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Original Research Article

Genetic Characterization of Edible Fig (*Ficus carica* L.) Genotypes Grown in Siirt Region Based on *Trn*l-F Region*

Bahar Özdemir[1], Serdar Altintaş[2], Koray Özrenk[1†] and Ferit Çelik[3‡]

[1]Department of Horticulture, Faculty of Agricultural, Siirt University, Siirt, TURKEY
[2]Department of Agricultural Biotechnology, Faculty of Agricultural, Siirt University, Siirt, TURKEY
[3]Department of Horticulture, Faculty of Agricultural, Van Yüzüncü Yıl University, Van, TURKEY

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ABSTRACT

In this study, molecular characterization of fig genotypes grown in Siirt province was provided based on the *trn*L-F region. Phylogenetic trees created using NJ and UPGMA methods formed a total of 10 groups for both regions. The genotypes 10, 16, 5 and 4 from the same location were successfully separated from others, while genotype 13 of the same location were grouped in a different cluster. When the phylogenetic trees are examined, the groups differ from each other. In conclusion, genotypes with diversity should be studied in depth with further evaluations.

Keywords: Siirt, Fig, *Ficus carica* L., Molecular Systematic, *trn*L-F

INTRODUCTION

There are more than 1400 species in the *Moraceae* family, including figs (*Ficus carica* L.). The *Ficus* genus contains about 700 species (Watson and Dallwitz, 2004). Although fig is a subtropical fruit, with high adaptability it can also widely grown in the temperate climate zone (Kaşka *et al.* 1990). Fig production has been done since ancient times. North Anatolia, the coastal regions of the Black Sea, the Caucasus in the south of the Caspian Sea, Iran, Iraq, India, and the southwest of Arabia are the places where fig cultivation has been made since ancient times. America, South Africa and Australia are new cultural centers of figs (Kabasakal, 1990). It has been accepted as an exotic fruit in places where it is not cultivated (Aksoy *et al.* 2007).

Herodotus, in the sources he wrote in 484, stated that fig culture in Anatolia dates back to ancient times as much as human history (Çalışkan and Polat, 2012). Figs are described as a sacred fruit in almost all of the biblical religions. When we look at fig gene centers, Turkey has a special position due to being at the intersection of the East and the Mediterranean. As a result of having diverse gene resources, Anatolia is known as the homeland of figs as well as many other plant species (Demir, 1990; Ağaoğlu *et al.* 2001).

Our country has a rich variety of figs due to its climate, soil, and ecological diversity. It ranks first in the world in terms of breeding capacity. Figs produced in the Aegean Region, especially in Aydın, are known for their high quality in the world (Aksoy *et al.* 2001). Table figs were not as popular as dried figs in fig production (Aksoy *et al.* 2001; Işın *et al.* 2004). In addition to being a delicious fruit, fig has rich content for vitamins, proteins, minerals, and fiber (Şen, 2009). Although there is a rich diversity of figs in Turkey, unfortunately, it does not see the value it deserves at edible fig production except Bursa. However, in recent years, understanding the nutritional value and importance of figs promoted, increasing the possibilities of carrying fresh fruits, developments of package suitable for fig fruits have facilitated the supply of table figs to the foreign market and are in demand.

Wild fig forms are widely grown in the entire Mediterranean basin of Anatolia, in the Aegean and Southeastern Anatolia regions. The Southeastern Anatolia region contains diverse rich fig forms. Various cultured and wild forms of fig are found in Siirt (Botansuyu Basin), Diyarbakır, Elazığ, Gaziantep, Kahramanmaraş, and in Ceyhan Basin. Therefore, the Southeastern Anatolia Region has a special position as a fig gene center (Ilgın, 1995).

The *trn*L intron group and the region between trnL and *trn*F (intergenic space) are widely used in plant systematics and are among on-coding DNA regions (Quandt *et al.* 2004). The intergenic space is located between the *trn*L

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† Corresponding Author: korayozrenk@hotmail.com

‡ Thesis Joint Advisor

(UAA) 3 'exon and the *trn*F (GAA) gene. The *trnL*-F region is amplified by PCR using special primers to determine the base polymorphism and to determine the relationship between species.

The *ITS* region of nuclear ribosomal DNA has been used for phylogenetic studies in angiosperms since the 1990s. *ITS1* and *ITS2* regions in the *ITS* region can be easily amplified by PCR and can be sequenced using universal primers. The degree of variation in *ITS1* and *ITS2* regions differ between plant groups. The *ITS* region is a more conserved region than the inner and outer transcribed regions of rDNA (Baldwin *et al.* 1995). In this study, *trnL*-F regions of fig genotypes were used by amplifying with PCR.

In this study, the genetic kinship characteristics of fig genotypes sampled from different locations of the Siirt province in the Southeast Anatolia region, and the differences between these characteristics were tried to determine using various parameters. As a result of the study, it was aimed to determine the phylogenetic structures of fig trees cultivated in Siirt province.

MATERIALS AND METHODS

The research was conducted in Siirt central district and Eruh locations. The material of the study consists of 17 fig genotypes that have been cultivated in the region for many years. Young leaves of the collected fig genotypes were taken into small bags for DNA isolation, transported to the laboratory in ice containers, and stored at -80 °C. Locations, where plant samples were taken, are presented in Table 1.

Table 1. Studied fig genotypes and their geographical origins.

Genotype No	District	Province
1	Eruh	Ormanardı
2	Eruh	Kavaközü
3	Eruh	Bağgöze
4	Siirt	Gökçebağ
5	Siirt	Gökçebağ
6	Eruh	Ormanardı
7	Eruh	Ormanardı
8	Eruh	Kaşıkyayla
9	Eruh	Kavaközü
10	Siirt	Gökçebağ
11	Eruh	Ormanardı
12	Eruh	Kemerli
13	Siirt	Gökçebağ
14	Eruh	Ormanardı
15	Eruh	Ormanardı
16	Siirt	Gökçebağ
17	Eruh	Kemerli

DNA Isolation and PCR (polymerase chain reaction) Stages

DNA was isolated according to CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle, 1987), with modifications (Karaca *et al.* 2005). The extracted DNAs were imaged by pouring in 1% agarose gel, and a nanodrop spectrophotometer was used to determine DNA purity and quality.

*Trn*L-F and *trn*L primers were used for the amplification of *trn*L-F region of genomic DNAs obtained (Table 2). The total reaction volume for PCR was prepared as 50 µl and reaction steps are given in Table 3.

A 1.5% agarose gel was prepared to observe the bands formed as a result of PCR and PCR products were run on this gel.

Table 2. Primers used in PCR and their properties.

Primary	Nucleotide Sequence	Tm Value
*trn*F-f	ATTTGAACTGGTGACACGAG	52.8 ºC
*trn*L-e	GGTTCAAGTCCCTCTATCCC	54.4 ºC

Table 3. PCR program used for *trn*L-F primers.

Step	Temperature	Time	Number of Loops
PreDenaturation	95 ºC	7 mins	1 loop
Denaturation	94 ºC	1 mins	35 cycles
Connecting	52 ºC	1 mins	35 cycles
Elongation	72 ºC	2 mins	35 cycles
Final Elongation	72 ºC	10 mins	1 loop
Final Hold	4 ºC	25 hrs	

Sequence Analysis and Drawing of PhylogeneticTrees

The MEGA6 computer software (Tamura et all., 2013) was used to learn the kinship relations of the species belonging to the genus *Ficus* whose sequences are aligned. Neighbor-Joining (NJ) method and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method were used in phylogenetic tree creation. *Antiaris toxicarica* was used as an outgroup in the study.

RESULTS AND DISCUSSION

The qualities of the obtained DNAs were determined by agarose-gel, and it was determined that necessary and sufficient DNA for PCR (polymerasechainreaction) was obtained. Image of the gDNAs (genomic DNA) of the study material on agarose gel (Figure 1A) and the image of PCR products are presented (Figure 1B).

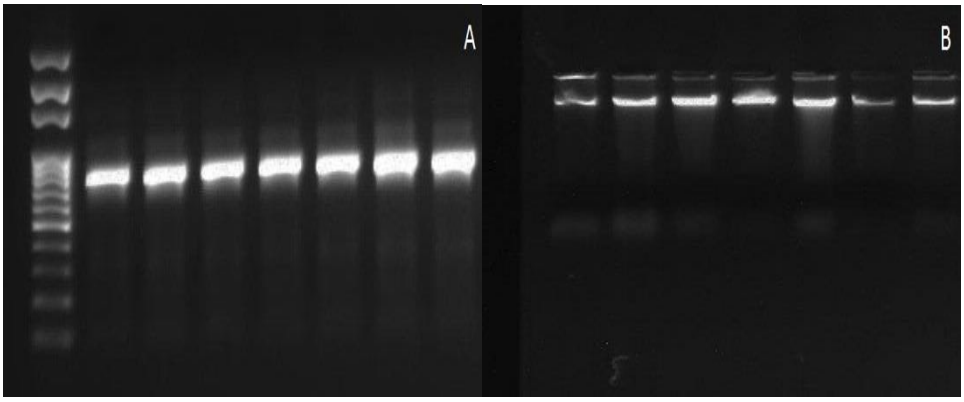

Figure 1. Gel image of 7 samples of PCR product (A) and genomic DNA (B) for *Ficus carica* L.

PCR products were read in one direction and the sequences obtained were analyzed with various software (Bioeditsequencher and MEGA-X). Sequence data of the regions used in the study were aligned using the ClustalXsoftware (Thompson *et al.* 1997) (Figure 2-3).

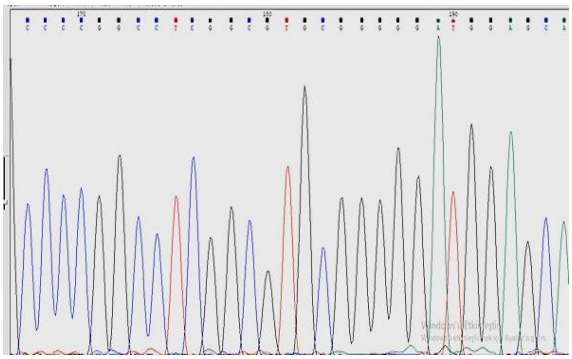

Figure 2. Part of the chromatogram image of the *trn*L-f region of the *Ficus carica* L. 2 sample.

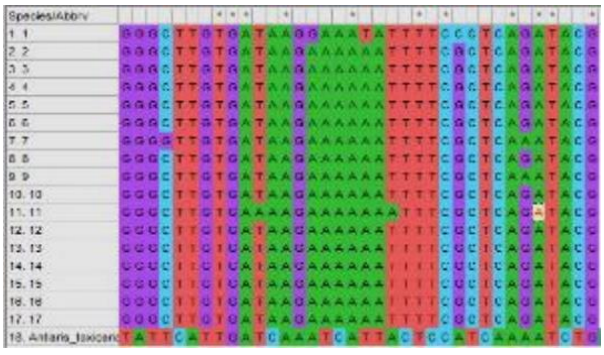

Figure 3. A portion of the aligned *trn*L-F sequences in the ClustaIW program.

Phylogenetic trees of the *trn*L-F region based on ML and NJ methods gave different results in separating individuals belonging to the same species. For example, genotypes 10, 16, 5, 4, and 17 were collected in a single group in the NJ method (Figure 4), while genotypes 10, 16, 5, 4 and 13 were collected in one group in the UPGMA method (Figure 5). While *Antiaris toxicarica* used as an outgroup in the NJ method formed a single group as expected, other genotypes formed a separate group among themselves.

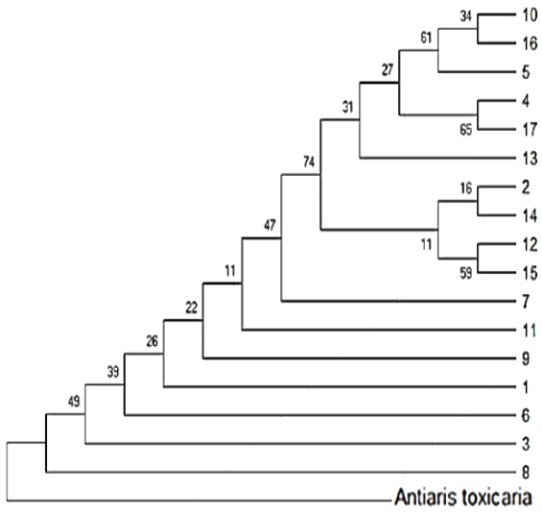

Figure 4. Tree of the *trn*L-F region as a result of Neighbor-Joining analysis (Numbers in the nodes of the phylogenetic tree show the bootstrap values)

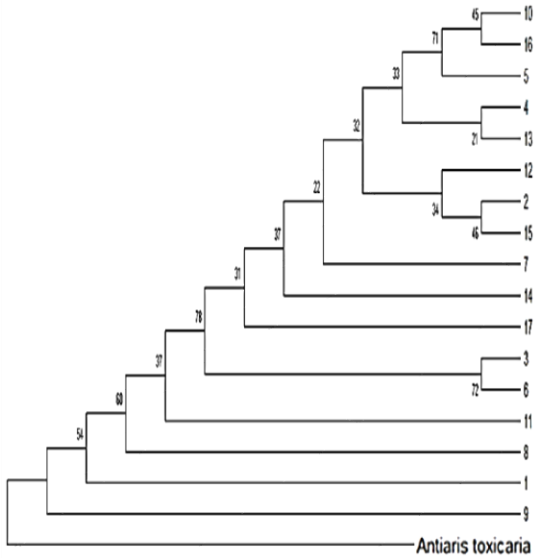

Figure 5. Tree of the *trn*L-F region as a result of UPGMA analysis (Numbers in the nodes of the phylogenetic tree show the bootstrap values)

Similarly, *Antiaris toxicarica* used as an outgroup in the UPGMA method formed a distinct group, while other genotypes formed a group among themselves. In both phylogenetic trees, genotypes 10, 16, 5, and 4 belonging to the same location were successfully separated from the others, while genotype 13 from the same location was formed a different group according to the method used. In this context, genotypes 10, 16, 5, 4, and 13 belonging to the same location were collected in a single group in the UPGMA method, while genotype 13 was located in a different place in the NJ method. Similarly, when the tree topography obtained as a result of each method is examined, it was seen that the branches are supported with different bootstrap values. For example; in the UPGMA method, branches are separated from each other with a bootstrap value of 54%, where as this rate was 49% in the NJ method. The bootstrap analysis is one of many analyzes that statistically reveal the reliability of phylogenetic relationships. This analysis is used to determine the most reliable branches in statistical terms using the parsimony criteri on from the branches of the obtained trees (Felsenstein, 1985). Bootstrap values ranging between 0-100% are defined as very strong if greater than 85%, strong between 70-85%, weak between 50% and 70%, and very weak if less than 50% (Kress, 2002).

The methodological articles published on DNA barcoding studies so far have focused on genome regions that will provide the most appropriate data from the taxonomists' point of view. For example; in the animal kingdom, mitochondrial cytochrome oxidase subunit 1 (COX-1) X 1 is thought to be a good candidate for barcoding studies. In the plant kingdom, on the other hand, it is much more difficult due to the very slow evolution of the mitochondrial and chloroplast genomes, which will ensure sufficient variation. In this case, the current strategy for taxonomists is to sequence various DNA regions that contain both nuclear and chloroplast fragments. In the study, the *trn*L-F region was successful in separating individuals belonging to different locations. However, this situation did not apply to the separation of individuals belonging to the same locations. Studies have shown that the rate of nucleotide change in plastid DNA is very low compared to the nuclear genome. In other words, while these sequences were uniform for all individuals obtained from a single variety, it should not be expected from all varieties belonging to the same species. The differences seen in our study may be partly due to this

situation. Also, the gene region used in characterization is important because the evolution processes and speeds of different regions belonging to different genomes differ from each other. For example; the *trn*H-psbA region belonging to the chloroplast genome is a relatively rapidly evolving region and is frequently used in species-level definitions. It was stated that the *trn*L-F region of the chloroplast genome we used was effective in genus-level definitions (Quandt *et al.* 2004).

CONCLUSIONS

Ficus carica L. genotypes used in the study were tried to be characterized by using the *trn*L-F region of the chloroplast genome. UPGMA and NJ methods, which are distance-based, were used to create dendrograms.

When the phylogenetic trees obtained are evaluated in terms of the methods used, it can be said that the phylogenetic tree created with the UPGMA method gives more successful results in separating the genotypes collected from different locations compared to the NJ method. However, when the genotypes belonging to a region were evaluated in both methods, the genotypes collected from the Eruh region were spread in different branches in the phylogenetic tree (when the genotypes belonging to this location were evaluated within themselves) (Figure 6).

The data obtained in the study conducted enabled the comparison and criticism of the fig genotypes sampled from the Siirt region at the molecular level. Considering that the samples used as the material in the study belong to a single species, we think that using different gene regions in combination or conducting supportive studies such as morphological characterization in addition to molecular characterization will give healthier results.

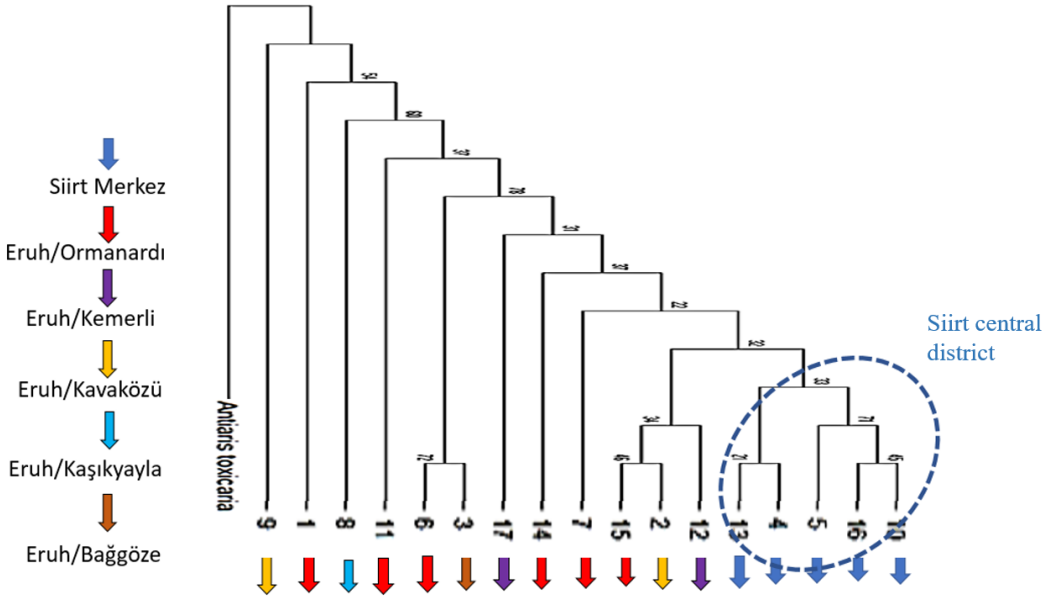

Figure 6. Tree and location markers formed as a result of UPGMA analysis of the *trn*L-F region.

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