Identification of Varietal Polymorphism in *Ficus carica* L. by RAPD (Randomly Amplified Polymorphic DNA) Markers

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Abstract

Since 1982, the Faculty of Agriculture at the Çukurova University has fig collection orchards containing various cultivars from Aegean, Southeast Anatolia, Marmara and Mediterranean regions. The Mediterranean area is mainly suitable for table fig production and these cultivars are named by their rind or flesh color such as yellow, green, purple, black etc. Therefore, there is confusion in identification of different cultivars. RAPD-markers allow genotype characterization to be done directly by DNA analysis. Random amplified polymorph DNA (RAPD) using twelve primers were characterized in thirty fig cultivars (*Ficus carica* L.). DNA was extracted from vegetative buds and amplified by PCR using random oligonucleotid primers. Genetic similarity values (Nei and Li, 1979) were calculated and UPGMA (Unweighted pair-group method analysis) cluster analysis was performed to generate a dendogram. The method, which is simple and rapid, should prove a useful system for the identification of fig cultivars.

INTRODUCTION

Turkey is the native origin of fig (*Ficus carica* L.), from which it was spread to other Mediterranean countries. Turkey produces almost 30 % of world fig production.

The common fig (*Ficus carica* L.) is a gynodioeous plant species, which grows and reproduces in the wild throughout the Mediterranean Basin (Kjellberg et al., 1987; Küden, 1997; 1999). The Mediterranean area is mainly suitable for table fig production and these cultivars are defined by their rind or flesh color such as yellow, green, purple, black etc. Therefore there exists confusion in evaluation of genetically different cultivars having the same colour (Küden, 1997; 1999). Molecular markers can successfully complement the identification and characterization of fig cultivars in addition to their morphological traits and agronomic performance, which usually influenced by the environmental conditions.

Traditional methods for cultivars identification are based on objective descriptions of the tree and fruit characteristics. However, these observations are time consuming and error-prone due to variations caused by environmental factors, which affect the expression of these characteristics (Guilford et al., 1997).

New technologies were introduced in the last years. Of these, trait markers can detect differences among trees directly at the DNA level. Analysis can thus be shifted from the tree phenotype to its genotype in fruit species.

Isoenzymes, RFLPs (Restriction Fragment Length Polymorphism), RAPDs (Randomly Amplified Polymorph DNA) and microsatellites (SSR) are the markers most widely used for cultivars identification.

Isoenzyme has been useful for cultivars identification. However, such variation has remained restricted to a few polymorph enzyme systems that are encoded by a limited number of loci.

Comparative analysis of isoenzyme patterns has been introduced as a supplementary method to identify cultivars and has been used to identify cultivars of many species including the common fig. Twenty-seven cultivars and seventeen enzymes were analysed and degree of electrophoretic similarity between cultivars was determined

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by calculating a similarity index value based on three enzyme systems: acid phosphates (AcPH), glutamate oxalacetate transaminase (GOT), and malate dehydrogenase (MDH) (Chessa et al., 1998). Although isozymes can be very useful in cultivars identification, they can be tissue specific and plant tissue must be sampled at comparable physiological states to obtain uniform and repeatable banding patterns. Furthermore, the limited number of isozyme loci available for examination may be insufficient for identification of many cultivars.

RFLP (Botstein et al., 1980) markers are co dominant and provide complete genetic information at a single locus. In addition to the special care needed in handling the radioactive probes, difficulties in extracting high quality DNA from many tree crop species has limited application of RFLP analysis (Warburton and Bliss, 1996). The discovery of polymerase chain reaction (PCR) has led to development of another genetic marker system for detecting DNA polymorphisms (Welsh and McClelland, 1990; Williams et al., 1990). This technique allows detecting extensive polymorphisms resulting from the unlimited number of arbitrary primers (Khadari et al., 1995).

In this study the genetic relationship among thirty fig cultivars and the level of polymorphism was assessed by using RAPD markers.

MATERIALS AND METHODS

Plant Material

The analysis performed on 30 fig cultivars (Table 1). Plant material was obtained from the research and experimental orchards of the University of Cukurova, Agricultural Faculty Horticultural Department (Kaşka et al., 1990).

DNA Isolation

Genomic DNA was extracted from vegetative buds according to Dellaporta et al. (1983). DNA concentration has been assessed using a spectrophotometer.

DNA Amplification

Fifteen 10-mer primers which were polymorph in fig were used to generate RAPD markers. Arbitrary decamer oligonucleotids purchased from Operon Technologies (Almeda, CA, USA). Amplification reaction were done in 25 µl volumes containing 10 X Taq DNA polymerase buffer, 200 µM each of dATP, dCTP, dGTP, dTTP, 30 ng of the primer 1 unit of Taq DNA polymerase and 20 ng of fig DNA. The mixtures were assembled at 0^0C, and then transferred to termal cycle, precooled at 4^0C.

Amplifications were performed Perkin Elmer thermal cycle (model; 9600; Perkin Elmer Applied Biosystems, Inc., Foster City, CA.) programmed according to Khadari et al. (1995).

Electrophoresis in Agarose Gel

The amplification products were separated by electrophoresis in 2 % agarose gels in 1x TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH:8.0) for 3 h at 70 volts. The gel stained with ethidium bromide and the fragment patterns were photographed (667, Polaroid) under UV light for further analysis.

Data Analysis

Bands on agarose gels were scored as present or absent and pair wise similarity matrix was constructed using the Dice coefficient : 2c / (a+b+2c) (Sneath and Sokal, 1973). The similarity values were calculated as the doubled number of bands (2c) shared between two patterns divided by the sum of all the bands in the same patterns, which comprises bands present in one of two patterns (a), bands present in the other one (b) and shared bands (c).

RESULTS AND DISCUSSION

Thirty fig cultivars were screened for RAPD markers using a set of 12 primers in a PCR-based DNA amplification procedure. Among 58 bands generated by 12 selected primers, 21 (36 %) were constant and corresponded to monomorphic loci. The remaining 37 variable bands were selected as RAPD markers (Table 2) The number of amplified DNA products was cultivars and primer dependent. Although the number of bands for each primer varied from 1 to 9 with an average of 4.8 bands per primer, the sizes of the amplified DNA bands were ranged from 200 to 1600 bp (Table 2).

Cluster analysis of genetic relationship was conducted to generate the dendogram of the fig types and the cultivars studied (Fig.1)

The analysis of 30 fig cultivars using 12 RAPD primers allowed us to distinguish all cultivars except Mor 3 and Mor 4 cultivars.

Based on 58 fragments a similarity matrix was generated using Dice coefficient of Nei and Li (1979). Based on dendogram, the genotypes can be separated to two major groups with a similarity value of 0.80.

The group 1 occurred as a big group and divided into two groups in itself (Group 1.1 and 1.2). Group 1.1 was also divided into two groups. The subgroup of 1.1.1 consists of black and purple fig cultivars (Mor 3, Mor 4, Bursa Siyahı, etc.), as well as green and yellow figs (01-IN-05, Bardakçı, Köfte, Kış inciri etc.). Group 1.1.2. consists of only yellow and green cultivars.

The group 1.2. consists of selected cultivars from Cukurova region, which contains black cultivars as well as yellow or green ones. The separation of these cultivars in one group was related to the geographic distribution of the population and relatively limited gene flow.

Group 2 was constituted only from Sultan Selim and İzmir Bardakçı, which might be considered as relatives.

This study demonstrated that RAPD markers are useful tools for the fig varietal identification. The advantage of this technique is to have ability to detect extensive polymorphisms, simplicity, rapidity and no need of radioisotopes (Khadari et al., 1995).
In conclusion, Turkey is the main genetic origin of fig in the world. Various kinds of cultivars can be found in different parts of the country with different or similar names. This study must be continued with more experiments to identify and characterize these cultivars in molecular basis.

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Tables

Table 1. List of fig cultivars used in this study.

Number	Name	Number	Name
1-	01-IN 03	**16-**	Şaranpol
2-	01-IN-04	**17-**	Patlıcan
3-	01-IN-05	**18-**	Bardakçı
4-	01-IN-06	**19-**	Köfte
5-	01-IN-07	**20-**	Karabakunya
6-	01-IN-08	**21-**	Kaya İnciri
7-	01-IN-09	**22-**	Sultan Selim
8-	01-IN-10	**23-**	İzmir Bardakçı
9-	01-IN-11	**24-**	Kış İnciri
10-	01-IM-01	**25-**	Kuş İnciri
11-	01-IM-02	**26-**	Gök Lop
12-	33-IN-04	**27-**	Kuşadası
13-	Mor-3	**28-**	Akça 1
14-	Mor-4	**29-**	Kara Yaprak
15-	Kara Hünü	**30-**	Bursa Siyahı

Table 2. Primers with the number of amplified products.

Primer[a]	Sequence (5'—3')	No. of total DNA products
OPA -11	CAATCGCCGT	1
OPA -16	AGCCAGCGAA	8
OPA -18	AGGTGACCGT	4
OPH -11	CTTCCGCAGT	5
OPH -18	GAATCGGCCA	7
OPK -17	CCCAGCTGTG	3
OPX -05	CCTTTCCCTC	3
OPX -09	GGTCTGGTTG	3
OPX -11	GGAGCCTCAG	6
OPY -04	GGCTGCAATG	9
OPY -11	AGACGATGGG	7
OPY -14	GGTCGATCTG	2

[a]Marker notation refers to the kit (last letter) and the primer (-number) purchased from Operon Technologies (OP).

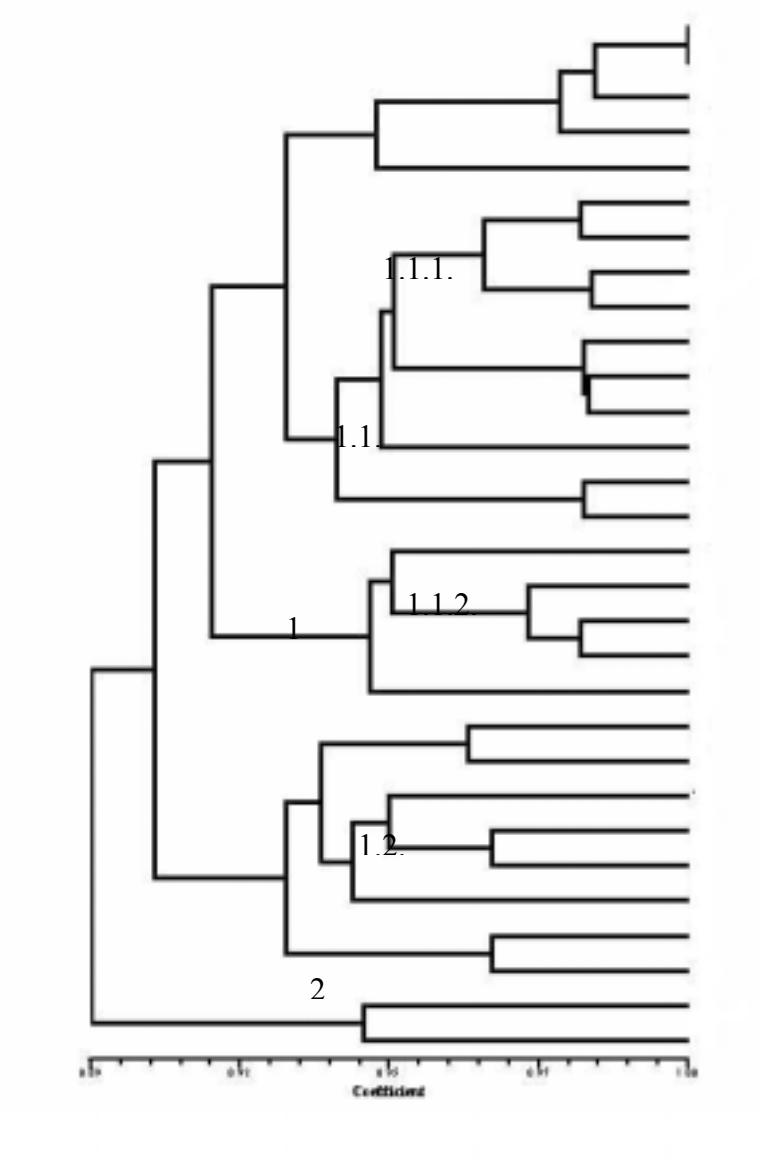

Fig. 1. Dendogram of the genetic relationship of 30 fig cultivars based on 12 RAPD primers.