Evaluation of Genetic Diversity among Persian Fig Cultivars by Morphological Traits and RAPD Markers

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Abstract. *Ficus carica* L. is one of the most ancient fruit trees cultivated in Persia (Iran). The conservation and characterization of fig genetic resources is essential for sustainable fig production and food security. Given these considerations, this study characterizes the genetic variability of 21 edible *F. carica* cultivars in the Fars Province using random amplified polymorphic DNA (RAPD) markers. The collected cultivars were also characterized for their morphological features. A total of 16 RAPD primers produced 229 reproducible bands, of which, 170 loci (74.43%) were polymorphic with an average polymorphic information content (PIC) value of 0.899. Genetic analysis using an unweighted pair-group method with arithmetic averaging (UPGMA) revealed genetic structure and relationships among the local germplasms. The dendrogram resulting from UPGMA hierarchical cluster analysis separated the fig cultivars into five groups. These results demonstrate that analysis of molecular variance allows for the partitioning of genetic variation between fig groups and illustrates greater variation within fig groups and subgroups. RAPD-based classification often corresponded with the morphological similarities and differences of the collected fig cultivars. This study suggests that RAPD markers are suitable for analysis of diversity and cultivars' fingerprinting. Accordingly, understanding of the genetic diversity and population structure of *F. carica* in Iran may provide insight into the conservation and management of this species.

Fig (*F. carica* L., $2n = 26$) is an ancient crop species belonging to the Moraceae family, which originates from the Mediterranean basin (Berg, 2003). Dating back to 11,200–11,400 years ago, carbonized figs from an early Neolithic site in the Jordan Valley revealed that fig was the first cultivated plant during the early Neolithic Revolution, preceding cereal domestication (Kislev et al., 2006). From there, fig domestication spread to

western Asia, other Middle Eastern countries, and across the globe (Aradhya et al., 2010).

Ficus carica L. is morphologically gynodioecious but is functionally dioecious, with pollination between caprifig and edible figs (Kjellberg et al., 1987). Fig contains hollow receptacles called syconium. Based on their pollination behavior and floral biology, they are classified into common, Smyrna, San Pedro, and caprifig types. Caprifig is functionally a male fig and has been categorized as primary type, whereas common-type is an advanced and commercial cultivar, and has pistillate flowers producing parthenocarpic fruits. Smyrna and San Pedro figs are known as intermediate types and require pollination for common fruiting. The San Pedro type is the exception, and develops early parthenocarpic fruits on older branches (Aradhya et al., 2010).

Dried fig fruit contains high amounts of crude fiber (5.8%, w/w) and polyphenols (Vinson et al., 2005). Moreover, it contains various vitamins, minerals, and amino acids, making it an important crop worldwide for both dry and fresh consumption. Southern Arabia, western Asia (including Mesopotamia), Anatolia, Transcaucasia, Persia, and regions of the Middle East are the centers of fig diversity (Condit, 1947). Fig propagation in other growing regions of the world, and their long history of domestication with different cultivars, have resulted in ambiguity of their taxonomy. More than 700 fig varieties are known, but some specific ecotypes and their genetic relationships are still undefined. Iran is one of the most important diversity centers of wild and edible fig in the world, with 42,000 ha devoted to fig cultivation. More than 95% of fig orchards are located in the Fars Province (Safaei et al., 2008). The varietal relationships of synonyms and homonyms of edible figs (Smyrna figs) in this region has not yet been reported. Evaluation of genetic diversity and identification of fig cultivars in the Fars Province are critical for the conservation of superior genotypes, breeding, mass production, and improving the quality of cultivars that have potential for trading in local and global markets.

Because of intraspecies variations in vegetative traits, it is difficult to differentiate genotypes based exclusively on external structure, particularly for leaf and fruit characteristics. Morphological traits may differ across years, growth conditions, and environment as these traits are extremely susceptible to genotype-environment interactions. Molecular markers are reliable tools for screening biodiversity among germplasm collections. Some of these molecular tools, such as simple sequence repeat (SSR), RAPD, inter SSR, and restriction length polymorphism, have been widely used for identifying various fig cultivars, landraces, structures, and differentiation in fig collections, and population diversity. (Amel et al., 2004; Dalkilic et al., 2011; Khadari et al., 2001, 2005; Papadopoulou et al., 2002). RAPDs provide sufficient polymorphism to determine within species similarity for local cultivars, genetic diversity, phylogenetic analysis, genotyping, and genetic relationships. RAPD method is fast, inexpensive, and does not require prior knowledge of the genome's sequence. This study aims to elucidate the genetic relationships between different local edible fig cultivars in the Fars Province. Objectives include the 1) identification of 21 edible fig accessions grown in the Fars state (southwestern Iran) and 2) evaluation of genetic relationships among these accessions using RAPD assay.

Materials and Methods

Plant material and morphological characteristics. The study presented here was conducted on 21 edible fig ecotypes

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listed in Table 1. The samples were collected between 2012 and 2013 from the agricultural and natural resources research stations of Estahban, Khafr, and Kazerun counties in the Fars Province of Iran. Leaf and fruit morphological traits were recorded in July at the time of fruit ripening. The leaf samples were first washed with tap water and surfaces sterilized with 10% (v/v) Clorox® solution for 5 min. Afterward, they were rinsed three times with distilled water and blotted dry with paper towels and stored at -80 °C or used immediately for DNA extraction. Morphological traits of fig cultivars were evaluated by fig descriptors, as recommended by the International Union for the Protection of New Varieties of Plants (UPOV, 2010). According to these guidelines, a total of 78 phenotypic characteristics represent well-defined traits used to describe fig cultivar/accessions. These morphological traits are considered as conventional tools for identification

of new, economically appealing cultivars. In this study, leaf length (LL; cm), leaf width (LW; cm), petiole length (PL; cm), number of leaf lobes (NLL), central lobe shape (CLS), shape of leaf base (SLB), shape of fruit, fruit size (FS), fruit skin color, fruit pulp internal color (FPIC), and pulp cavity (PC) were measured for edible *F. carica* cultivar/accession from Fars Province. The experiment was repeated 10 and 25 times for leaf and fruit traits, respectively (Tables 2 and 3). All samples were collected at the same level of visually determined physiological maturity.

DNA extraction and polymerase chain reaction (PCR) amplification. Genomic DNA was extracted from fresh, young leaves using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The quality and quantity of DNA was monitored by spectrophotometry and gel inspection. A total of 16 RAPD primers (MWG Operon Technologies, Ebersberg, Germany) were used for PCR

amplification (Table 4). PCR reactions were carried out in a total volume of 25 µL including 20 ng DNA templates, 1U Taq DNA polymerase (Vivantis, Chino, CA), 100 ng of each primer, 0.1 mM of deoxyribonucleoside triphosphates (dNTPs) mix, 1.25 mM of MgCl₂, and 2.5 µL of PCR buffer (10×). Amplification was performed in a PTC-100 thermal cycler system (T100 Thermal Cycler; Bio-Rad, Hercules, CA). The amplification reaction consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, and 72 °C for 2 min, and the final extension at 72 °C for 6 min. The amplified products were separated on 2% (w/v) agarose gels applied with 350 mL Tris/Borate/EDTA (TBE) buffer at 80 V for 60 min, stained with 2 mg·L⁻¹ ethidium bromide for 5 min, detained in sterilized distilled water for 3 min, and visualized under ultraviolet light and photographed.

Data analysis. The digital image files were analyzed using UVIDoc software Version 99.01 (UVitec Limited, Cambridge, UK). The experiment was repeated three times. Only reproducible, distinct, and well-separated RAPD fragments with high intensities ranging from 240 to 3500 bp were scored as present (1) or absent (0). Electrophoretic DNA bands of low visual intensity that could not be easily differentiated as present or absent were considered ambiguous markers and were not scored. The distance matrix was constructed using the NTSYSpc 2.02 software package (Rohlf, 1998). A dendrogram of genetic relationship was generated by clustering the data using an UPGMA. The cophenetic correlation coefficient was calculated, and the ability of the most informative primers to differentiate between varieties was assessed by calculating the percentage of polymorphic bands (PPB). The information content of each RAPD marker was computed as $PIC = 2 \sum_{i=1}^{k-1} \sum_{j=i+1}^k P_i P_j (1 - P_i P_j)$,

Table 1. Smyrna fig cultivars in the study.

No	Cultivar/accession	Place of origin	Location
1	Roghani	Estahban	29°07'36"N 54°02'32"E
2	Kale Gorbeie	Estahban	29°07'36"N 54°02'32"E
3	Payves Siyah	Estahban	29°07'36"N 54°02'32"E
4	Shah Anjeer Morvarid	Estahban	29°07'36"N 54°02'32"E
5	Sigotou	Estahban	29°07'36"N 54°02'32"E
6	Sabz Morvarid	Estahban	29°07'36"N 54°02'32"E
7	Pariyovee	Estahban	29°07'36"N 54°02'32"E
8	Barg Chenary	Estahban	29°07'36"N 54°02'32"E
9	Matti	Estahban	29°07'36"N 54°02'32"E
10	Kashky	Estahban	29°07'36"N 54°02'32"E
11	Charmee	Estahban	29°07'36"N 54°02'32"E
12	Rownoo	Estahban	29°07'36"N 54°02'32"E
13	Sabz	Estahban	29°07'36"N 54°02'32"E
14	Siah	Estahban	29°07'36"N 54°02'32"E
15	Shah Anjeer	Estahban	29°07'36"N 54°02'32"E
16	Atabaki	Estahban	29°07'36"N 54°02'32"E
17	Sefid	Khafr	28°56'10"N 53°18'36"E
18	Kanezak	Khafr	28°56'10"N 53°18'36"E
19	Payves	Kazerun	29°37'10"N 51°39'15"E
20	Ghane	Kazerun	29°37'10"N 51°39'15"E
21	Mambilee	Kazerun	29°37'10"N 51°39'15"E

Table 2. Leaf morphological characteristics of fig cultivars.

Cultivar/accession	LL (cm)	LW (cm)	PL (cm)	NLL	CLS	SLB
Roghani	13.9 ± 0.58 cd	14.1 ± 0.46 bc	4 ± 0.14 c	5	Lyrate	Strongly calcarate
Kale Gorbeie	15 ± 0.64 c	15.5 ± 0.51 b	5.4 ± 0.19 b	5	Lyrate	Strongly calcarate
Payves Siyah	7.5 ± 0.32 ghi	8.4 ± 0.27 ef	4.7 ± 0.17 c	Entire	—	Cordate
Shah Anjeer Morvarid	11.5 ± 0.48 def	10.5 ± 0.34 e	4.5 ± 0.16 c	5	Lyrate	Moderately calcarate
Sigotou	13.5 ± 0.57 cd	13 ± 0.42 c	6 ± 0.21 a	5	Linear	Strongly calcarate
Sabz Morvarid	12.7 ± 0.53 cde	14 ± 0.45 bc	3.1 ± 0.11 de	5	Lyrate	Strongly calcarate
Pariyovee	11.3 ± 0.47 def	7.8 ± 0.25 f	4.11 ± 0.15 c	5	Lyrate	Strongly calcarate
Barg Chenary	13.5 ± 0.57 d	10.6 ± 0.34 e	4.5 ± 0.16 c	5	Lyrate	Strongly calcarate
Matti	19.5 ± 0.82 a	19 ± 0.62 a	4.1 ± 0.15 c	5	Lyrate	Cordate
Kashky	17 ± 0.72 b	12.7 ± 0.41 c	3.87 ± 0.14 cd	3	Narrow rhombic	Cordate
Charmee	12.5 ± 0.53 cde	10.1 ± 0.33 e	2.7 ± 0.09 def	5	Lyrate	Strongly calcarate
Rownoo	11.8 ± 0.5 def	13.8 ± 0.45 bc	5.3 ± 0.19 b	5	Lyrate	Strongly calcarate
Sabz	16.8 ± 0.71 b	11.3 ± 0.37 d	4 ± 0.14 c	5	Lyrate	Strongly calcarate
Siah	19 ± 0.81 a	19 ± 0.62 a	4.5 ± 0.16 c	5	Lyrate	Strongly calcarate
Shah Anjeer	16.5 ± 0.69 bc	13.1 ± 0.43 c	4.5 ± 0.16 c	5	Lyrate	Moderately calcarate
Atabaki	13 ± 0.55 d	13 ± 0.43 c	5.3 ± 0.19 b	5	Broad rhombic	Cordate
Sefid	9 ± 0.38 gh	8 ± 0.26 f	3.5 ± 0.13 d	5	Lyrate	Cordate
Kanezak	8.9 ± 0.37 gh	7.6 ± 0.24 f	3.15 ± 0.1 de	3	Lyrate	Strongly calcarate
Payves	9.6 ± 0.41 g	8.2 ± 0.26 ef	2.5 ± 0.09 ef	3	Lyrate	Strongly calcarate
Ghane	13.3 ± 0.56 d	10.4 ± 0.34 e	3.5 ± 0.12 d	3	Broad rhombic	Strongly calcarate
Mambilee	8.9 ± 0.37 gh	7.5 ± 0.24 f	2.4 ± 0.08 ef	5	Linear	Strongly calcarate

LL = leaf length (cm); LW = leaf width (cm); PL = petiole length (cm); NLL = number of leaf lobes; CLS = center lobe shape; SLB = shape of leaf base. Different letters represent significant differences at $P < 0.05$.

Table 3. Fruit morphological characteristics of fig cultivars.

Cultivar/accession	SF	FS	FSGC	FPIC	PC
Roghani	Spherical	Small	Yellow-green	Red	Absent
Kale Gorbeie	Urceolate	Medium	Yellow-green	Red	Small
Payves Siyah	Pyriform	Small	Purple	Red	Small
Shah Anjeer Morvarid	Urceolate	Large	Yellow-green	Amber	Absent
Sigotou	Turbinate	Medium	Purple	Pink	Large
Sabz Morvarid	Spherical	Small	Green	Amber	Small
Pariyovee	Spherical	Medium	Green	Amber	Small
Barg Chenary	Urceolate	Large	Yellow-green	Amber	Small
Matti	Ovoidal	Small	Yellow	Amber	Large
Kashky	Spherical	Large	Purple	Pink	Absent
Charmee	Ovoidal	Large	Yellow-green	Pink	Absent
Rownoo	Spherical	Medium	Yellow-green	Yellow-White	Small
Sabz	Spherical	Medium	Green	Amber	Small
Siah	Spherical	Large	Purple	Red	Small
Shah Anjeer	Urceolate	Large	Yellow-green	Yellow-White	Absent
Atabaki	Spherical	Medium	Purple	Pink	Small
Sefid	Spherical	Small	Yellow-green	Amber	Small
Kanezak	Spherical	Small	Yellow	Red	Small
Payves	Spherical	Large	Yellow-green	Red	Absent
Ghanee	Spherical	Large	Purple	Pink	Small
Mambilee	Urceolate	Large	Yellow	Red	Small

SF = shape of fruit; FS = fruit size; FSGC = fruit skin ground color; FPIC = fruit pulp internal color; PC = pulp cavity.

Table 4. Randomly amplified polymorphic DNA polymorphism and amplification pattern in *Ficus carica*.

Primers	Sequence	Total no. of bands	Polymorphic bands	Polymorphism (%)	PIC	D
OPA02	TGCCGAGCTG	14	12	85	0.907	0.986
OPA05	AGGGGTCTTG	11	10	90	0.880	0.978
OPA16	AGCCAGCGAA	11	9	81	0.873	0.975
OPC17	TTCCCCCAG	14	12	85	0.911	0.987
OPH14	ACCAGTTGG	14	9	64	0.913	0.988
OPH18	GAATCGGCCA	20	10	50	0.941	0.994
OPH19	CTGACCAGCC	22	18	81	0.942	0.994
OPK17	CCCAGCTGTG	15	13	86	0.909	0.986
OPO3	CTGTTGCTAC	10	9	90	0.856	0.969
OPO13	GACAGGAGGT	14	11	78	0.898	0.983
OPX11	GGAGCCTCAG	12	9	75	0.889	0.981
OPY04	GGTCGCAATG	15	13	86	0.903	0.985
OPY07	AGAGCCGTCA	18	11	61	0.922	0.990
OPY11	AGACGATGGG	14	12	85	0.907	0.986
OPY13	CACCACCACC	16	8	50	0.926	0.991
OPY15	AGTCGCCCTT	9	4	44	0.854	0.965
Maximum		22	18	90	0.942	0.994
Minimum		9	4	44	0.854	0.965
Total		229	170	1,191	—	—
Average		14.31	10.6	74.43	0.899	1.1

PIC = polymorphism information content; D = discriminating power.

where P_i and P_j are the frequency of the i^{th} and j^{th} observation, respectively, and k represents the number of bands (Oliviera et al., 2006). $PIC_{av} = \sum PIC_i / N$ was calculated, where PIC_i is the PIC value of the i^{th} RAPD marker and N is the number of RAPD markers generated by an assay unit. Loci that are nonpolymorphic ($PIC = 0$) in the germplasm of interest were excluded from this calculation. Each DNA fragment visualized within the gel was considered as a single dominant RAPD marker locus. Only PPB with strong intensity were scored; each marker was identified by the primer combination and the band number as a suffix. The discrimination power of each RAPD marker was evaluated as $D = 1 - PI$ where the probability identity (PI) described by Pollefeys and Bousquet (2003) was calculated as $PI = \sum_i P_i^4 + \sum_{i=1}^n \sum_{j>i}^n (2P_i P_j)^2$. The data obtained by RAPD profile were used for

construction of a similarity matrix based on Jaccard's coefficient. The partitioning of molecular variance and correlation coefficients between the similarity matrix were analyzed according to Mantel (1967) using XLSTAT Pro version 7.5, a Microsoft Excel® add-in. Finally, a two-dimensional plot of 21 cultivars of *F. carica* was obtained using EIGEN procedures in the NTSYSpc version 2.1. The population structure and projection of phenotypic variation among cultivars were obtained through principal component analysis (PCA) to further support phenotypic variability. The results of the analysis are presented with graphs plotting the projections of the units onto the components and the loadings of the variables.

Results

Morphological analysis. Based on leaf morphological characteristics presented in

the study (Table 2; Fig. 1), there was high variation in quantitative traits. 'Matti' and 'Siah' genotypes presented the largest values for LL and LW, whereas 'Payves Siyah' and 'Mambilee' tended to have the smallest values for both parameters. The 'Sigotou' genotype has the longest PL (6 cm), which makes the abscission of the fruit easy and quick from the twig, and consequently maintains the fruit's integrity. As reported in Table 2, 'Payves Siyah' was the only variety which had the entire leaf. The rest of accession had three- or five-lobed leaves with different CLS (Fig. 1). Some of cultivars presented features of economic interest. Various cultivars, such as Shah Anjeer Morvarid, Barg Chenary, Siah, Kashky, Charmee, Shah Anjeer, Payves, Ghanee, and Mambilee, had the largest FS when compared with the other varieties in the study (Table 3; Fig. 1). Some cultivars, including Roghani, Kale Gorbeie, Peyvas Siah, and Barg Chenari, were juvenile (sapling) and no fruit was observed during the study, so no photographic record of their fruit was collected. However, they have been morphologically well characterized in collaboration with a fig research station team in the Fars Province, and their morphological traits are described in this study (Supplemental Fig. 1). The fruit skin ground color (FSGC) and FPIC of fig cultivars in this study were quite diverse (Table 3).

Genetic analysis and RAPD band patterns. From the 24 arbitrary RAPD primers initially screened, only 16 primers generated strong and reproducible amplification products, all of which displayed polymorphism. Genetic diversity among the fig cultivars in this study were detected using these 16 single decamer primers (Table 4; Fig. 2). A total of 229 loci were successfully generated with 170 (74.43%) of them polymorphic. Based on the data from the 16 assay units, the PIC_{av} was 0.899 (Table 4). Individual primers generated a number of bands varying from 9 (OPY15) to 22 (OPH19), with an average of 14.31 bands per assay unit (Table 4; Fig. 2). The PPB ranged from 4 (OPY15) to 18 (OPH19), with an average value of 10.6 bands per primer. None of the pair accessions exhibited identical band patterns. The maximum PIC value of 0.942 was observed using OPH19, whereas the minimum PIC value of 0.854 was obtained using OPY15. Indeed, the PIC value and PI showed that the OPH19 was more practical in comparison with the other primers used in this experiment as it produced more PPB (Table 4; Fig. 2). Discrimination power of each marker was estimated by the PI ranging from 0.965 to 0.994 which was nearly uniform (random) for the total of 170 PPB. The analysis was based on the principle that a band is considered to be polymorphic or monomorphic if it is present in some or all cultivars in the study. The relatedness of the fig varieties in the study was well established through the use of RAPD markers. Among the studied cultivars, the genetic similarity ranged from 0.514 to 0.839 (Table 5). The reliability and consistency of the UPGMA

Fig. 1. Leaf and fruit shapes of edible *Ficus carica* varieties in Fars Province. (A) 'Roghani', (B) 'Kale Gorbeie', (C) 'Payves Siyah', (D) 'Shah Anjeer Morvarid', (E) 'Sigotou', (F) 'Sabz Morvarid', (G) 'Pariyovee', (H) 'Barg Chenary', (I) 'Matti', (J) 'Kashky', (K) 'Charmee', (L) 'Rownoo', (M) 'Sabz', (N) 'Siah', (O) 'Shah Anjeer', (P) 'Atabaki', (Q) 'Sefid', (R) 'Kanezak', (S) 'Payves', (T) 'Ghanee', and (U) 'Mambilee'.

clustering method with the RAPD genetic similarity matrix was ensured by the high cophenetic correlation coefficient of $r = 0.737$. 'Shah Anjeer Morvarid' and 'Shah Anjeer' showed a high degree of genetic similarity (0.839). High genetic similarity (0.802) was also observed between cultivar Kashky and Charmee, which is likely due to the high level of intracultivar clonal similarity. The lowest genetic similarity (0.514) was observed between cultivars Kale Gorbeie and Sefid (Table 5).

Genetic variability and phylogenetic relationships. The dendrogram presents relationships among 21 fig cultivars based on the area of their diffusion and/or pedigree information (Fig. 3). Determination of the optimal number of clusters or number of acceptable clusters is an important feature in cluster analysis. The dendrogram contains five well-supported clusters/groups of edible figs (Fig. 3). The first cluster consists of Roghani and Kale Gorbeie cultivars, which are separated at a cutting value of 0.68. The second group contains Atabaki cultivar, which can be distinguished by a cutting point of 0.62 from the first group. The third cluster includes two distinct subgroups. The fig cultivars Payves Siyah, Siyah, Payves, Shah

Anjeer Morvarid, Shah Anjeer, Sigotou, Sabz Morvarid, Pariyovee, Rownoo, Sabz, Sefid, and Matti are categorized as the first subgroup. Among these cultivars, Shah Anjeer Morvarid and Shah Anjeer exhibited the highest genetic similarity (similarity coefficient of 0.84) in comparison with the other cultivars in this subgroup. Subsequently, the cultivars of Sabz Morvarid and Rownoo, with a similarity coefficient of 0.77, also show high genetic similarity. The second subgroup contains Kashky and Charmee cultivars with high genetic similarity (0.80), despite few morphological similarities between them. As shown in Fig. 3, the fourth cluster includes 'Ghane' and 'Mambilee' fig accessions. The position of Barg Chenary and Sigotou cultivars, at the extreme end of the dendrogram (the last cluster with similarity coefficient of 0.61), indicate that these are the most divergent cultivars in the study. These cultivars could be assessed as an out-group simply because they are the most divergent. The PCA analysis using RAPD data also supported the same clustering pattern (Fig. 4).

A PCA analysis (Fig. 5) using morphological data were constructed to illustrate the phenotypic relationships between the 21 cultivars. The two variables contributing to the

two principal components on the plan axes PC1 and PC2 accounted for 58.4% and 35.8%, respectively. The graphic representation of cultivars demonstrated on the plan axes (1 and 2) illustrated the significant opposition of cultivars 9 (Matti) and 14 (Siah) to the remaining cultivars, according to the first principal component. The leaves of these two cultivars are much larger in both length and width than the other cultivars. The second axis demonstrates a divergence of cultivar 3 (Payves Siyah), which is classified by very short LL. All other cultivars have LLs at least 1.4 cm longer than cultivar 3. The distribution of cultivars as depicted in the PCA appears to be independent of geographic origin.

Discussion

Genetic diversity analysis based on morphological parameters has many limitations due to the influence of various environmental factors. Molecular markers based on genetic diversity are accurate and practical for this analysis because they are unaffected by environmental conditions. The choice of the most appropriate genetic marker depends on the research objectives. The use of RAPD markers has been criticized for its poor reproducibility; however, various studies have confirmed that RAPD patterns are reliable and reproducible for intraspecific genetic diversity studies when the number of technical replicates is increased and laboratory practices are carefully performed (Schlag and McIntosh, 2012). Because RAPD molecular markers are DNA-based, more polymorphic, fast, and cheap, we elected to use it for exploring genetic relationships (diversity) of edible *F. carica* cultivars in the present study.

Some molecular markers, like amplified fragment length polymorphisms (AFLP) and SSR, have higher start-up costs. The average start-up costs required for AFLP and SSR are

Fig. 2. Randomly amplified polymorphic DNA profiles in agarose gel from 21 cultivars of *Ficus carica* using primers (A) OPH19 and (B) OPY15. M = molecular size ladder $\times$ 100 bp.

Table 5. Jaccard similarity coefficients matrix for 21 *Ficus carica* cultivars based on randomly amplified polymorphic DNA data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1.000																				
2	0.687	1.000																			
3	0.693	0.619	1.000																		
4	0.629	0.597	0.723	1.000																	
5	0.554	0.596	0.590	0.532	1.000																
6	0.601	0.524	0.671	0.697	0.591	1.000															
7	0.595	0.570	0.634	0.631	0.538	0.745	1.000														
8	0.630	0.636	0.686	0.603	0.712	0.592	0.595	1.000													
9	0.668	0.676	0.709	0.718	0.615	0.664	0.651	0.642	1.000												
10	0.657	0.605	0.708	0.690	0.573	0.618	0.595	0.698	0.694	1.000											
11	0.646	0.573	0.647	0.653	0.502	0.588	0.538	0.608	0.638	0.802	1.000										
12	0.649	0.571	0.692	0.664	0.567	0.771	0.642	0.622	0.715	0.675	0.619	1.000									
13	0.596	0.590	0.703	0.657	0.645	0.744	0.682	0.703	0.625	0.677	0.602	0.661	1.000								
14	0.661	0.602	0.756	0.729	0.553	0.657	0.601	0.682	0.697	0.696	0.659	0.661	0.700	1.000							
15	0.661	0.611	0.720	0.839	0.580	0.731	0.672	0.653	0.754	0.696	0.640	0.726	0.672	0.736	1.000						
16	0.648	0.593	0.631	0.583	0.569	0.529	0.548	0.650	0.585	0.647	0.568	0.602	0.603	0.623	0.642	1.000					
17	0.602	0.514	0.657	0.646	0.582	0.765	0.701	0.640	0.631	0.686	0.636	0.649	0.762	0.651	0.708	0.590	1.000				
18	0.662	0.576	0.704	0.659	0.554	0.659	0.619	0.655	0.672	0.661	0.587	0.645	0.646	0.729	0.710	0.625	0.644	1.000			
19	0.668	0.598	0.719	0.656	0.604	0.674	0.633	0.641	0.696	0.639	0.637	0.696	0.698	0.726	0.707	0.630	0.697	0.709	1.000		
20	0.630	0.521	0.716	0.680	0.577	0.654	0.597	0.578	0.666	0.621	0.583	0.640	0.624	0.686	0.686	0.611	0.639	0.688	0.666	1.000	
21	0.608	0.566	0.605	0.634	0.571	0.607	0.611	0.573	0.646	0.591	0.552	0.593	0.577	0.588	0.631	0.597	0.591	0.572	0.637	0.719	1.000

Sample ID: 1, 'Roghani'; 2, 'Kale Gorbeie'; 3, 'Payves Siyah'; 4, 'Shah Anjeer Morvarid'; 5, 'Sigotou'; 6, 'Sabz Morvarid'; 7, 'Pariyovee'; 8, 'Barg Chenary'; 9, 'Matti'; 10, 'Kashky'; 11, 'Charmee'; 12, 'Rownoo'; 13, 'Sabz'; 14, 'Siah'; 15, 'Shah Anjeer'; 16, 'Atabaki'; 17, 'Sefid'; 18, 'Kanezak'; 19, 'Payves'; 20, 'Ghane'; 21, and 'Mambilee'.

Fig. 3. Dendrograms obtained by unweighted pair-group method with arithmetic averaging cluster analysis based on Jaccard's coefficient of 21 *Ficus carica* cultivars using randomly amplified polymorphic DNA data. Sample ID: 1, Roghani; 2, Kale Gorbeie; 3, Payves Siyah; 4, Shah Anjeer Morvarid; 5, Sigotou; 6, Sabz Morvarid; 7, Pariyovee; 8, Barg Chenary; 9, Matti; 10, Kashky; 11, Charmee; 12, Rownoo; 13, Sabz; 14, Siah; 15, Shah Anjeer; 16, Atabaki; 17, Sefid; 18, Kanezak; 19, Payves; 20, Ghancee; and 21, Mambilee.

Fig. 4. Two-dimensional plot of 21 *Ficus carica* cultivars based on principal component analysis using a randomly amplified polymorphic DNA data. Sample ID: 1, Roghani; 2, Kale Gorbeie; 3, Payves Siyah; 4, Shah Anjeer Morvarid; 5, Sigotou; 6, Sabz Morvarid; 7, Pariyovee; 8, Barg Chenary; 9, Matti; 10, Kashky; 11, Charmee; 12, Rownoo; 13, Sabz; 14, Siah; 15, Shah Anjeer; 16, Atabaki; 17, Sefid; 18, Kanezak; 19, Payves; 20, Ghancee; and 21, Mambilee.

five times and 7.5 fold higher than RAPD, respectively (Dunham, 2011). RAPD molecular markers have been previously used for the genetic assessment of Palestinian fig genotypes and to determine the genetic variability in fig trees (Basheer-salimia et al., 2012; Rodrigues et al., 2012). Microsatellite markers were often preferred for characterizing different fig cultivars, including Egyptian, Spanish, and Tunisian figs, because they were codominant, hypervariable, and highly reproducible (Abou-Ellail et al., 2014; Achtak et al., 2009; Balas et al., 2014; Saddoud et al., 2005). However, microsatellite markers are locus-specific, thus requiring extensive genetic research (Agarwal et al., 2008).

In the present study, RAPD fingerprinting method was employed for its efficiency in disclosing the extent of polymorphism between 21 *F. carica* cultivars. The number of clusters was determined by taking into account only clusters that showed a genetic similarity higher than the overall mean value. The dendrogram obtained using the UPGMA method contains five well-supported clusters/groups of edible figs. An "acceptable cluster" is defined as, "a group of two or more genotypes where the within-cluster genetic distance is lower than the overall mean genetic distance and the between cluster distances are greater than the within-cluster distance of both clusters involved" (Sorkheh

et al., 2007). In the first group, which includes Roghani and Kale Gorbeie, leaf morphological traits NLL, CLS, and SLB and fruit traits FSGC and FPIC were identical in both cultivars, but their genetic similarity was only 0.68 (Table 5). These cultivars were domesticated in the same area (Estahban), and based on this analysis, the genetic diversity of *F. carica* cultivars in this area is relatively high. The cultivars Shah Anjeer and Shah Anjeer morvarid, from the third cluster, exhibited the highest similarity coefficient (0.839). Their morphological resemblance (Tables 2 and 3) also affirmed that these cultivars are quite similar and indicate that they might have some degree of inbreeding. Fig propagation is typically based on stem cuttings and this method has contributed to synonymy and homonymy, as somaclonal variation mostly occurs in sexually propagated fig species (Do Val et al., 2013). Subsequently, in the third group (first subcluster) both morphological and genetic similarities were observed for genotypes 'Sabz Morvarid' and 'Rownoo'. The high similarity coefficient (0.77) suggests few dissimilarities and close relatedness. Whereas, in the second subcluster, Kashky and Charmee cultivars also showed high genetic similarity coefficient but few similar morphological traits. There is no clear relationship to be ascertained between the two data sets, and in most instances, cultivars of morphological similarity may not necessarily demonstrate genetic convergence.

In this study, RAPD primers produced highly polymorphic and different loci for all fig cultivars analyzed. The results obtained show a high genetic diversity in Iranian accessions of the edible fig studied and no clear groupings based on geographical origin were observed, suggesting widespread exchange of plant material through vegetative propagation among different areas in the Fars Province. Previous studies of *F. carica* diversity typically investigate either the genetic or the phenotypic variation as it is difficult to establish a connection between the two. Disparity between morphological traits and RAPDs may be due to the presence of RAPD polymorphisms in both coding and noncoding genomic regions. As only a small portion of the genome is protein coding, it is very likely that the identified polymorphisms are within in noncoding regions (Persson and Gustavsson, 2001).

Conclusion

To conserve fig genetic resources and assess genetic diversity among Smyrna figs in Iran, we have collected and characterized 21 important fig accessions native to the Fars Province. RAPD markers based on genomic DNA of *F. carica* provided phylogenetic information that determined the genetic relationship of *F. carica* varieties in this study. RAPD results clearly revealed that *F. carica* cultivars could be differentiated from each other and in a few cases, RAPD-based classification corresponded with morphological similarities. The results of this study have demonstrated how integrated morphological

Fig. 5. Two-dimensional plot of 21 *Ficus carica* cultivars based on principal component analysis using a morphological data. Sample ID: 1, Roghani; 2, Kale Gorbeie; 3, Payves Siyah; 4, Shah Anjeer Morvarid; 5, Sigotou; 6, Sabz Morvarid; 7, Pariyovee; 8, Barg Chenary; 9, Matti; 10, Kashky; 11, Charmee; 12, Rownoo; 13, Sabz; 14, Siah; 15, Shah Anjeer; 16, Atabaki; 17, Sefid; 18, Kanezak; 19, Payves; 20, Ghanee; and 21, Mambilee.

traits and genetic analysis can provide a biodiversity evaluation of *F. carica* genotypes. Furthermore, highly reproducible amplification profiles showed that RAPD markers were efficient for diversity assessment by performing a high level of polymorphism (74.43%) across all varieties. More investigation is warranted to reveal a clear correlation between *F. carica* genetic variability and morphological trait similarity. Fig cultivars in the Fars Province are genetically diverse, and conservation of these genetic resources is therefore extremely important for breeding purposes and improving the fruit quality.

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Supplemental Fig. 1. Leaf and fruit shapes of (A) 'Roghani', (B) 'Kale Gorbeie', (C) 'Peyvas Siah', and (D) 'Barg Chenary'.