Molecular characterization of autochthonous Turkish fig accessions

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Abstract

Turkey is one of the main genetic centers for fig tree, *Ficus carica* L. The genetic variabilities of 76 fig accessions from Hatay province of Turkey were evaluated by analysis of 10 simple sequence repeats (SSR) loci. The number of alleles revealed by SSR analysis ranged from 3 to 12 alleles per locus with a mean value of 6.8. A total of 68 alleles were detected by SSR and the average heterozygosity was higher than the expected one. In addition, seven random amplified polymorphic DNA (RAPD) primers detected a total of 68 clear and reproducible bands, 55 of which were polymorphic, so it was possible to effectively characterize these fig accessions with either marker techniques. In both marker systems, Mantel's correlation between similarity scores and cophenetic values was moderately high (0.90 for RAPD and 0.87 for SSR), which demonstrated that the clustering patterns fitted the data well. The clusters obtained using these types of markers were independent. This study indicated that there is great genetic variability among local fig accessions, making them a valuable genetic source for incorporation into potential breeding programs especially for table fig selections.

Additional key words: *Ficus carica*; genetic diversity; RAPD; SSR.

Resumen

Caracterización molecular de accesiones autóctonas de higo de Turquía

Turquía es uno de los principales centros genéticos de la higuera, *Ficus carica* L. Se evaluó, mediante análisis de 10 loci de microsatélites o repeticiones de secuencias simples (SSR), la variabilidad genética de 76 accesiones de higuera muestreadas en la provincia Hatay de Turquía. El número de alelos revelados por análisis SSR varió de 3 a 12 por locus con una media de 6,8; se detectaron en total 68 alelos y la heterocigosidad promedio fue mayor que la heterocigosidad esperada. Además, siete cebadores RAPD detectaron un total de 68 bandas claras y reproducibles, de las cuales 55 fueron polimórficas, por lo que estas accesiones de higuera pudieron ser eficazmente caracterizadas utilizando ambas técnicas. Tanto con SSRs como con RAPDs, la similitud y las correlaciones cofenéticas mediante la prueba de Mantel fueron moderadamente altas (0,90 para RAPD y 0,87 para SSR), lo que demuestra que los patrones de agrupamiento se ajustan bien a los datos. Los grupos obtenidos con este tipo de marcadores fueron independientes. Este estudio encontró una gran variabilidad genética entre accesiones autóctonas de higuera, lo que las hace una fuente valiosa para su incorporación potencial en programas de mejora, especialmente para higos de mesa.

Palabras clave adicionales: diversidad genética; *Ficus carica*; RAPD; SSR.

Introduction

Fig (*Ficus carica* L.) is a crop known since ancient times and used for fruit production (Beck & Lord, 1988). The common fig (2n = 2x = 26) belongs to the order Urticales, family Moraceae, with over 1400 species classified into about 40 genera (Watson & Dallwitz, 2004). The *Ficus* species are gynodioecious and

Abbreviations used: H_e (expected heterozygosity); H_o (observed heterozygosity); NTSYSpc (numerical taxonomy and multiware analysis system software); PCA (principal component analysis); PI (probability of identity); RAPD (random amplified polymorphic DNA); SSR (simple sequence repeat); UPGMA (unweighted pair-group with arithmetic mean).

functionally dioecious. Trees producing only ovule-bearing fruit are functionally female whereas trees producing only pollen are functionally male. Figs are typically pollinated by pollen-carrying wasps (Kjellberg *et al.,* 1987). *F. carica* is one of the most important fruit species well-adapted to the various regions of Turkey and many Mediterranean countries. Fig trees are widely distributed throughout Turkey in regions near the Black Sea, Marmara, the Aegean and the Mediterranean coasts, southern Anatolia and in the interior valleys of central Anatolia. Fig cultivation on the coastal part of the Mediterranean region in Turkey has a promising future for the fresh fig export. In this region, Hatay province has many fig accessions important for potential breeding studies (Polat & Caliskan, 2008). Turkey is a leading country in fig production with 270,830 t of total figs production and 26% share, and exports 134,061 t, representing 36% of total world fig exports (FAO, 2007; http://faostat.fao.org/site/567/default.aspx#ancor). A number of cultivated and wild forms of fig, with a great diversity of color, shape, and flavor can be found in Turkey, where they are used primarily for fresh consumption. Today, figs are an important crop worldwide for either dry or fresh consumption, and are a valuable source of minerals and vitamins, amino acids, antioxidants, and total phenols (Vinson, 1999).

For several decades, the quantity of figs produced in Turkey has fluctuated significantly due to vulnerability to biotic and abiotic stresses and loss of agricultural land to intensive urbanization. As a consequence of these trends, severe genetic erosion has threatened local fig germplasm. Moreover, at present, the actual number of cultivars is difficult to estimate due to errors in cultivar identification and naming. Therefore, it became essential to establish a research program aimed at the evaluation and preservation of fig germplasm in Turkey.

Previous studies have used morphometric traits and isozymes to demonstrate the significant phenotypic and genetic variability present in Turkish fig germplasm (Eroglu, 1982; Ilgın & Kuden, 1998; Aksoy *et al.,* 2003; Uzun *et al.,* 2003; Çalişkan & Polat, 2008). Plant morphological characteristics are generally influenced by environmental conditions; and the discriminant ones are limited in number and do not allow the separation of the phenotypes into distinct groups (Khadari *et al.,* 2003; Baraket *et al.,* 2009; Soriano *et al.,* 2011). Therefore, the derived characterizations are not suitable to establish reference genotypes for fig breeding programs. To overcome these limitations, large-scale DNA-based PCR methods using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers have been successfully designed for fig accessions. Also, more powerful DNA-based methods have been performed and their efficiency has been proven in the description of the polymorphisms within and between species (Cabrita *et al.,* 2001; Salhi-Hannachi *et al.,* 2006; Ikegami *et al.,* 2009). In the present study, RAPD and SSR markers were used to characterize local fig accessions from the Hatay province of Turkey and to provide a molecular database for fig breeding.

Material and methods

Plant material and DNA isolation

This study was conducted with the 76 local fig accessions listed in Table 1. These accessions were selected for development of table fig cultivars. Accession code number was used for sequence number of figs in the principal components analysis. Cultivar 'Sarılop' was the reference cultivar for SSR analyses. Leaf samples from these accessions were collected from Hatay Province, which is located in the Mediterranean region of Turkey. The accessions 'Bardak' and 'Dolap' are San Pedro-type figs and the other accessions are Smyrna-type or common figs.

DNA was extracted using the procedure described by Lefort *et al.* (1998). DNA quality and quantity were assessed on a 1% (w/v) agarose gel stained with ethidium bromide. The concentration and purity of the extracted DNA were analyzed using a NanoDrop® ND-1000 spectrophotometer.

SSR and RAPD analysis

Determination of microsatellite polymorphism was performed using ten different SSR markers: FCUPO27-4, FCUPO38-6, FCUPO66-7, FCUPO68-1 (Bandelj *et al.,* 2007), MFC1, MFC2, MFC8 (Khadari *et al.,* 2001), LMFC25, LMFC30 (Giraldo *et al.,* 2005), and FM4-70 (Zavodna *et al.,* 2005). The selection of the fig SSRs was based on their high polymorphism information content. These primers target regions of simple and complex microsatellite repeats. Using a thermocycler (Biometra®, Goettingen, Germany) PCR was performed with 200 ng (6 μL) of DNA as template. Each reaction included 0.5 μL primer, 0.5 mM of each dNTP (1 μL), 0.5 units of GoTaq (0.07 μL) (Promega, Madison, WI, USA), 25 mM MgCl$_2$ (1 μL) (Promega,

Table 1. Origin of local fig accessions collected in the eastern Mediterranean region of Turkey

Accession Number	Accession name	Location	Accession Number	Accession name	Location
1	Şebli	Samandağ	39	Sarı 2	Altınözü
2	Siyah 5	Samandağ	40	Fahli	Altınözü
3	Zırhıni	Samandağ	41	Allene Karası	Altınözü
4	Bığrasi 1	Samandağ	42	Siyah 2	Altınözü
5	Tınesvit	Samandağ	43	Armut Sapı	Altınözü
6	Mor 3	Antakya	44	Beyaz Fahli	Altınözü
7	Sütlü Sarı	Antakya	45	Kandamık	Altınözü
8	Bığrasi 2	Antakya	46	Sultani 1	Altınözü
9	Mersinli	Antakya	47	Kıreni 1	Altınözü
10	Sultani 2	Antakya	48	Sehli 1	Altınözü
11	Meryemi 2	Yayladağı	49	Siyah 1	Belen
12	Siyah 7	Yayladağı	50	Ahmediye	İskenderun
13	Kırmızı 2	Yayladağı	51	Meryemi 1	Belen
14	Ramlı 2	Yayladağı	52	Büyük Siyahlop	İskenderun
15	Sultani 3	Yayladağı	53	Bakras 3	Belen
16	Siyah 8	Yayladağı	54	Bığrasi 4	İskenderun
17	Ramlı 1	Yayladağı	55	Şeble 2	İskenderun
18	Lopkara 1	Yayladağı	56	Fransavi	İskenderun
19	Şibili	Yayladağı	57	Şami	İskenderun
20	Siyah 6	Yayladağı	58	Sıhle	İskenderun
21	Lopkara 2	Yayladağı	59	Hılvıni	İskenderun
22	Beyaz İncir	Yayladağı	60	Burnu Kızıl	İskenderun
23	Dolap	Yayladağı	61	Şeble 1	İskenderun
24	Karagöz	Yayladağı	62	Bakrasi 5	İskenderun
25	Bardak	Yayladağı	63	Sarı 4	Dörtyol
26	Siyah 4	Antakya	64	Halep İnciri	Dörtyol
27	Sarı 5	Antakya	65	Baldır İnciri	Hassa
28	Mor 2	Antakya	66	Payas	Hassa
29	Mor 1	Antakya	67	Gud Yeniği	Hassa
30	Kırmızı 1	Antakya	68	Sarı 3	Hassa
31	Erkenci	Antakya	69	Siyah 3	Hassa
32	Yeşil İncir	Antakya	70	Mor 4	Hassa
33	Kıreni 2	Altınözü	71	Sarı 6	Hassa
34	Mor 5	Altınözü	72	Sarı 1	Kırıkhan
35	Sehli 2	Altınözü	73	Kabak 1	Kırıkhan
36	Kuruye 2	Altınözü	74	Kabak 2	Kırıkhan
37	Fetike	Altınözü	75	Mor 6	Kırıkhan
38	Kuruye 1	Altınözü	76	Kilis İnciri	Belen

Madison, WI, USA), and 5X PCR buffer (2 μL) in a reaction volume of 11.1 μL. The amplification was initiated at 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. Forward primers for each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). Amplicons were separated by multiplexing by the use of different fluorescent dyes on different PCR products. PCR amplification was performed separately for each SSR locus and then the products run in the same lane. PCR products were first checked on a 2% (w/v) agarose gel in 1X TBE buffer at 100 V for 40 min and stained with 10 mg mL^{-1} ethidium bromide. PCR products were diluted in sample loading solution (20 μL SLS) and standards from the GenomeLab DNA Standard-400 (0.5 μL) were included. A total of 231 samples were loaded for electrophoresis in three groups according to fragment size and fluorescent dyes. 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 the Beckman CEQ DNA

Analysis Software (version 8.0). At each run, 'Sarılop' was included as reference.

RAPD analysis was carried out with an initial screening of 50 decamer primers (Operon Technologies). Seven primers yielded clear and reproducible bands which were used to identify genetic variation among fig accessions used in the study. PCR amplification for RAPD was performed in a thermocycler (Biometra® PCR System). Amplifications were performed according to Ergul *et al.* (2002) in a total volume of 25 µL containing 100 ng of DNA, 5U Taq polymerase, 0.3 µM primers, 2.5 mM dNTPs, 25 mM $MgCl_2$, and 10X PCR buffer (Fermentas, CA). Amplified samples were loaded on 1.2% agarose gels (mixture of 0.4% SeaKem LE agarose and 0.8% Nu Sieve GTG agarose, FMC Corporation) in 1X TBE buffer, and run at 100 V for 4 h. The molecular sizes of the amplification products were estimated using the 100 bp Plus DNA Ladder (Fermentas, CA). The analyses were duplicated to ensure the reproducibility of the banding patterns.

Data analysis

For RAPD marker analysis, bands were scored as either present or absent to generate a set of binary data. The complement of the Jaccard similarity coefficient (Sneath & Sokal, 1973) was used to assess dissimilarity as described by Leal *et al.* (2010) as a measure of genetic distance between accessions. Cluster analysis of RAPD markers of 76 accessions was performed using NTSYS-pc, Version 2.02 (Rohlf, 1998). A dendrogram was constructed based on Jaccard's similarity data applying the unweighted pair group method (UPGMA).

As to SSR 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 (calculated as $PI = \Sigma(p_i)^4 - \Sigma\Sigma(2p_ip_j)^2$ where p_i is the frequency of the ith allele), and presence of identical genotypes were determined for each locus using IDENTITY version 1.0 software (Wagner & Sefc, 1999), as described by Paetkau *et al.* (1995). Microsat version 1.5 (Minch *et al.,* 1995) was used to calculate the proportion of shared alleles using the ps option [option 1- (ps)] (Bowcock *et al.,* 1994) to assess genetic distances between individuals as described by Tangolar *et al.* (2009). Data were then converted to a similarity matrix and a dendrogram was constructed via the unweighted pair-group with arithmetic mean (UPGMA) method (Sneath & Sokal, 1973), using the

Numerical Taxonomy and Multiware Analysis System (NTSYSpc) software, version 2.0 (Rohlf, 1998).

To obtain estimates of the magnitudes of differences between the dendrograms constructed based on SSR and RAPD data, cophenetic value matrices were computed for each dendrogram, and these cophenetic matrices were compared by the Mantel's test correspondence test using the NTSYSpc numerical taxonomy package, version 2.0 (Rohlf, 1998). The EIGEN of NTSYSpc was used to perform cluster analysis based on Principal Component Analysis (PCA) (Chakravarthy & Naravaneni, 2006).

Results and discussion

SSR analysis

Genetic diversity of 76 fig accessions grown in Hatay, in addition to one reference cultivar ('Sarılop'), was investigated using ten SSR markers, and a total of 68 alleles (Table 2). The number of alleles per locus ranged from 3 for LMFC25 (the least informative locus, PI: 0.0912) to 12 for FCUPO38-6 (the most informative locus, PI: 0.7132) with an average of 6.8. These values, on per locus, were consistent with data reported by Khadari *et al.* (2004), while they were higher than those reported by Ikegami *et al.* (2009). The mean H_o and H_e were determined to be 0.71 and 0.68, respectively. The H_o values for loci MFC1, FCUPO27-4, FCUPO66-7, LMFC25, and LMFC30 were higher than H_e values. These results were similar to those of Khadari *et al.* (2001) and Giraldo *et al.* (2005). Levels of heterozygosity observed for all loci analyzed in this study were higher than those found in other fig studies (Giraldo *et al.,* 2005; Bandelj *et al.,* 2007). The frequency of null alleles (r) at the MFC2, FCUPO38-6, FCUPO68-1, and FM4-70 loci was the lowest (Table 2).

The similarity index values among accessions ranged from 0.15 to 1.00. The largest distance value was observed between the 'Beyaz Fahli' and 'Mor 5' accessions (0.15) (data not shown). Cluster analysis based on genetic distances detected by SSR marker analysis revealed that these 76 accessions can be divided into four main groups (Fig. 1). Group A includes 62 accessions and is correlated with Group B, with a similarity index value of 0. 42. The majority of the accessions of group A had an assortment of various morphological characteristics, such as black fruit skin color ('Siyah 3' and 'Siyah 4'), green-yellow fruit skin color ('Sultani 1', 'Sultani 2' and

Table 2. Simple sequence repeat (SSR) loci, number of alleles (n), expected heterozygosity (H_e), observed heterozygosity (H_o), probability of identity (PI), and frequency of the null allele (r) for 76 fig accessions analyzed

Locus	Allele size (bp)	Number of alleles (n)	Expected heterozygosity (H_e)	Observed heterozygosity (H_o)	Probability of identity (PI)	Frequency of null alleles (r)
MFC1	159-191	5	0.620	0.909	0.3095	–0.178
MFC2	156-188	6	0.711	0.597	0.2166	0.066
MFC8	172-182	4	0.545	0.545	0.4099	–0.000
FCUPO27-4	184-206	10	0.830	0.857	0.0912	–0.014
FCUPO38-6	150-184	12	0.854	0.720	0.7132	0.061
FCUPO66-7	143-163	6	0.675	0.844	0.2276	–0.100
FCUPO68-1	153-177	9	0.762	0.727	0.1360	0.020
LMFC25	212-222	3	0.409	0.519	0.5442	–0.078
LMFC30	239-261	8	0.712	0.779	0.2044	–0.038
FM4-70	192-206	5	0.663	0.610	0.3027	0.032
Total	143-222	68				
Mean		6.8	0.678	0.710	0.3155	

'Sultani 3'), or purple fruit skin color ('Şeble 1' and 'Şeble 2'). The greatest similarity was observed among figs with purple skin color in Group A. 'Mor 3', 'Siyah 7', 'Siyah 6', 'Kırmızı 1', 'Kuruye 2' 'Kuruye 1', and 'Sehli 1' accessions were found to have very close genetic relationships in subgroup A3. 'Kabak 1' and 'Kabak 2' accessions were found to be similar and grouped together in the main group (A1) which also included 'Sarı 1'. In Group B, 'Bığrasi 2', 'Bakras 3', 'Bığrasi 4' and 'Bakrasi 5' accessions were morphologically very similar to each other, and RAPD analysis of these also revealed similar results. Group C contained the fig accessions 'Allene Karası' and 'Siyah 2', with a similarity index value of 0.86, while 'Beyaz Fahli' had a similarity index value of 0.56 with Group C members. 'Allene Karası' and 'Siyah 2' accessions have black fruit skin colors, whereas 'Beyaz Fahli' has green-yellow fruit skin color. 'Hılvıni' accession appears separate from all the rest (Group D). The similarity index value between 'Hılvıni' and Group C was 0.38, which indicates a certain genetic relationship between these fig accessions.

RAPD analysis

From seven arbitrary RAPD primers tested, a total of 68 bands were amplified, and 55 of them were polymorphic. A total of 10 fragments were amplified using OPC04 and OPO14 primers, while only four were amplified with the OPB02 primer, with a mean of 7.86 bands per primer. All primers produced polymorphic products and banding patterns. Primers OPO14,

OPC20, and OPH3 generated more than 80% polymorphic bands, while OPBO2, OPB08, OPC04, and OPF05 primers showed 75-80% polymorphism (Table 3).

The analysis using seven RAPD primers permitted to distinguish all 76 accessions, except 'Kabak 1' and 'Kabak 2'. Four major groups were identified by the cluster analyses (Fig. 2). Groups C and D include 'Armut Sapı' and 'Şeble 1', respectively, while the other fig accessions are predominantly grouped in the first cluster (Group A), which is comprised of three different subgroups (A1 to A3). Group A1 includes 59 accessions, subdivided into eight subgroups (A1.1 to A1.8). The accessions 'Bığrasi 1', 'Bığrasi 4', 'Bığrasi 2' and 'Bakras 3' were included in the subgroup A1.1. The accessions 'Kabak 1' and 'Kabak 2' are included in A1.2 together with 'Sarı 1'. A close relationship is found between the accessions 'Sultani 2' and 'Sultani 3' (A1.2) and 'Kuruye 1' and 'Meryemi 1' (A1.3). The accessions 'Siyah 5', 'Siyah 7', 'Zırhıni', 'Mor 3', and 'Tinesvit' with purple skin colors are included in the second subgroup (A2) together with 'Sütlü Sarı' with yellow skin color. Six accessions were found in Group B. The genetic distance between fig accessions ranged from 0.44 to 1.00. 'Kabak 1' and 'Kabak 2' were found to be the closest (1.00), whereas 'Şeble 1', 'Siyah 7' and 'Yeşil İncir' were the most distant ones (0.44) (data not shown).

Combined RAPD and SSR analysis

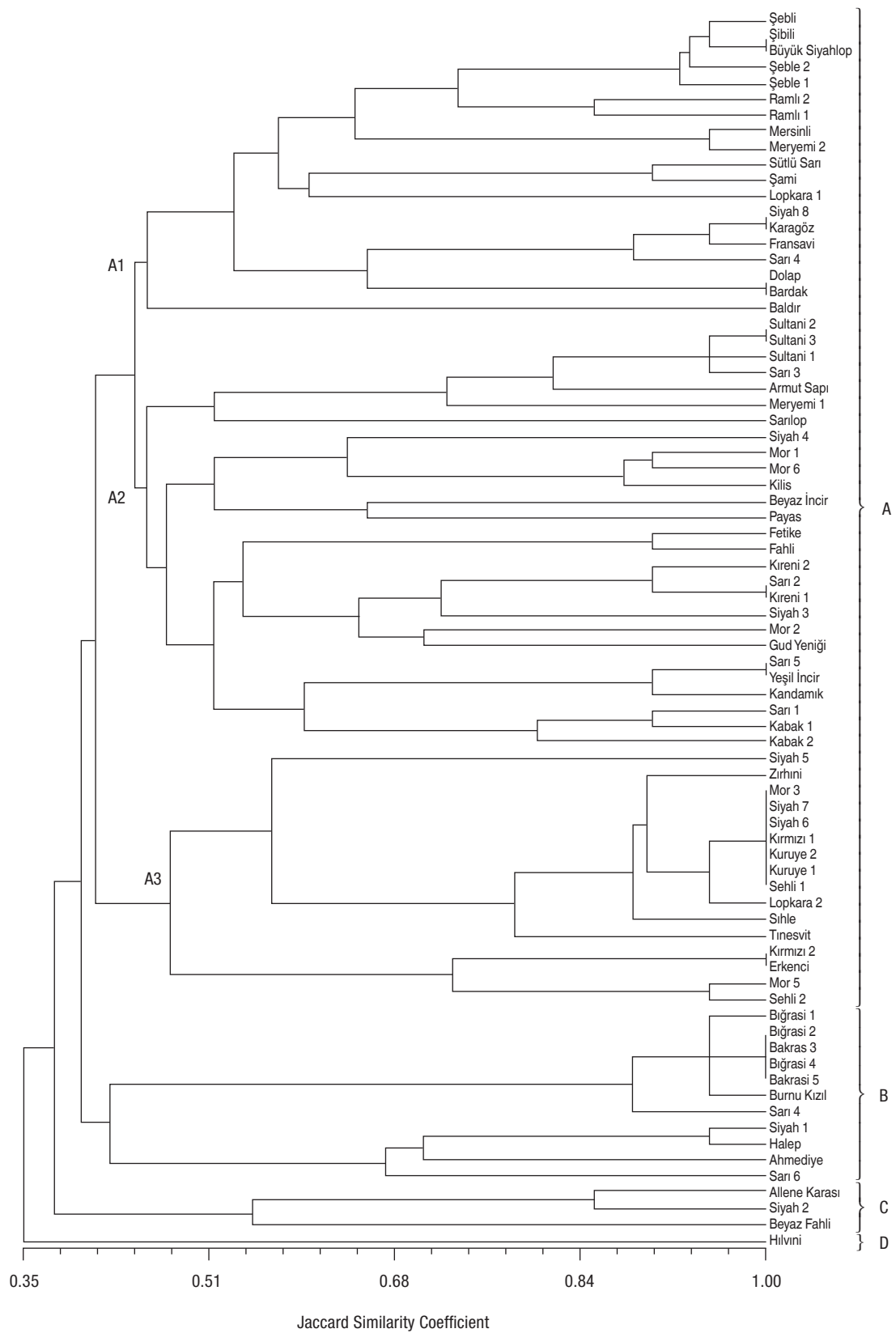The combined dendrogram (Fig. 3), constructed using the combined data from both types of molecular markers,

Figure 1. Dendrogram of genetic similarity among the analyzed fig accessions based on SSR data.

Table 3. Results of seven arbitrary primers in RAPD analysis for fig accessions from the Mediterranean region of Turkey

Primer	Sequence (5'-3')	Total bands	Polymorphic bands	Polymorphism ratio (%)
OPB02	TGATCCCTGC	5	4	80.0
OPB08	GTCCACACGG	12	9	75.0
OPC04	CCGCATCTAC	13	10	76.9
OPC20	ACTTCGCCAC	8	7	87.5
OPF05	CCGAATTCCC	9	7	77.8
OPH3	AGACGTCCAC	9	8	88.9
OPO14	AGCATGGCTC	12	10	83.3
Total		68	55	
Mean		9.71	7.86	81.3

was dissimilar to those obtained separately with each marker. However, there were some similarities which led to a better representation of the relationship for some accessions. The Mantel test was used to compare the similarity matrixes. The correlation coefficient of similarity matrixes of RAPD and SSR were 0.82. The cophenetic correlation found in this study between the dendrogram and the similarity values was moderate for SSR ($r = 0.87$), and high for RAPD ($r = 0.90$). Cophenetic correlation values in our study were higher than those found by Ikten *et al.* (2010) (SSR = 0.61), and Ikegami *et al.* (2009) (RAPD = 0.68 and SSR = 0.80).

Similarity index values derived from RAPD and SSR data of fig accessions varied between 0.60 and 0.99. The dendrogram defined one large group (A) and three small clusters (B, C, and D). Group A includes 74 accessions and subdivided into two subgroups (A1 and A2). Group A1 includes 66 accessions, subdivided into five subgroups (A1.1. to A1.5). Group A.1.1 also subdivided into five subgroups (A.1.1.1 to A.1.1.5). Some accessions were common to both RAPD and SSR analysis, including the group containing the 'Bığrasi 1', 'Bığrasi 4', 'Bığrasi 2', 'Bakras 3', and 'Bakrasi 5' (A1.2); the 'Sultani 2' and 'Sultani 3' (A1.1.3) accessions; and the group containing the 'Sarı 1', 'Kabak 1' and 'Kabak 2' (A1.1.4); 'Zırhini', 'Mor 3', 'Siyah 7', 'Siyah 6', 'Kırmızı 1', 'Kuruye 1', 'Lopkara 2', 'Kuruye 2', 'Sehli 1', and 'Tinesvit' accessions (A1. 4). Indeed, the 'Bığrasi 1', 'Bığrasi 2', 'Bığrasi 4', and 'Bakras 3' accessions; the 'Sultani 2' and 'Sultani 3' accessions; and the 'Kabak 1' and 'Kabak 2' accessions possess similar fruit quality characteristics (fruit size, shape, skin color, and total soluble solids), and could possibly be the same accessions with different local names. The 'Zırhini', 'Mor 3', 'Siyah 6', 'Siyah 7',

'Kırmızı 1', 'Kuruye 1', 'Lopkara 2', 'Kuruye 2', 'Sehli 1', and 'Tinesvit' accessions were also closely linked with regard to fruit characters, such as purple fruit skin color, intermediate size, and total soluble solids greater than 20%. However, these accessions had some distinct characters such as fruit skin cracks, fruit neck, and stalk shape (data not shown).

On the other hand, 'Dolap' and 'Bardak', which are San Pedro-type accessions, were grouped in the same small cluster in subgroup A1.5. As shown in Fig. 4, the 'Beyaz İncir' and 'Siyah 4' (Group B), 'Armut Sapı' (Group C) and 'Şeble 1' (Group D) accessions were separated from other accessions. These results showed that similarities, homonymies (accessions that share the same name but have a different genetic profile), and synonymies (accessions with the same SSR profile but a different traditional name) appear to be common in fig, and that distinct names probably arose due to the transport of plant material among districts and villages of Hatay province in the Mediterranean region. On the other hand, slight phenotypic differences could be related with selection of phenotypically diverse clones through the accumulation of mutations (Giraldo *et al.,* 2005).

These results showed that RAPD and SSR are available and informative for molecular characterization of fig accessions. The clustering patterns obtained from two types of marker data showed a different level of discrimination. Although, RAPD and SSR primers were able to amplify all DNA templates, each type of marker detected different types of genetic variation. RAPD markers may detect mutations at many locations within an entire genome, but microsatellites typically detect mutation at particular loci, often within repetitive DNA (Baranek *et al.,* 2006). SSR and RAPD markers have similar marker index values but contrasting data for fig discrimination.
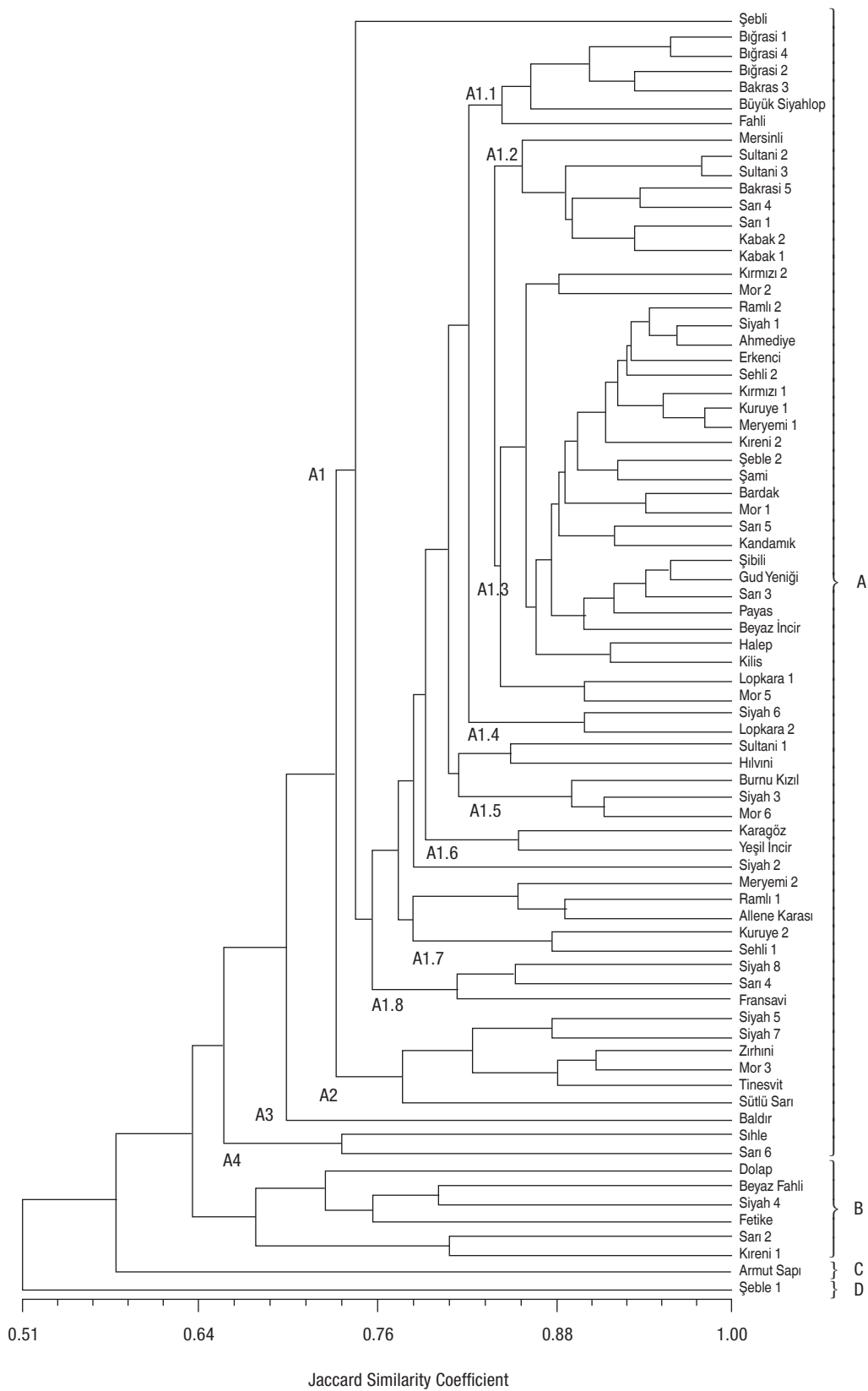
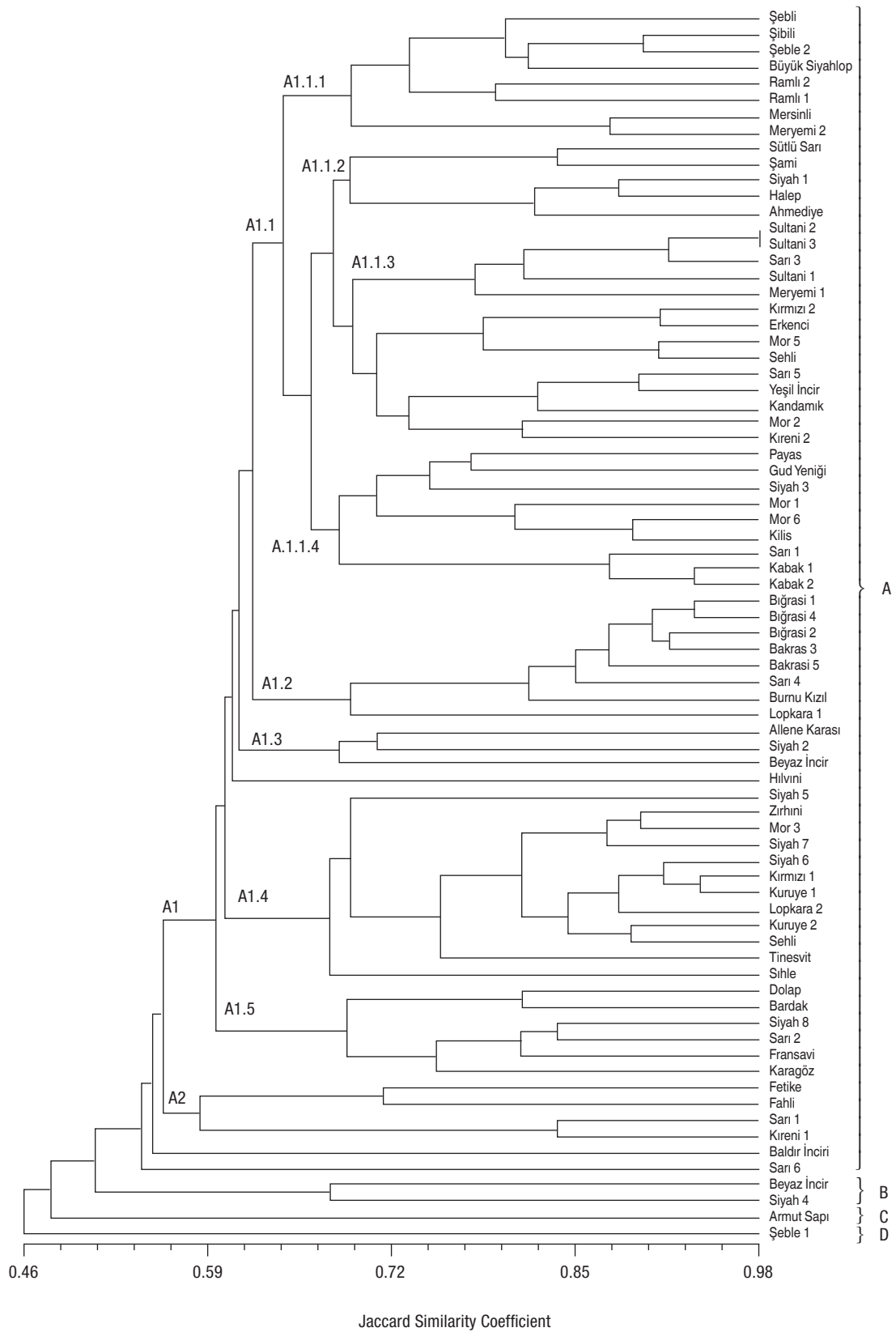**Figure 2.** Dendrogram of genetic similarity among the analyzed fig accessions based on RAPD data.

Figure 3. Dendrogram of genetic similarity among the analyzed fig accessions using both RAPD and SSR data.
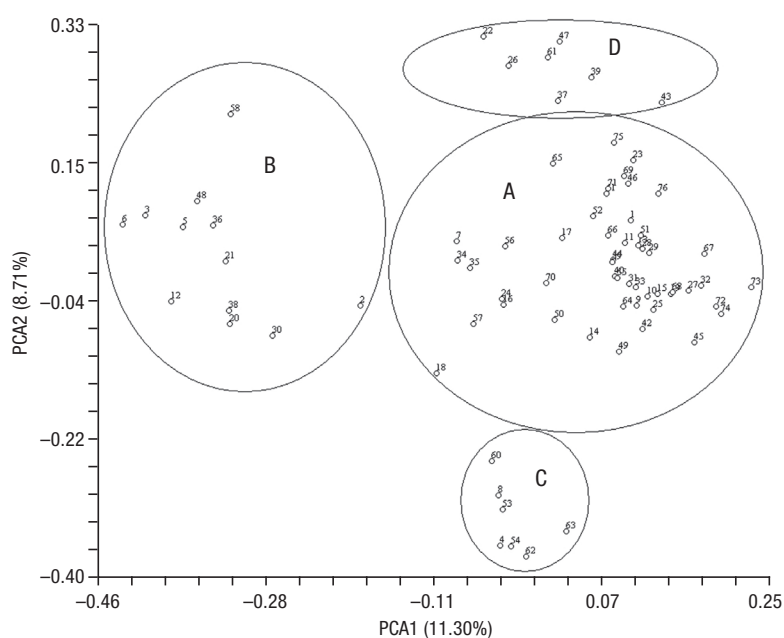
Figure 4. Principal components analysis grouping of 76 fig accessions based on RAPD and SSR markers.

Salhi-Hannechi *et al.* (2005) reported that considerable variation was observed among 18 Tunisian fig accessions with seven RAPD primers. Also, Khadari *et al.* (2003) showed that nine RAPD primers were not suitable to distinguish 30 fig cultivars whereas six SSR primers were sufficient for identification. Actually, our results displayed that ten SSR primers can be suitable for genetic diversity among fig accessions while seven RAPD primers not sufficient for discrimination studies. Therefore, molecular researches studied with RAPD primers on numerous fig accessions as many as primers should be used.

The similarity index that was obtained from Jaccard's similarity coefficients on SSR and RAPD was subjected to PCA analysis. The contributions of PCA 1, PCA 2, and PCA 3 were 11.30, 8.71 and 8.11%, respectively. These three eigen vectors accounted for 28% of the total observed variation, a value lower than those reported by Ikegami *et al.* (2009) (38%) and Ikten *et al.* (2010) (38%). Fig. 4 shows the distribution of cultivars according to the first two components (PCA 1 and PCA 2). The results indicated that 76 accessions could be divided into four main groups (A, B, C, and D). SSR markers correlated to axis 1 contribute the most to similarity in Group B and Group C.

The present study developed the first molecular database for figs in the Mediterranean region of Turkey. SSR and RAPD assays showed different results. In general, for genetic diversity studies, the use of different molecular genetic methods should be considered in advance and selected based on the anticipated degree of genetic diversity among the accessions to be analyzed. Our results indicate that SSR or RAPD are powerful tools to identify genetic diversity among fig accessions, being SSRs the most efficient. Future germplasm collections and introductions should include molecular analysis in order to optimize genetic diversity and avoid duplication in the present collection. In conclusion, we have found that this rich genetic diversity would be useful for development of table figs in regional and national fig breeding programs.

Acknowledgements

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