort Sci 133: 69-77.

Giraldo E, Lopez-Corrales M, Hormaza JI, 2010. Selection of the most discriminating morphological qualitative

variables for characterization of fig germplasm. J Amer Soc Hort Sci 135: 240-249.

Ikegami H, Nogata H, Hırashima K, Awamura M, Nakahara T, 2009. Analysis of genetic diversity among European and Asian fig varieties (*Ficus carica* L.) using ISSR, RAPD and SSR markers. Genet Resour Crop Evol 56: 201-209. https://doi.org/10.1007/s10722-008-9355-5

IPGRI and CIHEAM, 2003. Descriptors for figs. Int Plant Genet Resour Inst (IPGRI, Rome, Italy, and International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Paris, France.

Khadari B, Hochu J, Santoni S, Kjellberg F, 2001. Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus Ficus. Molec Ecol Notes 1: 191-193. https://doi.org/10.1046/j.1471-8278.2001.00072.x

Kislev ME, Hartmann A, Bar-Yosef O, 2006. Early domesticated fig in the Jordan Valley. Science 321: 1372-1374. https://doi.org/10.1126/science.1125910

Landguth EL, Fedy BC, Oyler-McCance SJ, Garey AL, Emel SL, Mumma M, Wagner HH, Fortin MJ, Cushman SA, 2012. Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. Mol Ecol Resour 12: 276-284. https://doi.org/10.1111/j.1755-0998.2011.03077.x

Lefort F, Lally M, Thompson D, Douglas GC, 1998. Morphological traits microsatellite fingerprinting and genetic relatedness of a stand of elite oaks (*Q. robur* L.) at Tuallynally, Ireland. Silvae Genet 47: 5-6.

Minch E, Ruiz-Linares A, Goldstein D B, Feldman M, Cavalli-Sforza LL, 1995. *Microsat* (vers 1.4d): a computer program for calculating various statistics on microsatellite allele data. Stanford Univ, Ca, USA.

Mori K, Shirasawa K, Nogata H, Hirata C, Tashiro K, Habu T, Kim S, Himeno S, Kuhara S, Ikegami H, 2017. Identification of RAN1 orthologue associated with sex determination through whole genome sequencing analysis in fig (*Ficus carica* L.). Sci Rep 7: 1-11.

Paetkau D, Calvert W, Stirling I, Strobeck C, 1995. Microsatellite analysis of population structure in Canadian polar bears. Molec Ecol 4: 347-354. https://doi.org/10.1111/j.1365-294X.1995.tb00227.x

Podgornik M, Vuk I, Vrhovnik I, Mavsar DB, 2010. A survey and morphological evaluation of fig (*Ficus carica* L.) genetic resources from Slovenia. Sci Hortic 125: 380-389. https://doi.org/10.1016/j.scienta.2010.04.030

Rodriguez M, Rau D, O'Sullivan D, Brown AHD, Papa R, Attene G, 2012. Genetic structure and linkage disequilibrium in landrace populations of barley in Sardinia. Theor Appl Genet 125: 171-184. https://doi.org/10.1007/s00122-012-1824-8

Rohlf F, 2004. NTSYS-pc: Numeric Taxonomic Systems. Applied Biostatistics Inc. Version 2.20. Setoukat, NY, USA.

Salhi-Hannachi A, Chatti K, Saddoud O, Mars M, Rhouma A, Marrakchi M, Trifi M, 2006. Genetic diversity of different Tunusian fig (*Ficus carica* L.) collections revealed RAPD fingerprints. Hereditas 143: 15-22. https://doi.org/10.1111/j.2005.0018-0661.01904.x

Sneath PHA, Sokal RR, 1973. Numerical taxonomy. Freeman, San Francisco, Ca, USA.

Song BH, Windsor AJ, Schmid KJ, Ramos-Onsins S, Schranz ME, Heidel, AJ, Mitchell-Olds, T, 2009. Multilocus patterns of nucleotide diversity, population structure and linkage disequilibrium in Boechera stricta, a wild relative of Arabidopsis. Genetics 181: 1021-1033. https://doi.org/10.1534/genetics.108.095364

Storey WB, Condit IJ, 1969. Fig (*Ficus carica* L.) In: Outlines of perennial crop breeding in the tropics, Misc paper 4; Ferwerda FP & Wit F (eds). Landbouwhogeschool, pp: 259-267.

Stover E, Aradhya M, 2008. Fig genetic resources and research at the US national clonal germplasm repository in Davis, California. Acta Hortic 798: 57-68. https://doi.org/10.17660/ActaHortic.2008.798.6

Ulas B, Fiorentino G, 2010. Preliminary archaeobothanical analyses from the site of Yumuktepe. J Arche Art 135: 1-10.

Wright S, 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420. https://doi.org/10.1111/j.1558-5646.1965.tb01731.x