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

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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 unmixxed, 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); Fs (fixation index); Hₑ (expected heterozygosity); Hₒ (observed heterozygosity); HW (Hardy-Weinberg equilibrium); LD (linkage disequilibrium); NYSYSpe (numerical taxonomy and multivariate analysis system software); PCR (polymerase chain reaction); PI (probability of identity); SSR (simple sequence repeat); UPGMA (unweighted pair-group with arithmetic mean).

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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 (Gall & 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’, ‘Kićık 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’maras), 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 very 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 sequence repeat) markers previously characterized in fig, namely LMFC18, LMFC23, LMFC24, LMFC25, LMFC27 and LMFC30 (Giraldo et al., 2005); FCUPO08-2, FCUPO38-6, FCUPO044, FCUPO068-1 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 MgCl2 (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* | AGF* | NLL* | Accession | Fruit size | AMF* | AGF* | NLL* |
|-----------|------------|------|------|------|-----------|------------|------|------|------|
| Adana01   | Medium     | High | Medium | 7    | K’maras01 | Small      | Very low | Medium | 3    |
| Adana02   | Medium     | Very high | Very High | 5    | K’maras02 | Small      | Very low | Medium | 5    |
| Adana03   | Medium     | Very high | Very High | 5    | K’maras03 | Small      | Very low | Medium | 3    |
| Adana04   | Medium     | Low   | Low   | 3    | K’maras04 | Small      | Very low | High   | 5    |
| Adana05   | Small      | High  | High  | 3    | K’maras05 | Small      | Medium | Medium | 5    |
| Adana06   | Small      | Medium | Medium | 5    | K’maras06 | Medium     | High   | Very low | 4    |
| Adana07   | Medium     | Very low | Low   | 3    | K’maras07 | Small      | Medium | Low    | 4    |
| Adana08   | Small      | Very high | High  | 5    | K’maras08 | Small      | High   | Very low | 4    |
| Adana09   | Medium     | High  | Medium | 5    | K’maras09 | Medium     | Medium | High   | 4    |
| Adana10   | Medium     | Very high | High  | 3    | K’maras10 | Small      | Low    | High   | 3    |
| Adana11   | Small      | Medium | Medium | 5    | K’maras12 | Medium     | Medium | Medium | 3    |
| Adana12   | Medium     | Very high | Medium | 3    | K’maras14 | Small      | Very low | Very low | 3    |
| Hatay01   | Medium     | Medium | High  | 7    | K’maras15 | Medium     | Medium | Medium | 3    |
| Hatay02   | Very large | Low   | High  | 7    | K’maras16 | Large      | Low    | Medium | 5    |
| Hatay03   | Small      | Medium | Medium | 7    | K’maras17 | Small      | Medium | Medium | 4    |
| Hatay04   | Medium     | High  | High  | 5    | K’maras18 | 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    |
| Hatay07   | Medium     | High  | High  | 5    | Mersin03  | Medium     | High   | High   | 7    |
| Hatay08   | Small      | Medium | High  | 3    | Mersin04  | Medium     | Very high | Medium | 1    |
| Hatay09   | Small      | Low   | Medium | 3    | Mersin05  | Medium     | High   | High   | 4    |
| Hatay10   | Medium     | Very high | Very high | 5    | Mersin06  | Medium     | High   | Very low | 5    |
| Hatay11   | Small      | High  | Medium | 3    | Mersin07  | Small      | Medium | High   | 4    |
| Hatay12   | Medium     | Medium | High  | 3    | Mersin08  | Medium     | High   | Medium | 5    |
| Hatay13   | Medium     | Very high | Very high | 5    | Mersin09  | Medium     | High   | Medium | 5    |
| Hatay14   | Small      | High  | Medium | 3    | Mersin10  | Small      | Low    | Very low | 4    |
| Hatay15   | Medium     | Medium | High  | 3    | Mersin11  | Medium     | Very high | Medium | 3    |
| Hatay16   | Medium     | Very high | Medium | 5    | Mersin12  | Medium     | Very high | Medium | 5    |
| Hatay17   | Small      | Very high | Low   | 5    | Mersin13  | Large      | Very high | High   | 3    |
| Hatay18   | Medium     | Very high | High  | 5    | Mersin14  | Medium     | High   | High   | 3    |
| Hatay19   | Large      | Very high | Very high | 5    | Mersin15  | Small      | Very small | Very low | 5    |
| Hatay20   | Medium     | High  | Medium | 7    | Mersin16  | Small      | Very low | Very low | 5    |
| Hatay21   | Large      | High  | Medium | 5    | Mersin17  | Small      | Medium | Medium | 3    |
| Hatay22   | Medium     | Very high | Medium | 7    | Mersin18  | Medium     | High   | High   | 3    |
| Hatay23   | Medium     | Low   | Medium | 3    | Mersin19  | Very small | Low    | Medium | 3    |
| Hatay24   | Medium     | Medium | High  | 3    | Mersin20  | Small      | Low    | Medium | 4    |
| Hatay25   | Medium     | Medium | High  | 3    | Mersin21  | Small      | Medium | High   | 4    |
| Hatay26   | Large      | Low   | High  | 3    | Mersin22  | Small      | Medium | High   | 4    |
| Hatay27   | Medium     | Low   | Medium | 3    | Osmaniye01 | Very small | Very high | High   | 5    |
| Hatay28   | Medium     | Medium | Medium | 3    | Osmaniye02 | Very small | High   | High   | 5    |
| Hatay29   | Medium     | Medium | High  | 5    | Osmaniye03 | Very small | Very low | Very low | 3    |
| Hatay30   | Medium     | Medium | High  | 5    | Osmaniye04 | Small      | High   | Medium | 3    |
| Hatay31   | Medium     | Low   | Medium | 4    | Osmaniye05 | Medium     | Very high | Medium | 3    |
| Hatay32   | Large      | Low   | High  | 5    | Osmaniye06 | Medium     | Medium | Very high | 4    |
| Hatay33   | Medium     | Very high | Medium | 5    | Osmaniye07 | Small      | High   | Medium | 3    |
| Hatay34   | Medium     | Medium | High  | 3    | Osmaniye08 | Medium     | Very high | Medium | 3    |
| Hatay35   | Medium     | Low   | High  | 5    | Osmaniye09 | Medium     | Medium | Very high | 4    |
| Hatay36   | Medium     | Very high | High  | 5    | Osmaniye10 | Small      | Low    | High   | 5    |
| Hatay37   | Medium     | Medium | High  | 5    | Osmaniye11 | Small      | Medium | High   | 1    |
Table 1. Continued.

| Accession   | Fruit size | AMF* | AGF* | NLL | Accession   | Fruit size | AMF* | AGF* | NLL |
|-------------|------------|------|------|-----|-------------|------------|------|------|-----|
| 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 |

* AMF: number of male flower per profichi fruit. * AGF: number of gall flower per profichi fruit. * 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 (Hₑ) and observed (Hₒ) heterozygosity, estimated frequency of null alleles (r), probability of identity (PI) and presence of identical genotypes were evaluated for each locus using IDENTIFY version 1.0 software (Paetkau et al., 1995). The fixation index (Fst) is equal to (Hₑ-Hₒ)/Hₑ, where Hₑ and Hₒ indicate expected and observed heterozygosity (Wright, 1965). The PI was calculated as PI = Σ (pᵢ)ᵢ - ΣΣ (2pipᵢ)ᵢ, where pᵢ 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 (Nm) 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 Hₑ and Hₒ were 0.594 and 0.449, respectively. The Hₑ values for MFC1 and MFC-4 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
Genetic diversity and population structure in caprifigs using SSR markers

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

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 |
| FCUPO68-1| 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 |
| FCUPO70| 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 |
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 Mersin07 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’marcaş 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’marcaş or Osmaniye and Mersin (Table 5). Genetic similarity among the caprifig groups was evaluated using Nei’s
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’marash 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’marash 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’marash 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
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, ‘Sarılıop’, 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’maɾaş08 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 groupsa.

| Group     | Mersin | Adana | K’maɾaş | Osmaniye | Hatay | References |
|-----------|--------|-------|---------|----------|-------|------------|
| Mersin    | 1.000  |       |         |          |       |            |
| Adana     | 0.093 (90.7) | 1.000 |         |          |       |            |
| K’maɾaş   | 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 |

aValues represent $N_{m}$, and each value in parentheses indicates percentage of genetic similarity between that pair of groups.

Figure 2. Genetic relationships among the caprifig regional groups based on Nei’s (1972) genetic distance.
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.
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’maras 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 $F_{st}$ values between the Osmaniye and K’maras 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’maras 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.

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