Romanian Biotechnological Letters *Vol. 22, No. 2, 2017*

ORIGINAL PAPER

Molecular Identification of Fig Cryptic Virus and Fig Fleck-Associated Virus in Turkey

Received for publication, March 26, 2015
Accepted, January 10, 2016

EMİNUR ELÇİ[1*], TUĞÇE HANÇER[2], KADRİYE ÇAĞLAYAN[3]
[1]*University of Ömer Halisdemir, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, Niğde, Turkey*
[2]*University of Mustafa Kemal, Faculty of Agriculture, Department of Field Crops, Hatay, Turkey*
[3]*University of Mustafa Kemal, Faculty of Agriculture, Department of Plant Production, Hatay, Turkey*
***Address correspondence to:** University of Ömer Halisdemir, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, 51240, Niğde, Turkey*
Tel.: +90388225 4478; Email: eminur@gmail.com

Abstract

Recently, several new viruses infecting fig trees were identified. To assess the presence, distribution and genetic diversity of Fig cryptic virus (FCV) and Fig fleck-associated virus (FFkaV) in fig trees of Turkey, a total of 65 fig samples, which show yellowing, chlorotic, necrotic spots and vein clearing symptoms, were collected from Aegean and Mediterranean regions, which are the most important fig growing regions of Turkey, in spring 2014 and tested by molecular analysis. After cDNA synthesis, FCV and FFkaV specific primer sets of RNA dependent RNA polymerase (RdRp) regions were used for reverse transcription-polymerase chain reaction (RT-PCR) analysis and the PCR products were directly sequenced. The obtained sequences were analyzed and nucleotide sequence analysis confirmed the FCV and FFkaV infections. According to the results, some of the fig trees are infected in Turkey by FCV and FFkaV with an incidence of 20 % and 9.2 %, respectively. BLAST analysis of both FCV and FFkaV has shown high identity with Italian isolates (FCV|ref|NC015494.1 and FFkaV|ref|NC015229.1). For FFkaV, the phylogenetic analysis, constructed from partial RdRp nucleotide sequences, clustered the isolates based on their geographical origin. While the correlation between FFkaV isolates and regions was very high, no correlation between collection regions and FCV isolates was observed. It can be concluded that, fig trees from the most important fig growing regions of Turkey are infected by FCV and FFkaV and it is instrumental to overview of the viral control strategies for fig plantations in Turkey.

Keywords: *Ficus carica* L., FCV, FFkaV, RT-PCR, RdRp, sequencing.

1. Introduction

Fig (*Ficus carica* L.) has been cultivated since ancient times through the Eastern Mediterranean to the Southern Asia region. The main production countries are mainly Mediterranean and Asian countries such as Turkey, Egypt, Algeria, Morocco and Iran. Turkey is one of the leading producers and exporters for the fig fruit and total fig production reached 274.535 tons in 2012 that corresponds to one of the largest fig economies in the world [1].

Recently, several viruses infecting fig trees have been identified from various fig growing countries and they belong to *Closteroviridae*, *Bunyaviridae*, *Flexiviridae*, *Partitiviridae*, *Tymoviridae* and *Caulimoviridae* families [2-3-4-5-6-7-8-9-10]. The complexity of viruses cause induces of yield and fruit deformations on figs. Also, due to the propagation methods of fig, viruses can widely spread to the healthy plants.

Fig cryptic virus (FCV) is one of the previously identified viruses and it is a member of the family *Partitiviridae* of the genus *Deltapartitivirus*. It has a bipartite genome; RNA-1 contains one open reading frame (ORF) encoding a 54 kDa protein and comprising RNA dependent RNA polymerase (RdRp) domain, RNA-2 has also single ORF coding coat protein (CP). Its prevalence was reported in Albania, Algeria, Italia, Lebanon, Syria, Tunisia and Iran with an incidence ranging from 4.5% to 18.5% [6-11]. Also, it was detected with an infection rate of 15% in fig trees of Turkey [12].

Fig fleck-associated virus (FFkaV) is also one of the recently identified viruses which belong to family *Tymoviridae* of the genus *Maculavirus*. Its genome consists of a positive sense, single stranded RNA (ca.7046 na) which has two ORF encoding replication-associated proteins, the coat protein (p24) and putative movement proteins (MP). It was detected in various Mediterranean countries such as Albania, Algeria, Italia, Lebanon, Syria, Tunisia and Iran with an incidence ranging from 8.6% to 25% [6-11]. However, there had not been any report on FFKaV infections in Turkey, yet.

Up to now, there are only a few reports published on fig infecting viruses in Turkey [12-13-14-15]. The aim of this study was to detect FFkaV in different fig-growing regions of Turkey and determine the distribution and phylogenetic relationships of FCV and FFKaV isolates in Turkey.

2. Materials and Methods

The leaf samples of fig trees, which show virus-like symptoms, were collected from some of the fig-growing regions of Turkey in spring 2014. Totally, 65 trees were tested; 15 trees from each of İzmir, Aydın and Bursa provinces, respectively, and 20 trees from Hatay province in Turkey (Table 1). Positive samples were kindly supplied by Dr. Angelantonio Minafra (Bari, Italy).

Table 1. Fig cryptic virus (FCV) and Fig fleck-associated virus (FFkaV) incidence in different fig-growing provinces of Turkey.

Sampled Province	No. of Tested Trees	No. of Infected Trees by FCV	No. of Infected Trees by FFkaV	*Total Infections (%)*
İzmir	15	2 (13%)	3 (20%)	*33%*
Aydın	15	2 (13%)	1 (6.6%)	*20%*
Bursa	15	1 (6.6%)	1 (6.6%)	*13%*
Hatay	20	8 (40%)	1 (5%)	*45%*
Total	***65***	***13 (20%)***	***6 (9.2%)***	***29%***

Total RNAs were extracted from leaf samples by using the RNeasy Plant mini kit (Qiagen Sci., Hilden, Germany) after homogenization of tissue samples in liquid nitrogen following the manufacturer's instructions. The yield and quality of total RNAs were estimated by using a NanoDrop spectrophotometer (Thermo Sci., USA) and RNAs were stored at –80ºC. cDNA was synthesized from total RNAs using random primers with the Super Script Choice System for cDNA synthesis (Invitrogen, ThermoFisher Sci., Massachusetts, USA). The synthesized cDNAs were used as template in PCR analysis. The PCR was carried out with 3 µl of cDNA, 0.5 µl of 200 µM dNTP, 1 µl of 25 mM $MgCl_2$, 2.5 µl of 5x PCR buffer and 0.5 µl of 10 µM of each virus specific primers with 0.25 µl of 5 units/µl Taq DNA polymerase (Promega Corp., Fitchburg, WI, USA). FCV and FFkaV specific primers (R1-s 5'-TCGGATTGTCTTTGGAGAGG-3', R1-a 5'-CGCATCCACAGTATCCCATT-3', ca. 353 bp and D8-s 5'-TCAATCCCAAGGAGGTGAAG-3', D8-a 5'-ACACGGTCAATGAGGGAGTC-3', ca.

270 bp) were used for amplification of a part of RNA that encodes viral RdRp genes, respectively [6-7]. PCR reactions were performed at 35 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min; and a final extension for 10 min at 72°C. The PCR products were analyzed using an automated capillary electrophoresis at Fragment Analyzer ™ (Advanced Analytical Technologies, Inc. Ames, USA).

The PCR products were directly sequenced for both directions using the ABI 3730 Automated Genetic Analyzer at MedSanTek Company (İstanbul, Turkey) after PCR purifications. The obtained sequences were assembled and analyzed with the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI). Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06 [16] for the partial RdRp regions of FCV and FFkaV. All the RdRp sequences of FCV and FFkaV isolates deposited in GenBank (NCBI) were included to the phylogenetic analysis. Multiple alignments of nucleotide sequences were performed with the algorithm CLUSTAL W [17] and the neighbor-joining method was used to construct phylogenetic tree with the program MEGA 6.06 [16] with p-distance method based on 500 bootstrap replicates. The pairwise distance method was used to estimate the evolutionary distances between sequences by computing the proportion of nucleotide differences between each pair of sequences based on the Kimura two-parameter model (Kimura 1980) [17].

3. Results and Conclusions

Although, there are no studies on symptomatology of FFkaV and no visible symptoms were reported with FCV infected plants yet, symptomless and leaf samples with virus-like symptoms were collected. Based on the field observations, most of the leaf samples showed mosaic spots, vein clearing and yellowing symptoms. The molecular detection of FCV and FFkaV in fig trees with those symptoms could be due to the mixed infections and complex mechanism of the fig viruses. Previously, mixed infections of FCV by various viruses on the fig trees were reported in Turkey [12]. Based on this report, the tested 13 FCV infected trees from İzmir were also infected by *Fig mosaic virus* (FMV) and Fig badnavirus-1 (FBV-1) and five of them were also infected by Fig latent virus 1 (FLV-1) in addition to FMV and FBV-1. However, the detected one FCV infected tree was not mixed infected by any other tested fig viruses out of the tested two FCV infected trees from Hatay [12]. In this study, none of the tested trees were infected both FCV and FFkaV. Moreover, most of the fig viruses are recently identified and there are still questions about virus-symptoms relations. Based on RT-PCR results, RNAs were amplified ~ 353 bp products of FCV and it was detected in all the tested provinces with an incidence ranging from 6.6 % to 40% (Table 1, Figure 1A).

Table 2. The nucleotide and amino acid identities of Fig cryptic virus (FCV) and Fig fleck-associated virus (FFkaV) isolates with reference Italian isolate based on BLAST analysis.

Isolate Name	Acc. No.	Collection Region	Nucleotide identity (%)	Amino acid identity (%)
FFKAV33	KT267253	İzmir	* 90	* 99
FFKAV34	KT267254	İzmir	* 90	* 96
FFKAV36	KT267255	Aydın	* 90	* 99
FFKAV28	KT267256	Hatay	* 90	* 96
FFKAV35	KT267257	Bursa	* 90	* 99
FFKAV37	KT267258	İzmir	* 90	* 99
FCV1	KT267259	Hatay	** 99	** 100
FCV3	KT267260	Hatay	** 99	** 100

FCV4	KT267261	İzmir	** 85	** 70
FCV5	KT267262	Hatay	** 99	** 99
FCV6	KT267263	Hatay	** 99	** 98
FCV7	KT267264	Hatay	** 99	** 98
FCV8	KT267265	Hatay	** 99	** 97
FCV47	KT267266	İzmir	** 99	** 97
FCV48	KT267267	Hatay	** 98	** 95
FCV50	KT267268	Hatay	** 99	** 94
FCV52	KT267269	Bursa	** 99	** 96
FCV58	KT267270	Aydın	** 99	** 99
FCV59	KT267271	Aydın	** 99	** 99

** Identity with reference FFKaV isolate (KC331993.1for nucleotide and YP004300302.1 for amino acid identity)*
*** Identity with reference FCV isolate (FR687854.1 for nucleotide and YP004429258.1 for amino acid identity)*

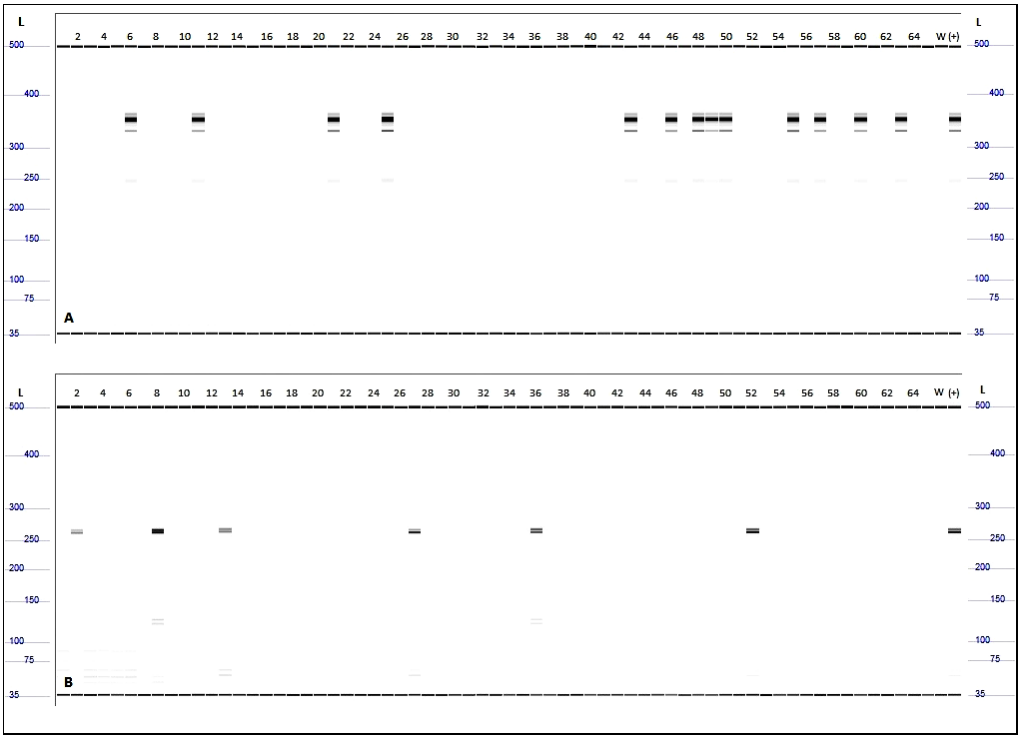

Figure 1. PCR amplifications of Fig Cryptic Virus (FCV) and Fig Fleck-associated Virus (FFkaV) in different 65 fig trees, A: Fragment Analyzer results of PCR products of FCV, B: Fragment Analyzer results of PCR products of FFkaV, L: 35-500 bp Ladder (Advanced Analytical Technologies, Inc. Ames, USA), 1-15: Samples collected from İzmir, 16-30: Samples collected from Aydın, 31-45: Samples collected from Bursa, 46-65: Samples collected from Hatay. W: Water control, (+): Positive control.

A tree from Bursa, 2 trees from both Aydın and İzmir provinces were infected by FCV among the 15 trees per region, respectively. The most infected trees were observed with a 40% incidence in Hatay, which is located in the Southern part of Turkey. Result showed that, 20% of the tested plants were infected by FCV. This result is similar to the report from Elbeaino &

al. (2011a) and our previous report [12]. Elbeaino & al. reported the findings about FCV infections with an incidence of 18.5% in six different Mediterranean fig-growing countries. Also, Ale-Agha and Rakhshandehroo (2014) detected FCV in the 9 trees out of 197 tested trees with an incidence of 4.5% in Iran. BLAST analysis resulted in more than 98% nucleotide identity and 94% amino acid identity with the reference Italian isolate (Acc. no. FR687854 and YP004429258.1), with the exception on one isolate (Table 2). The FCV isolate from İzmir was 85% identical with the reference Italian isolate (Acc. no. FR687854). Based on the phylogenetic analysis, the reference Italian isolate (FCV|ref|NC015494.1) was clustered together with FCV isolate from Aydın, which is very close to Italy. However, one isolate from Bursa province and one isolate from İzmir province were clearly grouped apart from all FCV isolates with the maximum distance of 0.282 and 0.164 (Table 3).

Table 3. Genetic distance matrix derived from partial sequence of RNA-dependent RNA polymerase (RdRp) gene of Fig cryptic virus (FCV) isolates collected from different fig-growing regions of Turkey.

FCV1F_HTY																					
FCV3F_HTY	0.017																				
FCV4F_IZMIR	0.157	0.164																			
FCV5F_HTY	0.007	0.017	0.157																		
FCV6F_HTY	0.021	0.024	0.164	0.024																	
FCV7F_HTY	0.028	0.028	0.167	0.031	0.024																
FCV8F_HTY	0.014	0.003	0.164	0.017	0.028	0.031															
FCV47F_IZMIR	0.003	0.017	0.153	0.010	0.021	0.028	0.014														
FCV48F_HTY	0.031	0.035	0.178	0.035	0.028	0.038	0.038	0.028													
FCV50F_HTY	0.014	0.024	0.153	0.007	0.031	0.035	0.024	0.017	0.042												
FCV52F_BURSA	0.258	0.272	0.404	0.265	0.275	0.282	0.268	0.254	0.272	0.268											
FCV58F_AYDIN	0.017	0.028	0.164	0.017	0.038	0.038	0.024	0.021	0.049	0.017	0.275										
FCV59F_AYDIN	0.017	0.028	0.157	0.021	0.035	0.035	0.028	0.021	0.045	0.024	0.275	0.014									
FCV|ref|NC015494.1	0.024	0.035	0.167	0.024	0.045	0.045	0.031	0.028	0.052	0.024	0.275	0.007	0.021								
FCCV|ref|NC009519.1	0.481	0.484	0.540	0.484	0.495	0.495	0.481	0.481	0.495	0.484	0.585	0.467	0.477	0.467							
FCV-Algeria|FR776009.1	0.024	0.031	0.167	0.017	0.042	0.045	0.031	0.028	0.049	0.024	0.275	0.014	0.021	0.007	0.474						
FCV-Italy|FR776008.1	0.031	0.038	0.174	0.024	0.049	0.052	0.038	0.035	0.056	0.031	0.282	0.021	0.028	0.014	0.481	0.007					
FCV-Lebanon|FR776007.	0.028	0.038	0.171	0.028	0.049	0.049	0.035	0.031	0.056	0.028	0.279	0.014	0.028	0.007	0.474	0.014	0.017				
FCV-Syria|FR776006.1	0.049	0.056	0.192	0.042	0.066	0.070	0.056	0.052	0.073	0.049	0.293	0.038	0.045	0.031	0.491	0.024	0.024	0.031			
FCV-Tunusia|FR776005.1	0.031	0.042	0.174	0.031	0.052	0.052	0.038	0.035	0.059	0.031	0.282	0.014	0.028	0.007	0.470	0.014	0.014	0.010	0.035		
FCV-Albania|FR776004.1	0.031	0.042	0.171	0.031	0.052	0.052	0.038	0.035	0.059	0.031	0.282	0.014	0.028	0.007	0.467	0.014	0.021	0.014	0.038	0.014	

The depth or complete genome analysis is suggested, although, it is suspected that there is a variation on RdRp fragment (353 bp) of FCV genome of those isolates. The FCV sequences, deposited in GenBank with accession numbers of FR776004.1, FR776005.1, FR776006.1, FR776007.1, FR776008.1, FR776009.1 from Albania, Tunisia, Syria, Lebanon, Italy and Algeria were used for the genetic diversity analysis and they were separately clustered. The selected out-group control *Fragaria chiloensis cryptic virus* (FCCV-Acc. no. NC009519.1) obtained from GenBank was distinctly separated from all isolates, as expected (Figure 2).

According to phylogenetic analysis of 13 Turkish FCV isolates, some correlation between isolates and geographic regions was observed. In addition, genetic distance matrix shows the similarity between the isolates. Comparing with FCV infections, FFkaV infections were detected very low in the same surveyed provinces. FFkaV was detected in 6 trees out of the tested 65 trees collected from four provinces with an incidence of 9.2% (Table 1). One sample from each of Aydın, Bursa and Hatay provinces and 3 samples from İzmir were amplified a DNA fragment with a size about 270 bp using with the PCR, and the specific primer for FFkaV (Figure 1B). While FCV infections were detected high in Hatay, FFkaV infections were found very low compared to the other sampled regions. Similar to our results, the studies on FFkaV infections in Albania, Algeria, Italia, Lebanon, Syria, Tunisia and Iran have shown an incidence ranging from 8.6% to 25% [6-11]. BLAST analysis of obtained sequences resulted in 90% nucleotide identity and more than 96% amino acid identity with the reference Italian isolate (Acc. no. NC015229.1 and YP004300302.1) for all the detected

isolates (Table 2). This identity ratio shows the similarities and differences of the isolates between two different countries.

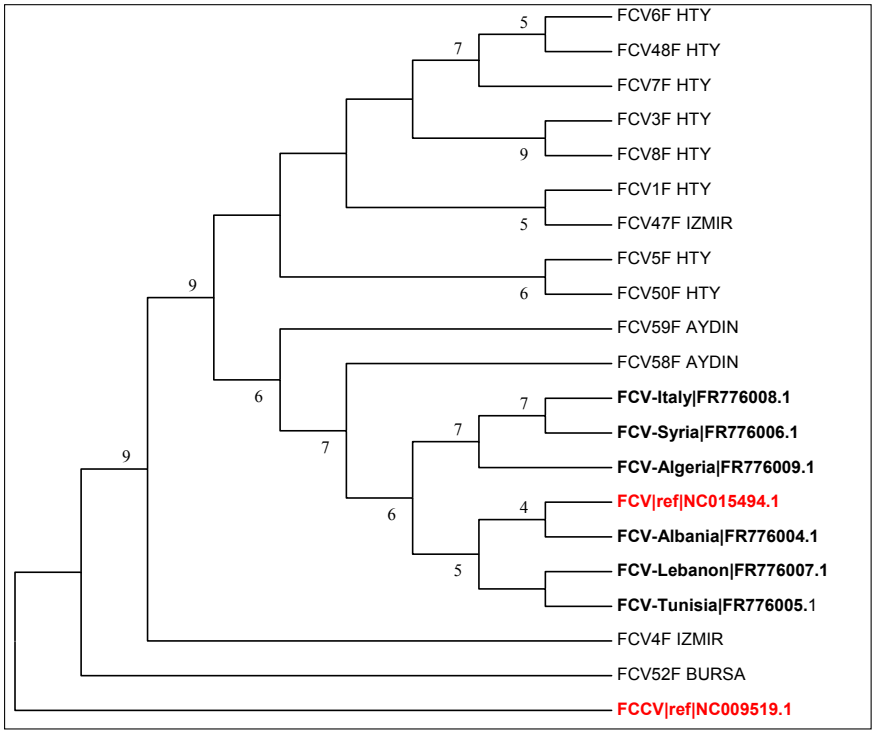

Figure 2. Phylogenetic tree constructed with partial sequences of Fig cryptic virus (FCV) isolated from fig leaves. Numbers at the nodes represent the percentages determined by the bootstrap analysis with 500 replicates and only values higher than 50% are shown. FCV sequences (FR776004.1, FR776005.1, FR776006.1, FR776007.1, FR776008.1, FR776009.1) from Albania, Tunisia, Syria, Lebanon, Italy, Algeria and reference isolate (RefSeq. Acc. no. NC015494.1) deposited in GenBank (NCBI) were used for the analysis and they are highlighted in bold. *Fragaria chiloensis cryptic virus* (FCCV-RefSeq-Acc. no. NC009519.1) was used as out-group control.

Based on the phylogram derived from partial RdRp sequences of FFkaV, the isolates from İzmir, Aydın and Bursa were clustered together, whereas the only isolate from Hatay was separated apart from them. FFkaV sequences from Iran deposited in GenBank (Acc. No. KC331992, KC331993, KC331994, KC331995, KC331996, KC331997, KC331998, KC331999) were included for the genetic diversity analysis and they were distinctly separated from all Turkish FFkaV isolates. Also, the reference FFkaV isolate (Acc. no. NC015229.1) was distinctly separated from all Turkish and Iranian isolates. It shows that there is different evolutionary events and genetic bottle neck constraint involved in the formation of FFkaV population worldwide. *Grapevine fleck virus* (GFkV- Acc. no. AJ309022.1) was selected for out-group control and separated from all FFkaV isolates (Figure 3). Genetic distance matrix analysis also confirms the correlation between isolates (Table 4). Based on the phylogenetic analysis and genetic distance matrix, it can be concluded that there is a correlation between isolates and collection regions (Figure 3). From the phylogram for FCV and FFkaV it is also

indicated that divergent isolates of these viruses are present in Turkey. The distinctly separated out-group controls, both for FCV and FFkaV phylogeny analysis that, confirm the accuracy of results.

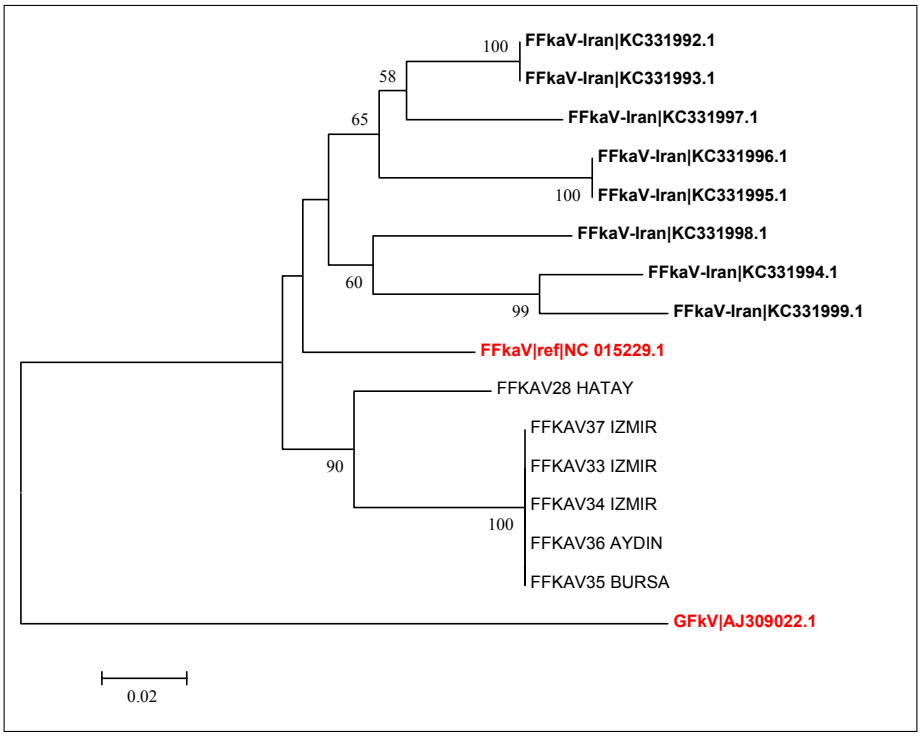

Figure 3. Phylogenetic tree constructed with partial sequences of Fig fleck-associated virus (FFkaV) isolated from fig leaves of Turkey. Numbers at the nodes represent the percentages determined by the bootstrap analysis with 500 replicates and only values higher than 50% are shown. FFkaV sequences from Iran deposited in GenBank (NCBI, Acc. No. KC331992, KC331993, KC331994, KC331995, KC331996, KC331997, KC331998, KC331999) and reference isolate (Acc. no. NC015229.1) which are highlighted in bold were used for the analysis. *Grapevine fleck virus* (GFkV-Acc. no. AJ309022.1) was used as out-group control.

Table 4. Genetic distance matrix derived from partial sequence of RNA-dependent RNA polymerase (RdRp) gene of Fig fleck-associated virus (FFkaV) isolates collected from different fig-growing regions of Turkey.

FFkaV-Iran|KC331997.1															
FFkaV-Iran|KC331996.1	0.100														
FFkaV-Iran|KC331994.1	0.118	0.136													
FFkaV-Iran|KC331999.1	0.127	0.131	0.054												
FFkaV-Iran|KC331998.1	0.109	0.118	0.100	0.127											
FFkaV-Iran|KC331995.1	0.100	0.000	0.136	0.131	0.118										
FFkaV-Iran|KC331992.1	0.063	0.077	0.140	0.136	0.113	0.077									
FFkaV-Iran|KC331993.1	0.063	0.077	0.140	0.136	0.113	0.077	0.000								
FFKAV33_IZMIR	0.127	0.136	0.140	0.154	0.122	0.136	0.104	0.104							
FFKAV34_IZMIR	0.127	0.136	0.140	0.154	0.122	0.136	0.104	0.104	0.000						
FFKAV36_AYDIN	0.127	0.136	0.140	0.154	0.122	0.136	0.104	0.104	0.000	0.000					
FFKAV28_HATAY	0.118	0.118	0.140	0.149	0.122	0.118	0.118	0.118	0.072	0.072	0.072				
FFKAV35_BURSA	0.127	0.136	0.140	0.154	0.122	0.136	0.104	0.104	0.000	0.000	0.000	0.072			
FFKAV37_IZMIR	0.127	0.136	0.140	0.154	0.122	0.136	0.104	0.104	0.000	0.000	0.000	0.072	0.000		
FFkaV|ref|NC_015229.1	0.100	0.104	0.131	0.113	0.109	0.104	0.095	0.095	0.100	0.100	0.100	0.100	0.100	0.100	
GFkV|AJ309022.1	0.267	0.271	0.285	0.281	0.285	0.271	0.276	0.276	0.271	0.271	0.271	0.258	0.271	0.271	0.267

In this study, the results provide evidence that fig trees of Turkey are infected by FFkaV and low genetic variation was observed between partial RdRp fragments of Turkish FFkaV isolates. Phylogenetic analysis revealed that, there is a high correlation between FFkaV isolates and geographical origin. Moreover, phylogeny and genetic diversity of FCV isolates were analyzed and no correlation between the geographic origins of FCV isolates was observed. The presence of possible vectors in the region or the interactions between the several viruses involve in FMD can resulted in this variation. Although, the biological and epidemiological implications of genetic diversity of these two viruses are not yet to be elucidated worldwide, these results demonstrate the situation of them and reveal the importance of viral control strategies for fig plantations in Turkey.

¶References

1. FAO. Food and agricultural organization of the United Nations. Internet Resource: *http://faostat.fao.org/default.aspx.* (verified Aug 10. 2014) (2012).
2. T. ELBEAINO, M. DIGIARO, A. DE STRADIS, G.P. MARTELLI. Partial characterization of a closterovirus associated with a chlorotic mottling of fig. *J Plant Pathol.* 88:187-192 (2006).
3. T. ELBEAINO, E. CHOUERIRI, C. HOBEIKA, M. DIGIARO. Presence of Fig leaf mottle-associated virus and in Lebanese fig orchards. *J Plant Pathol.* 89:409-411 (2007).
4. T. ELBEAINO, M. DIGIARO., A. DE STRADIS, G.P. MARTELLI. Complete nucleotide sequence of four RNA segments of Fig mosaic virus. *Arch Virol.* 154:1719-1727 (2009).
5. T. ELBEAINO, K. HEINOUN, M. DIGIARO, G.P. MARTELLI. Fig mild mottle-associated virus. a novel closterovirus infecting fig. *J Plant Pathol.* 92:165-172 (2010).
6. T. ELBEAINO, R.A. KUBAA, M. DIGIARO, A. MINAFRA, G.P. MARTELLI. The complete nucleotide sequence and genome organization of Fig cryptic virus. a novel bipartite dsRNA virus infecting fig. widely distributed in the mediterranean basin. *Virus Genes.* 42:415-421 (2011a).
7. T. ELBEAINO, M. DIGIARO, G.P. MARTELLI. Complete sequence of Fig fleck-associated virus. a novel member of the family Tymoviridae. *Virus Res.* 161:198-202 (2011b).
8. J.J. WALIA, N.W. SALEM, B.W. FALK. Partial sequence and survey analysis identify a multipartite. negative-sense RNA virus associated with fig mosaic. *Plant Dis.* 93:4-10 (2009).
9. G. GATTONI, A. MINARA, M.A. CASTELANO, A. DE STRADIS, S. BOSCIA, T. ELBEAINO, M. DIGIARO, G.P. MARTELLI. Some properties of Fig latent virus. a new member of the family Flexiviridae. *J Plant Pathol*. 91:555-564 (2009).
10. I.E. TZANETAKIS, A.G. LANEY, K.E. KELLER, R.R. MARTIN. New viruses found in fig exhibiting mosaic symptoms. In: Julius-Kühn-Archiv. 427 (ed) Proc 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. 5-10 July 2009. Neustadt. Germany. 2010. pp 79-82.
11. G.N. ALE-AGHA. and F. RAKHSHANDEHROO. Detection and molecular variability of Fig fleck-associated virus and Fig cryptic virus in Iran. *J Phytopathol.* 162:417-425 (2014).
12. E. ELÇİ, C. ULUBAS SERCE, M. GAZEL, K. CAGLAYAN. Molecular detection and comparative sequence analysis of viruses infecting fig trees in Turkey. *J Plant Pathol.*. 160:418-423 (2012).
13. B.K. CAGLAR, H. FİDAN, M.E. GÜLDÜR, T. ELBEAİNP. The prevalence of three viruses infecting fig in southern Turkey. *J Phytopathol.* 159:181-183 (2010).
14. K. CAGLAYAN, E. ELÇİ, C. ULUBAS SERCE, K. KAYA, M. GAZEL, V. MEDINA. Detection of Fig mosaic virus in viruliferous eriophyid mite Aceria ficus. *J Plant Pathol.*. 94:629-634 (2012).
15. E. ELÇİ, C. ULUBAS SERCE, K. CAGLAYAN. Phylogenetic analysis of partial sequences from Fig mosaic virus isolates in Turkey. *Phytoparasitica.* 41:263-270 (2013).
16. K. TAMURA, D. PETERSON, N. PETERSON, G. STECHER, M. NEI, S. KUMAR. MEGA5: molecular evolutionary genetics analysis using maximum likelihood. evolutionary distance. and maximum parsimony methods. *Mol Bio Evol*. 28:2731–2739 (2011).
17. M.A. LARKIN, G. BLACKSHIELDS, N.P. BROWN, R. CHENNA, P.A. MCGETTIGAN, H. MCWILLIAM, F. VALENTIN, I.M. WALLACE, A. WILM, R. LOPEZ. Clustal W and Clustal X version 2.0. *Bioinformatics.* 23:2947-2948 (2007).
18. M. KIMURA. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111-120 (1980).