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Genetic Discrimination of Egyptian Fig Cultivars Revealed by RAPD Fingerprints

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ABSTRACT

Ficus carica L., well adapted to the Mediterranean climate, is ubiquitous in Egypt. In spite of the large possibilities of its adaptation to the Egyptian climate, its cultivation remains traditional. In Egypt, these species is represented by a large number of varieties which are facing genetic erosion. To save these genetic resources some of the Egyptian varieties had been studied using molecular markers. Nine RAPD markers were used to survey the genetic diversity in Egyptian fig (*Ficus carica* L.). Seven fig cultivars originating from diverse geographical areas were analyzed. As a result, considerable genetic diversity was detected among the studied *F. carica* accessions. The relationships among the seven varieties were studied by cluster analysis. The dendrogram showed two main groups composed of cultivars. Our data proved that RAPD markers are useful for germplasm discrimination as well as for investigation of patterns of variation in fig. Since this designed procedure has permitted to establish a molecular database of the reference collections, the opportunity of this study is discussed in relation to the improvement and rational management of fig germplasm. Results indicated that RAPD is useful, rapid and accurate technique for studying genetic diversity and germplasm characterization of *Ficus carica* some cultivars. There is a wide spectrum genetic variation among studied fig varieties, these variation could be an effective factor in breeding program.

Key words: *Ficus carica*, genetic variation, RAPD, finger print markers, egyptian, fig cultivars, genetic studies

INTRODUCTION

Fig is one of the most adapted fruit to Mediterranean basin (Zohary and Hopf, 2000). Fig plants belonged Moraceae family. Genus *Ficus*, includes approximately 400 monoecious and 350 gynodioecious (Parrish *et al*., 2004) or contains around 800 species (Al Malki and Elmeer, 2010). Large number of cultivated and wild figs can be found with great diversity of color, shape and flavor, especially among the cultivars grown for fresh consumption (Koyuncu *et al*., 1998). Many countries set up a new strategy to increased productivity and quality of fig crop for its highly nutritive values. In Egypt, several fig cultivars (Sultany, Aboudi, white adcy, Gizy, Black mission, conadric and Katoda) planted in several areas (North coast, Sinai, oases and south of Egypt). Some of these cultivars introduced from ancient time to Egypt and vegetative propagated. As

consequences of undocumented or uncertified reproduction procedures of these cultivars, exchange these cultivars among nurseries with uncorrected labeled and due to numerous of synonymy (several denomination for the genotype) and homonymy (several genotypes under the same denomination), pomological characterization is insufficient to distinguish between these cultivars (Khadari *et al*., 2004). Besides, data based on the use of morphological traits, particularly those concerning the fruits, are either sensitive to the environmental conditions or limited to the fruit production season. Also, isozyme characterization results may be differed according to environmental conditions. Since then, genetic markers based on DNA polymorphisms have been developed and became routinely common tool employed for germplasm characterization, random amplified polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and microsatellites or Simple Sequence Repeat (SSR) (Hafid *et al*., 2009). One of the most widely tested techniques seems to be the random amplified polymorphic DNA (RAPD) method based on the Polymerase Chain Reaction (PCR) (Williams *et al*., 1990).

Since, RAPD has a high accuracy in distinguishing genotypic variants, it considered as powerful tool (Santoni *et al*., 2000; Trifi *et al*., 2000; Amadou *et al*., 2001; Al-Khalifah and Askari, 2003; Khadari *et al*., 2003; Rajora and Rahman, 2003; Onguso *et al*., 2004). Also, Egyptian fig cultivars are found in a varied environmental conditions (North coast, Upper of Egypt and Sinai peninsula), with different water quality, soils and under these varied circumstance a wide spectrum genetic variation could be found. In this sense, the present study as one of serial and sequential studies that aim to investigate the usefulness of Egyptian Fig cultivars in light of comparison based on agronomical (productivity and quality) and molecular analysis. Finally, setting up database for Fig crop could be used in the future to develop the crop by breeders.

MATERIAL AND METHODS

Plant material: Polymerase Chain Reaction (PCR) analysis was carried out by using the genomic DNA from the seven Egyptian fig cultivars collected from different areas (private farm in north coast of Egypt, (white adcy), Faculty of Agricultur's farm, Alexandria University(Sultany), Desert research center, Ameria region (Aboudi, Black mission, conadria and Katoda) and Agriculture research center, Giza (Gizy). Fig cultivars included four local cultivars (Sultany, Aboudi, White Adcy and Gizy) and three imported fig cultivars (Black mission, Conadria and Katoda).

DNA extraction: Frozen young fresh leaves (500 mg) were ground to a powder in a mortar with liquid nitrogen. The DNA's from genotypes were extracted using CTAB extracting buffer according (Saghai-Maroof *et al*., 1984) and stored at -20°C until use.

Determination of DNA concentration by UV spectroscopy: Spectrophotometer was employed at (260 nm) to measure DNA concentration. The concentration of DNA (which used in RAPD reaction) was calculated, according to Sambrook *et al*. (1989), assuming that DNA at a concentration of 50 g mL^{-1} had an optical density of 1 at 260 nm as follows:

$$\text{DNA concentration} = \frac{OD260 \times 50 \text{ dilution factor} \times 50 \text{ mgmL}^{-1}}{1000}$$

Primers and DNA amplification: Nine Primers (Table 1), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, Ebgland HP79 NA), were tested in this experiment to identify fig varieties.

Table 1: Nine primer sequences used in identification of fig cultivars

Primer code	Sequence
OPA-01	CAG GCC CTT C
OPA-02	TGC CGA GCT G
OPA-04	AAT CGG GCT G
OPA-05	AGG GGT CTT G
OPA-11	CAA TCG CCG T
OPA-16	AGC CAG CGA A
OPA-18	AGG TGA CCG T
OPA-19	CAA ACG TCG G
OPA-20	GAC CAA TGC C

PCR reactions were performed in a 25 L volume reaction mixture containing: 20 ng of total cellular DNA, 100 M of primer (1 .l), 5.l of 5X green Taq DNA polymerase buffer, 5 IU L^{-1} of GoTaq DNA polymerase (0.25.l) (Promega), 10 mM of each dNTP (0.5.l), 25 mM $MgCl_2$ (4 L), up to 25.l by nuclease-free water. PCR was performed in a DNA thermocycler (Biometra, Germany). Samples were first heated at 94°C for 3 min and subjected to 35 cycles of the following cycle: 45 sec at 94°C, 45 sec at 37°C, 1.5 min at 72°C. A final step of 5 min at 72°C was always run. PCR reaction was tested on 1.6% agarose (Genetics) gel and 100 bp DNA Ladder H3 RTU (Genetics) was used as the standard marker.

Data handling and cluster analysis: Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Pairwise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to Jaccard (1908). The similarity coefficients were, then, used to construct dendograms, using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivartiate Analysis System), version 1.80 (Applied Biostatistics Program).

RESULTS

The random amplified polymorphic DNA-polymerase chain reaction (RAPDPCR) was used for identification and molecular characterization of seven fig species, four local (Gizy, Sultany, White adcy and Aboudi) and three imported cultivars (Black mission, Conadria and Katoda) as shown in Fig. 1. A total of nine primers were screened for their ability to generate consistently amplified band patterns and to assess polymorphism in the tested fig varieties. Nine primers amplified a total of 111 reproducible DNA bands with an average of 12.3 DNA bands per primer as shown in Table 2. Their sizes ranged between 100-2500 bp. All the primers displayed polymorphic amplicons.

Also it could be noticed that generated bands varied from primer to another whereas OP-A19 produce high number of bands (16) comparing with other primes, meanwhile the lowest number of bands produced from primer OP-A05 (5). Other primers varied between this ranges of bands number. Of the total amplicons (111 bands), only 72 (~65%) were polymorphic for studied fig varieties, with an average of 8 bands per primer. The remaining 39 (~35%) bands of the total 111 bands were monomorphic. Primers OPA-19, OPA-16 and OPA-20 produced 9, 8 and 7 monomorphic bands, respectively. However, OPA-02 and OPA-04 primers generate 0 monomorphic bands. In regard the polymorphism percentage, it was differed among used primers;

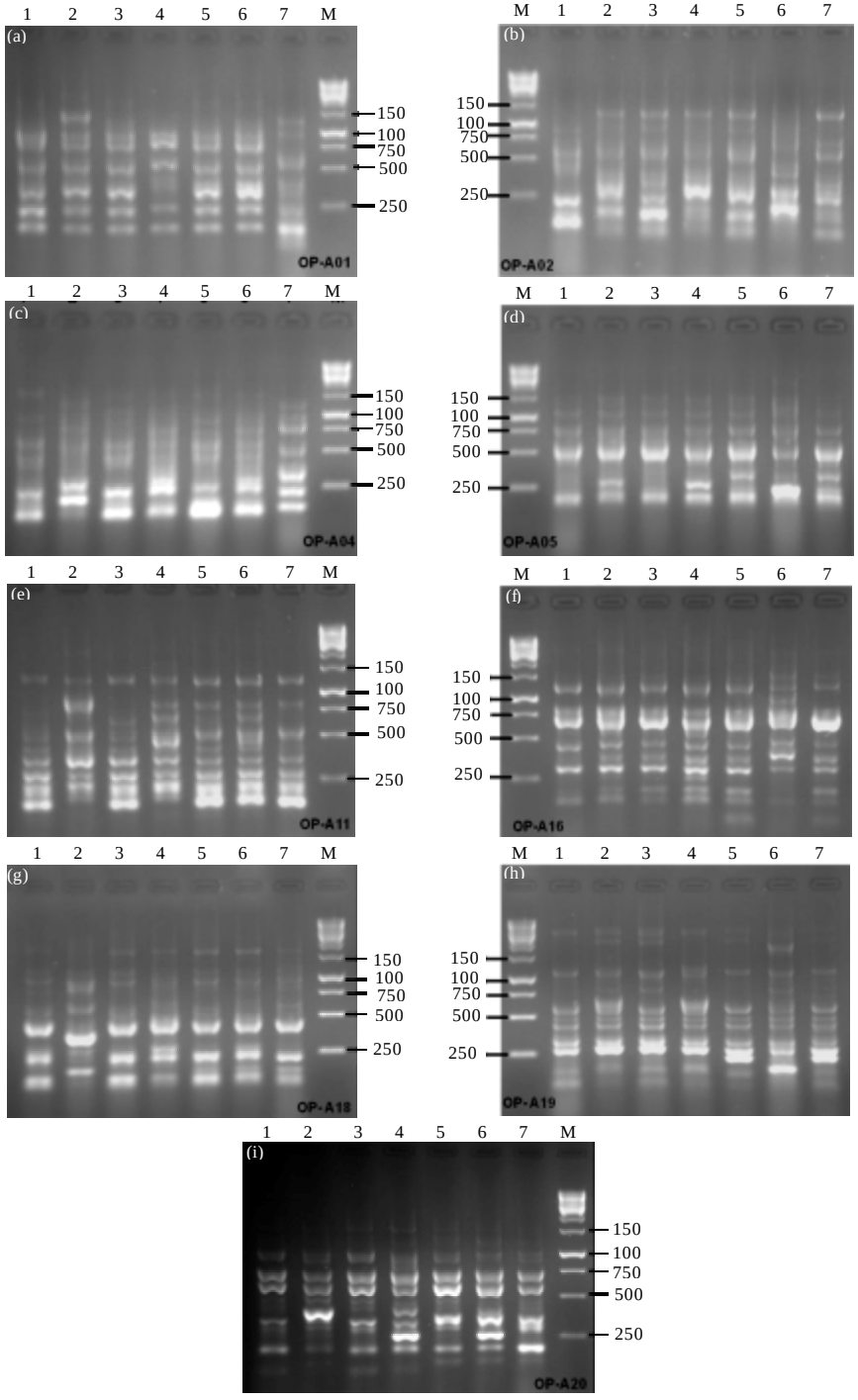

Fig. 1(a-i): RAPD pattern of (a): OPA01, (b): OPA02, (c): OPA11, (d): OPA16, (e): OP18, (f): OPA19 and (g): OPA 20, primers with 7 fig cultivars grown in Egypt whereas 1: Balck mission, 2: Gizy, 3: Sultany, 4: White Adcy, 5: Aboudi, 6: Conadria and 7: Katoda

Table 2: Genetic polymorphism between seven fig species

Primers	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism percentage (%)
OP-A01	11	3	8	73
OP-A02	14	0	14	100
OP-A04	13	0	13	100
OP-A05	8	4	4	50
OP-A11	11	4	7	64
OP-A16	13	8	5	39
OP-A18	11	4	7	64
OP-A19	16	9	7	44
OP-A20	14	7	7	50
All primers	111	39	72	65

Table 3: Total bands produced from each primer for the seven fig species

	Primers								
Species	OP-A01	OP-A02	OP-A04	OP-A05	OP-A11	OP-A16	OP-A18	OP-A19	OP-A20
Black mission	6	8	6	7	8	9	8	13	10
Gizy	7	5	4	6	6	10	9	12	10
Sultany	6	6	6	6	8	9	6	11	10
White adcy	7	5	7	6	7	9	7	11	11
Aboudi	7	8	8	5	9	10	7	11	10
Conadria	7	8	8	6	10	9	7	12	11
Katoda	7	7	6	5	8	9	8	9	10

Table 4: Genetic similarity matrix between seven fig cultivars with RAPD markers based on Jaccard's coefficients

Species	Black mission	Gizy	Sultany	White adcy	Aboudi	Conadria	Katoda
Black mission	1.00						
Gizy	0.62	1.00					
Sultany	0.81	0.56	1.00				
White adcy	0.56	0.49	0.57	1.00			
Aboudi	0.74	0.55	0.74	0.63	1.00		
Conadria	0.72	0.56	0.74	0.72	0.82	1.00	
Katoda	0.62	0.55	0.61	0.54	0.71	0.62	1.00

the OPA-02 and OPA-04 primers produced the highly polymorphism percentage (100%), meanwhile OPA-16 and OPA-19 produced the lowest polymorphism percentage 39 and 44%, respectively.

In regard to fig cultivars, as shown in Table 3, most of fig cultivars showed high number of bands (ranged from 11 to 13) with OPA-19 primer except Katoda cultivar that gave the highest number of bands (10) with OPA-20 primer. That indicates, primers OP-A19, OP-A16 and OP-A20 were the most effective primers to discriminate genetic variation among the fig cultivars under study.

Genetic similarity: One of the present study goals was to investigate the RAPD marker efficiency in determining, accurately, the genetic relationship between fig Egyptian cultivars.

Nine primers were used to construct a similarity matrix (Table 4) for seven fig cultivars. Simple matching coefficient ranging from 0.49 to 0.82 suggested a broad genetic base for fig genotypes. The genetic similarity estimates of the 21 pairwise comparisons among the genotypes, based on the 72 polymorphic and 39 monomorphic bands.

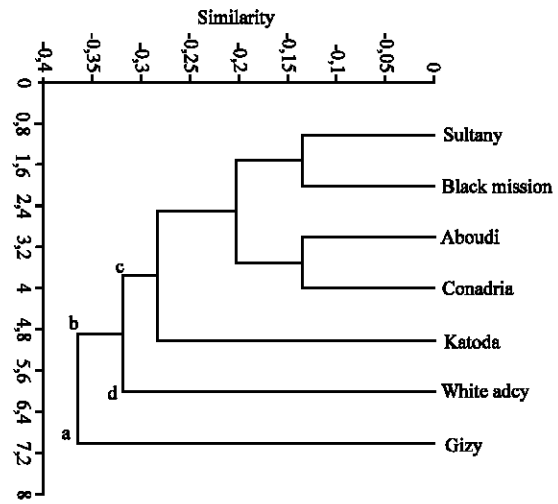

Fig. 2: Dendrogram of genetic relationships among the studied fig cultivars

The similarity values showed clearly substantial differences among the fig varieties. The genetic similarity ranged from 0.49 to 0.82. The large genetic diversity that resulted in this study may be due to the different areas from which the genotypes were collected. Some distinctive varieties showed high genetic similarity with each other, such as Aboudi and Conadria (0.82) and between sultany and Black mission (0.81). On the contrary, some varieties displayed low genetic similarity such Gizy and the rest studied fig cultivars (0.49-0.62) and particularly white adcy (0.49). This distanced could be good sign for breeder.

The dendrogram tree of the studied fig cultivars were performed using UPGMA cluster analysis of Jaccard genetic similarity coefficients generated from nine RAPD markers (Fig. 2). The results of fig cultivars analysis revealed a high diversity between these cultivars. Two main clusters could be observed. The first cluster (a) included Gizy cultivar, while the second one (b) included remain cultivars. The second Cluster divided into two sub-clusters (c and d). The (d) cluster includes only White Adcy, meanwhile (c) cluster divided into two groups. First group included both of Sultany and Black mission and the other group included both of Aboudi and Conadria.

DISCUSSION

The goal of this study was to appraise the usefulness of RAPD-PCR as an approach to routine assessment of the genetic richness of fig species while circumventing the need for a common or single genetic marker. RAPD markers allow more precise identification and determination of the genetic relationships among individuals (Van Heusden and Bachmann, 1992) and provide an estimate of population structure (Gabrielsen *et al*., 1997; Martin *et al*., 1997). In addition, RAPD has created the opportunity for fine-scale genetic characterization of germplasm collections because it is highly polymorphic and are not readily influenced by environmental conditions (Geuna *et al*., 2003; Hokanson *et al*., 2001; Oraguzie *et al*., 2001). RAPD markers have been previously used for cultivar characterization in fig (DeMasi *et al*., 2003; Elisiario *et al*., 1998; Khadari *et al*., 1995). Our results enable researchers to distinguish or identify among particular genotypes under investigation by using a specific RAPD marker or the combination of more than 1 RAPD marker.

Farooq and Azam (2002) and Wang *et al.* (2009) reviewed that a new generation of DNA markers such as randomly amplified polymorphic DNA (RAPD), were introduced into the modern plant breeding systems. Some of this marker is relatively simple, easy to use, automatable, dominant, near infinite in number and is comparatively faster to assay. Frequent application of such markers systems transformed the classical/conventional plant breeding programmes into Marker-assisted Plant breeding or plant molecular breeding. The levels of polymorphism generated by the nine random primers in our study (Table 1) showed more variance among the local and imported genotypes cultivated in Egypt. This variation could be useful for improvement certain characterization.

The dendrogram (Fig. 2) clustered the genotypes into variable groups and subgroups and showed efficiency in identifying genetic variability. Moreover, four of local and imported cultivars (Aboudi and Conadria and Black mission and sultany) were clustered together that they possessed narrow genetic background and indicating that they may be produced from one origin genotype. That means, one of these genotypes is the common ancestor and the others were produced by evolution during their presence in different origins to be adapted with environmental conditions. That is in agreement with Pathak *et al.* (2010) and Abd-El-Hady *et al.* (2010) who reported that the genotypes from the same geographical region were grouped into different clusters.

The different genotypes may have the same pedigree and common ancestor, at least for one of the parent. In the future, it is important to perform cell hybridization between the long genetic distance fig genotypes by using cell fusion to improve fig characterization. In conclusion, the molecular genetic analysis used in the present study successfully distinguished among different fig cultivars. The study could recommend the use of RAPDs as a rapid and accurate method of identification to facilitate classification of figs species for the development of germplasm and breeding programs in *Ficus carica*.

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