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Prevalence and phylogeny of fig viruses in the South Croatian Adriatic Region

Mate ČARIJA[1], Katarina HANČEVIĆ[1,*], Tomislav RADIĆ[1], Emanuel GAŠI[1], Mira RADUNIĆ[1,2]

[1] *Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia*
[2] *Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Svetošimunska 25,10000 Zagreb, Croatia*
*Corresponding author. E-mail: Katarina.Hancevic@krs.hr

Summary. Fig viruses are major challenges for fig production, and may be widely present in Croatia. A survey was carried out to determine the most economically important viruses of fig trees in the South Croatian Adriatic Region, by analyzing 28 fig genotypes from field sites and a national fig collection. Using RT-PCR and specific primers, five viruses were detected, including: fig badnavirus 1 (FBV-1) in all the assessed samples, fig mosaic virus (FMV) (55% of samples), fig leaf mottle-associated virus 1 (FLMaV-1) (44%), fig fleck-associated virus (FFkaV) (17%), and fig mild mottle-associated virus (FMMaV) (10% of samples). Most of the sampled trees were infected by multiple viruses, and only five harbored only FBV-1. Sequencing and phylogenetic analyses of two representative sequences for each of these viruses confirmed their identities and showed close relationships with Mediterranean isolates, indicating their regional dissemination. This study has provided new information of fig virus presence in the South Croatian Adriatic Region, is the first to report prevalence of FLMaV-1, FMMaV and FFkaV in Croatian fig germplasm, and to determine virus phylogenetic relationships. Virus monitoring in fig plantations and in certified propagation material, and integrated disease management strategies, are required to protect fig production in Croatia.

Keywords. *Ficus carica* L., Fig mosaic disease, Mediterranean region, molecular characterization.

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ORCID:
MČ: 0000-0003-4004-7105
KH: 0000-0001-7993-7450
TR: 0000-0002-1633-6516
EG: 0000-0002-2554-3162
MR: 0000-0003-3929-0239

INTRODUCTION

Fig (*Ficus carica* L.) is an important cultivated perennial fruit species, which originated from the Middle East and Southwest Asia (Kislev *et al*., 2006). This plant is renowned for its rich, nutritional fruit, and became widespread in the Mediterranean basin during the Phoenician, Greek and Roman eras (Bakarić *et al*., 1989; Kislev *et al*., 2006; Zohary *et al*., 2012). Fig is also cultivated in the East Adriatic Coast region, where many unique genotypes have been identified (Radunić *et al*., 2025). In Croatia, figs are mostly grown on the margins of family gardens in combination with other fruit species, olives, grapevines, vegetables, and aromatic plants. Commercial fig orchards

are also present but rare (Radunić *et al*., 2025). The latest available data from 2019 showed that Croatia's annual fig production is approx. 800 tons (FAO stat, 2019).

Fig plants are susceptible to several virus pathogens that can significantly impact growth, fruit quality and yields. The most important virus disease impacting fig trees is fig mosaic disease (FMD), which was first described by Condit and Horne (1933). Symptoms of FMD include vigour reduction of fig trees, the leaves with chlorotic and yellowish spots and mosaic patterns developed on the leaves and fruit (Preising *et al*., 2021). Impacts on tree physiological processes have been little investigated, but recent studies indicate that even in asymptomatic plants, photosynthesis is impaired, along with organic acid biosynthesis (Pedrelli *et al*., 2023).

Several viruses have been identified in trees showing FMD (Preising *et al*., 2021). The main cause of the disease is considered to be fig mosaic virus (FMV, *Emaravirus*; Vončina *et al*., 2015), but other viruses may also be involved in disease etiology. Fig leaf mottle-associated virus 1 (FLMaV-1, *Closterovirus*; Elbeaino *et al*., 2006), fig badnavirus 1 (FBV-1, *Badnavirus*; (Laney *et al*., 2012)), fig mild mottle associated virus (FMMaV, *Closterovirus*; Elbeaino *et al*., 2010), and fig fleck-associated virus (FFkaV, *Maculavirus*; Elbeaino *et al*., 2011) have also been associated with FMD. While a survey of two fig viruses (FMV and FBV-1) contributing to FMD development has been conducted in the northern coastal regions of Croatia (Vončina *et al*., 2015), there are no data on the presence, prevalence and diversity of fig viruses in the South Croatian Adriatic Region, where a large number of unique fig genotypes occur (Radunić *et al*., 2025).

Since the most common mode of virus spread occurs through the main modes of fig propagation (cuttings and grafts), infection rates of fig plants are high. Some viruses (e.g. FMV) are also transmitted by eriophyid mites (e.g., *Aceria ficus*; Caglayan *et al*., 2012). Although uncertainties remain regarding the causal agents of FMD (Elbeaino, 2022), FMV was included in the list of Regulated non-quarantine pests to help prevent spread of this virus through vegetative propagation material (European Commission, 2019). Detecting the presence of major fig viruses and understanding their etiology are essential for developing effective strategies for virus detection, certification of propagation material, and integrated disease management in fig cultivation.

The present study assessed the sanitary (virus) status of 28 fig samples from nine locations in the South Croatian Adriatic Region. From selected positive samples, Sanger sequencing and phylogenetic analyses were carried out to assess their relationships with sequences reported in previous studies.

MATERIALS AND METHODS

Thirteen fig samples were collected from different fig orchards in the South Croatian Adriatic Region, and 15 samples were collected from the fig germplasm collection that is currently being developed at the Institute for Adriatic Crops and Karst Reclamation, in Split. The samples were collected from the nine locations shown in Figure 1.

Total nucleic acid extraction and virus detection

Total nucleic acid (TNA) extractions were each carried out from 150 mg of fresh leaf tissue, using the method of Alsaheli *et al.* (2020). For detection of RNA viruses, each leaf extract was purified from any remaining DNA using TURBO DNA-free™ Kit (Invitrogen), according to the manufacturer's instructions. Reverse transcription was performed on 500 ng of RNA template using M-MLV reverse transcriptase (Invitrogen) with additions of 100 units of RNase inhibitor (Invitrogen) and 5 μM random nonamers (Sigma Aldrich). For the detection of DNA virus (FBV-1), total nucleic acid was used directly as the template without DNase treatment and reverse transcription.

Virus detection was carried out with the primers listed in Table 1, using the following PCR conditions: denaturation at 94°C for 2 min, followed by 35 cycles each at 94°C for 30 sec, primer-specific annealing temperature (Table 1) for 45 sec, and elongation at 72°C for 60 sec, and final elongation at 72°C for 7 min. All PCR products were later analyzed by agarose gel electrophoresis.

Phylogenetic analyses

Two representative isolates of each obtained virus were sequenced by Macrogen Europe Inc. (Amsterdam, Netherlands), and were subsequently deposited in the GenBank under the accession numbers PV942097 to PV942106. Sequences obtained were aligned in Clustal X 2.1 (Larkin *et al*., 2007) and analyzed in Mega 5 (Tamura *et al*., 2011). Phylogenetic trees were constructed using the neighbour-joining method and Tamura–Nei evolutionary model. Bootstrap analysis was based on 1,000 repetitions, and other sequences were obtained using NCBI Blast tool.

RESULTS

Sanitary status

The most prevalent virus detected in all the tested samples was FBV-1, and the least prevalent was FMMaV,

Figure 1. Sampling sites for assessing the virus sanitary status of fig trees in the South Croatian Adriatic Region. The inset (top right) shows the position of the study area within the Mediterranean region.

Table 1. Primers used in RT-PCR and PCR for assessing the sanitary (virus) status of fig trees in the South Croatian Adriatic Region.

Virus	Primer sequence	Protein coding region[a]	Annealing temperature	Reference
Fig badnavirus 1	F: GCTGATCACAAGAGGCATGA R: TCCTTGTTTCCACGTTCCTT	MP	55°C	Tzanetakis *et al.* (2010)
Fig mosaic virus	F: CGGTAGCAAATGGAATGAAA R: AACACTGTTTTTGCGATTGG	RdRp	55°C	Elbeaino *et al.* (2009)
Fig leaf mottle-associated virus 1	F: CGTGGCTGATGCAAAGTTTA R: GTTAACGCATGCTTCCATGA	HSP70h	55°C	Elbeaino *et al.* (2006)
Fig fleck associated virus	F: TCAATCCCAAGGAGGTGAAG R: ACACGGTCAATGAGGGAGTC	RdRp	60°C	Elbeaino *et al.* (2011)
Fig mild mottle associated virus	F: AAGGGGAATCTACAAGGGTCG R: TATTACGCGCTTGAGGATTGC	HSP70h	60°C	Elbeaino *et al.* (2010)

[a]MP = movement protein; RdRp = RNA dependent RNA polymerase; HSP70h = heat shock protein 70 homologue.

identified in 10.35 % of the samples. FMV was detected in 55.17% of the samples while FLMaV-1 and FFkaV were detected in 44.83% and 17.24%, respectively (Table 2.). Most of the samples were infected with multiple viruses and only five samples were singly infected with FBV-1. The most common coinfection occurring was FBV-1 and FMV, either alone or together with other viruses.

Phylogenetic analyses

Sequences obtained from Sanger sequencing were aligned with homologous sequences using the NCBI Blast tool where all sequences available in the NCBI were used to construct the phylogenetic trees. The FMV sequences showed greatest similarity to those reported from Serbia and Bosnia and Herzegovina (Figure 2 a.). They clustered separately from other Croatian sequences of FMV which were obtained in previous research (Vončina *et al*. 2015).

FBV was the most uniform virus, as indicated by the number of clusters formed and sequences obtained that alligned closely to most of the sequences from NCBI BLAST, indicating the low genomic diversity of this virus (Figure 2 b). Sequences of FLMaV obtained in this

Table 2. Distribution of fig viruses in different fig tree varieties and sampling locations.

Sample ID	Fig variety	Location	FMV	FLMaV-1	FFkaV	FMMaV	FBV-1
FCO66	Unknown_Brač	Brač	+				+
FC01	Wild fig	IAC[a]	+				+
FC02	Zamorčica	IAC	+	+	+	+	+
FC03	Zimica	IAC	+	+	+	+	+
FC04	Petrovača bijela	IAC	+	+			+
FC016	Zamorčica	IAC	+	+			+
FC025	Mala Sušioka	IAC	+				+
FCO92	Melanzana nera	IAC		+			+
FCO87	Della Signora	IAC	+				+
FCO88	Dottata bianca	IAC		+			+
FCO93	Melanzana bianca	IAC	+	+			+
FCO91	Verdone	IAC	+				+
FCO90	Turca	IAC					+
FCO27	Modrulja	IAC					+
FCO82	Unknown_IAC82	IAC					+
FCO33	Unknown_IAC33	IAC					+
FCO64	Unknown_64	Kaštela	+	+	+		+
FCO94	Zamorčica	Kaštela	+		+		+
FCO95	Unknown_95	Kaštela	+	+			+
FCO57	Unknown_57	Kaštela		+			+
FCO96	Petrovača bijela	Klis					+
FCO67	Unknown_967	Lastovo	+				+
FCO71	Unknown_Lastovo	Lastovo	+				+
FCO86	Unknown_Mljet	Mljet					+
FCO85	Unknown_Plava	Opuzen		+	+	+	+
FCO59	Unknown_59	Solin	+	+			+
FCO77	Unknown_77	Šipan		+			+
FCO78	Unknown_78	Šipan					+

[a]Fig germplasm collection at the Institute for Adriatic Crops and Karst Reclamation.

study alligned closely to those obtained in studies conducted in Spain, Austria, Bosnia and Herzegovina, and Greece (Figure 2 c). FFkaV sequences showed the closest alignement with sequences from Austria, Italy and Palestine (Figure 2 d).

For FMMaV, the smallest number of reference sequences was available from NCBI BLAST (Figure 2 e). The sequences obtained in the present study clustered together in a separate microgroup, positioned close to Tunisian isolates and one Austrian isolate (Figure 2 e).

DISCUSSION

This study has provided an initial survey of fig viruses in the South Croatian Adriatic Region. In addition to confirming the widespread presence of FBV-1 and FMV, detections of FLMaV-1, FFkaV, and FMMaV in Croatian fig germplasm provide the first records of these viruses in this country. These results expand the known geographic distributions of these viruses, and show that the sanitary status of fig in Croatia is more complex than previously recognized.

Fig mosaic disease (FMD) symptoms were first reported in Croatian fig germplasm by Perišić (1952). However, identification of the infecting viruses was delayed until the application of modern diagnostic techniques to fig tree samples from the Northern Croatian coastal region (Istrian Peninsula) (Vončina *et al*., 2015). In the present study, samples from the South Croatian Adriatic Region were analyzed, and this confirmed presence of FMV and FBV-1 in this region. FMV was detected at lower incidence in the South Croatian Adriatic Region compared to the Istrian Peninsula (55.2% vs. 87%), while FBV-1 was found in all the assessed samples, which is consistent with previous results from Istria) (Vončina *et al*., 2015).

FBV-1 is known to integrate into the fig genome, suggesting a long-term coevolutionary relationship of

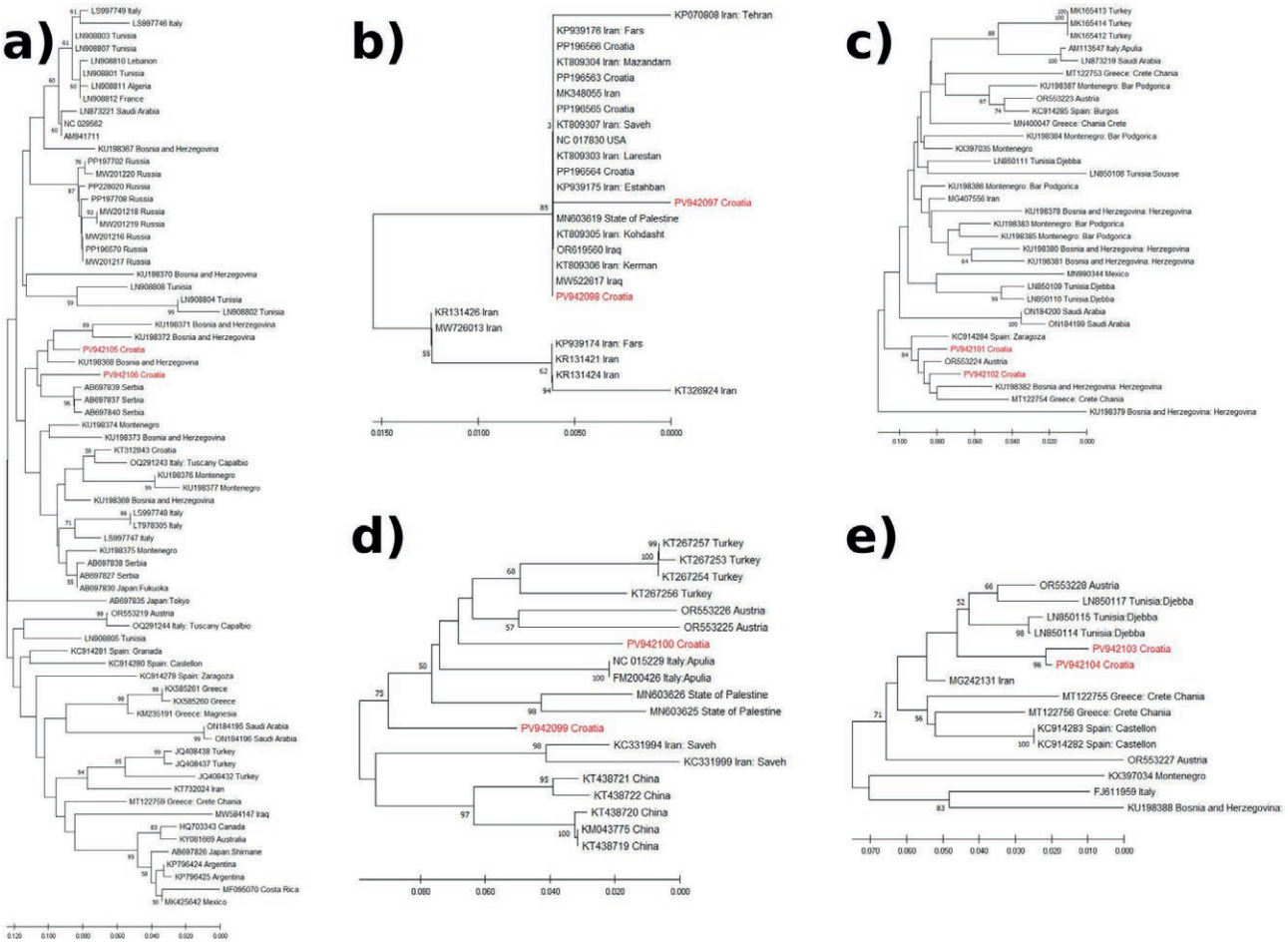

Figure 2. Neighbour-joining trees based on fig mosaic virus (a), fig badnavirus 1 (b), fig leaf mottle-associated virus (c), fig fleck-associated virus (d), and fig mild mottle-associated virus (e). Sequences obtained in the present study (in red font) are presented together with homologous sequences from NCBI BLAST. Bootstrap support values are shown at the branch nodes.

this virus with its host (Tzanetakis *et al*., 2010), and is confirmed to be internationally widespread (Preising *et al*., 2021). Of all viruses known to infect fig trees, FMV is the only one that is clearly associated with FMD development (Elbeaino, 2022) and impacts of this disease on fig tree physiological processes (Pedrelli *et al*., 2023). Spread of FMV may be facilitated by its ability to infect alternative hosts other than fig (Elbeaino *et al*., 2018). FMV has also been reported in neighboring countries, with incidences of 41% in Bosnia and Herzegovina and 26% in Montenegro (Delić *et al*., 2017).

FLMaV-1 was present in 44.8% of the tested samples, which is similar to the results of Delić *et al.* (2017) for Bosnia and Herzegovina and Montenegro. This proportion is different from the more general 22% of figs infected by FLMaV-1 virus proposed by Preising *et al.* (2021), but this indicates the widespread presence of this virus in the Balkan peninsula, probably mediated by the presence of its known vector *Ceroplastes rusci* (Yorganci and Açıkgöz, 2019) which was previously detected in Croatia (Croatian Agency for Agriculture and Food, 2018).

The low detection rates of FMMaV (10.4%) and FFkaV (17.2%) indicate sporadic presence of these viruses, although their pathogenic roles remain poorly understood and merit further study. The detection rate of FMMaV was consistent with similar findings in Syria (12.2%; Elbeaino *et al*., 2012), and slightly greater than reported in neighbouring Bosnia and Herzegovina (8.1%) and Montenegro (5.7%) (Delić *et al*., 2017). The incidence of FFkaV observed in the present study was greater than that reported in neighboring countries (8.2% in Bosnia and Herzegovina and 2.8% in Montenegro; Delić *et al*., 2017), yet it was comparable to the international average of 19% proposed by Preising *et al.* (2021).

Phylogenetic analyses showed that the Croatian virus isolates were genetically close to those reported

in neighboring and Mediterranean countries, indicating potential routes of virus dissemination via trade and historical plant movement. The complexity of dissemination of fig planting material was demonstrated for the Eastern Adriatic Coast by Radunić *et al.* (2025), where no clear patterns of origin at regional level were identified for local varieties, probably due to exchange of plant material which occurred through centuries in the Mediterranean basin. FMV isolates formed a microcluster with sequences of Serbian isolates, and similar clustering patterns were observed for other sequences obtained from Austrian, Palestinean, Greek, and Tunisian fig virus isolates. The FMV isolates obtained in the present study clustered separately from those reported previously (Vončina *et al*., 2015), indicating greater genomic diversity of FMV within Croatian fig germplasm. This highlights the regional nature of fig virus diversity, and emphasizes the need for coordinated phytosanitary regulations and certified propagation material across countries.

The present study has expanded knowledge of fig tree viruses in Croatia, and highlights the importance of comprehensive virus surveillance. Detection of mixed virus infections in the majority of samples underscores the complex nature of FMD, and the challenges it poses for fig cultivation. Overall, this study provides new knowledge of fig virus presence in the South Croatian Adriatic Region, and the first report of incidences of FLMaV-1, FMMaV, and FFkaV in Croatian fig germplasm, and as their phylogenetic relationships. These results provide a foundation for future research in fig, to investigate virus–host interactions, vector dynamics, and integrated disease management strategies. Because only two representative sequences per virus were analyzed, the genomic diversity of Croatian virus isolates from fig should be complemented in future research.

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