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New or Unusual Disease Reports

First report of virus detection in *Ficus carica* in Austria

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Summary. *Ficus carica* is one of the most ancient cultivated crops, and is grown mainly in the Mediterranean region. In Austria, due to milder winters and longer warm periods than normal, figs are becoming more productive and popular among private growers. For future propagation of some fig varieties, the phytosanitary status of eight fig accessions, representing four Austrian genotypes maintained in a varietal collection plot, was investigated using PCR assays for presence of eight fig-infecting viruses. The four fig trees were infected with fig mosaic virus (FMV), fig badnavirus 1 (FBV-1), fig leaf mottle-associated virus 1 (FLMaV-1), fig mild mottle-associated virus (FMMaV) and fig fleck-associated virus (FFkaV); whereas fig leaf mottle-associated virus 2 (FLMaV-2), fig latent virus 1 (FLV-1) and fig cryptic virus 1 (FCV-1) were not detected. The sequences of PCR amplicons obtained from different viruses and samples showed greatest nucleotide variability of 0.5% for FBV-1, 12% for FLMaV-1, 16.3% for FMV, 14% for FMMaV, and 15% for FFkaV, when compared to their homologues in GenBank. A phylogenetic tree for FMV constructed based on partial RNA1 sequences showed that the Austrian isolates were most closely related to previously described Spanish and Greek isolates. The different symptoms observed in the tested trees were mainly in similar to with those reported for FMV, the agent of fig mosaic disease. This is the first report on the presence of fig mosaic-associated viruses in Austria.

Keywords. Fig, mosaic disease, viruses, detection, phylogenetic analyses.

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INTRODUCTION

Fig (*Ficus carica* L.) is an ancient domesticated crop, grown since *ca*. 11000 years BP in the lower Jordan Valley (Kislev *et al*., 2006). Fig orchards are mainly cultivated in the Mediterranean basin, in Algeria, Egypt, Tunisia, Turkey, Iran, and Morocco (FAO, 2019). In southern Europe, fig trees are widespread due to the favourable climate, while in northern and central

Europe a few varieties withstand the low winter temperatures. In Austria, due to changing climatic conditions (milder winters and longer warm periods), figs are becoming popular and more productive among private growers.

Fig plants are normally resistant to many diseases, but they are propagated from cuttings and this facilitates the spread of virus and phytoplasma infections. Mosaic disease (MD) is a major disorder affecting figs in the wild environments, and this disease was first described in California by Condit (1933). Trees affected by MD show leaf symptoms including chlorotic spots, mottling, mosaic patterns, necroses, and deformation (Elbeaino, 2022). To date, 13 viruses associated with MD have been identified, of which only fig mosaic virus (FMV) has been was confirmed as an etiologic agent by fulfilment of Koch postulates (Elbeaino, 2022). Phytoplasma and viroid infections have also been reported from fig hosts (Alsaheli *et al*., 2020; Elbeaino, 2022).

The present study investigated the presence of viruses in eight fig accessions of four cultivars deposited in a fig genotype collection plot, where the plants had symptoms of MD. These plants included two of the cultivar 'Negronne', three of 'Pastilière', two of 'Rivers Brown Turkey', and one plant designated as 'Laimer'. 'Negronne', also known as mulberry fig, is a summer and autumn twice-bearing variety that is native to France, which has shiny and glossy leaves of variable shape on each tree, varying from completely unlobed to deeply incised with five lobes. This give the plants an attractive appearance, and winter hardiness down to -16°C. 'Pastilière' is the best fig variety for fresh consumption, and is also native to France with comparable winter hardiness. 'Rivers Brown Turkey' is a summer and autumn fig native to England, and is a winter hardy variety able to tolerate temperatures as low as -19°C (Seiler, 2022). 'Laimer' is a garden fig of unknown origin.

MATERIALS AND METHODS

Source of plant material and extraction of total nucleic acids

Eight fig accessions representing four genotypes (Table 1) were surveyed in spring and autumn 2022. Leaf samples were collected from symptomatic plants in both seasons (Figure 1). In addition, leaves from two Italian fig cultivars ('Marangiana Bianca' and 'Figazzano Incognita') infected with five viruses (FLMaV-1, FMMaV, FMV, FBV-1 and FFkaV) were provided by the University of Bari (Dr A. Minafra), and these were used as positive controls in the molecular assays. Total nucleic acids (DNA and RNA) were extracted from 100 mg samples of leaf vein tissues excised from symptomatic and asymptomatic leaves, using DNeasy Plant Pro Kit and RNeasy Plant Mini Kit (Qiagen).

RT-PCR and PCR assays

Reverse-Transcription Polymerase Chain Reaction assays (RT-PCR) were each carried out on 100 ng of TNA, using the Qiagen One-Step RT-PCR kit (Qiagen) according to the manufacturers' instructions, and

Table 1. List of fig viruses and their corresponding specific primers used in PCR\RT-PCR assays.

Virus	Primer	Primer sequences (5'–3')	Amplicon (bp)	Reference
FLMaV-1	N17-s N17-a	CGTGGCTGATGCAAAGTTTA GTTAACGCATGCTTCCATGA	350	Elbeaino *et al.* (2006)
FLMaV-2	F3-s F3-a	GAACAGTGCCTATCAGTTTGATTTG TCCCACCTCCTGCGAAGCTAGAGAA	360	Elbeaino *et al.* (2007)
FMMaV	LM3-s LM3-a	AAGGGGAATCTACAAGGGTCG TATTACGCGCTTGAGGATTGC	311	Elbeaino *et al.* (2010)
FLV-1	CPtr-s CPtr-a	CCATCTTCACCACACAAATGTC CAATCTTCTTGGCCTCCATAAG	389	Gattoni *et al.* (2009)
FMV	E5-s E5-a	CGGTAGCAAATGGAATGAAA AACACTGTTTTTGCGATTGG	302	Elbeaino *et al.* (2009)
FFkaV	D8-s D8-a	TCAATCCCAAGGAGGTGAAG ACACGGTCAATGAGGGAGTC	270	Elbeaino *et al.* (2011b)
FCV-1	R1-s R1-a	TCGGATTGTCTTTGGAGAGG CGCATCCACAGTATCCCATT	353	Elbeaino *et al.* (2011a)
FBV-1	1094F 1567R	ACCAGACGGAGGGAAGAAAT TCCTTGCCATCGGTTATCTC	474	Laney *et al.* (2012)

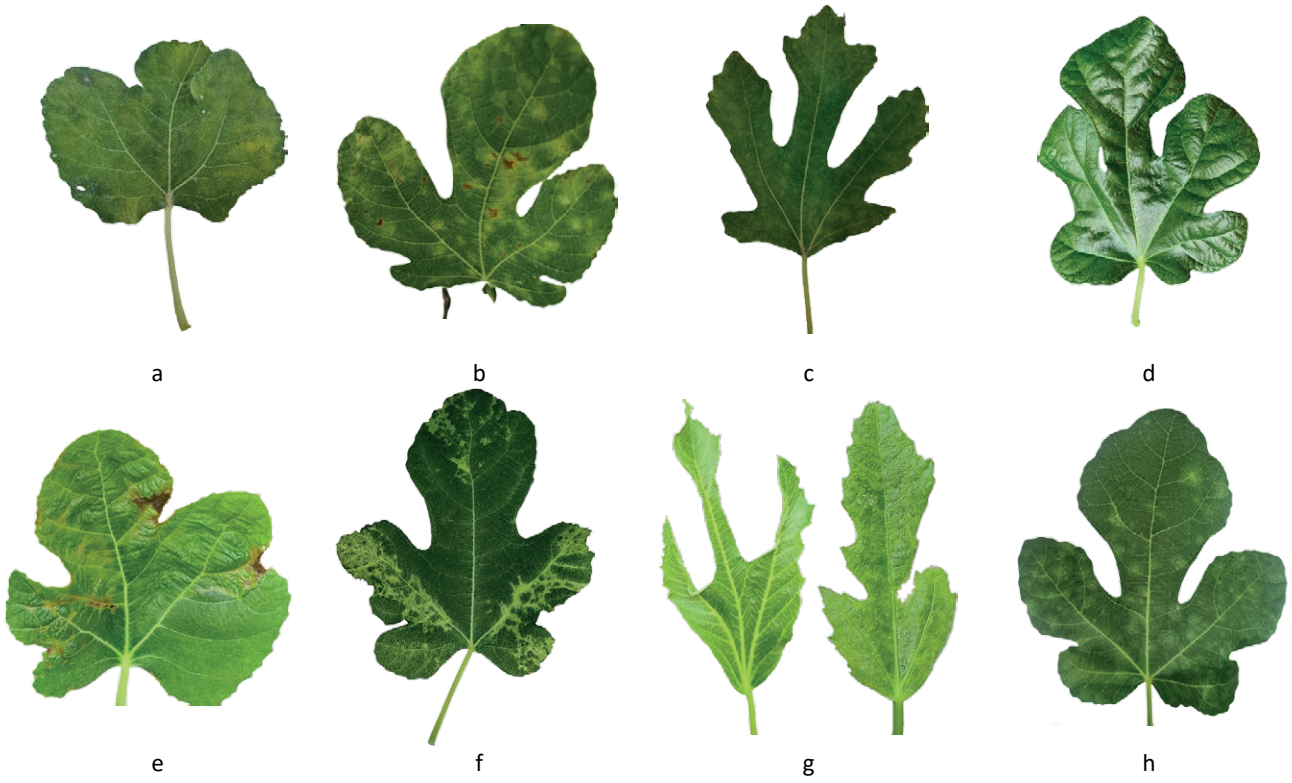

Figure 1. Leaves of Austrian fig plants of the cultivars 'Pastilière' (a and e), 'Rivers Brown Turkey' (b and f), 'Negronne' (c and g), and 'Laimer' (d, h), displaying typical MD leaf symptoms during Autumn 2022 (a to d) and Spring 2023 (e to h). The symptoms consisted of lobe deformations (a and e); vein clearing and mosaic (b and f), and leaf deformations (c and g); and leaf puckering and ringspots (d and h).

the specific primer pairs for each of eight fig-infecting viruses (Table 1). The one-step RT-PCR reaction was performed at 50°C for 30 min, 95°C for 15 min, followed by 40 cycles each at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR products were analyzed by agarose gel electrophoresis. FBV-1 detection was carried out with PCR, using HotStarTaq Master Mix (Qiagen), and the reactions were carried out at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 20 sec, and 72°C for 30 sec, and 10 min extension.

Sequence and phylogenetic analyses

PCR amplicons were sequenced bi-directionally using the sense and antisense primers specific to each virus (Eurofins Genomics and Microsynth AG). Multiple alignments of nucleotide sequences were made using Geneious Prime v.2023.1.1. Searches for homologies with nucleotides were conducted with the Blastn program (Altschul *et al.*, 1990). A phylogenetic tree for Austrian FMV isolates (Figure 2) was constructed using the NJPLOT package in Geneious Prime v.2023.1.1. The Neighbor-Joining algorithm, with p-distance method and bootstrap of 1000 replicates were used. European mountain ash ringspot-associated emaravirus (EMARaV, acc.no: NC 013105) was used as the outgroup species to root the tree.

RESULTS AND DISCUSSION

Detection of fig viruses

Based on the PCR and RT-PCR results, the viruses FLMaV-1, FMMaV, FMV, FBV-1 and FFkaV, were detected in the analyzed fig samples. All four fig genotypes were infected with at least three of these viruses. Of the viruses analyzed, FBV-1 and FLMaV-1 were the most prevalent, followed by FMV (Table 2). FLMaV-2, FLV-1 and FCV-1 were not detected in any of the samples. The analyses carried out in the two seasons were gave the same results, and these are summarized in Table 2.

At sequence levels, PCR amplicons generated from three fig genotypes infected with FBV-1 were

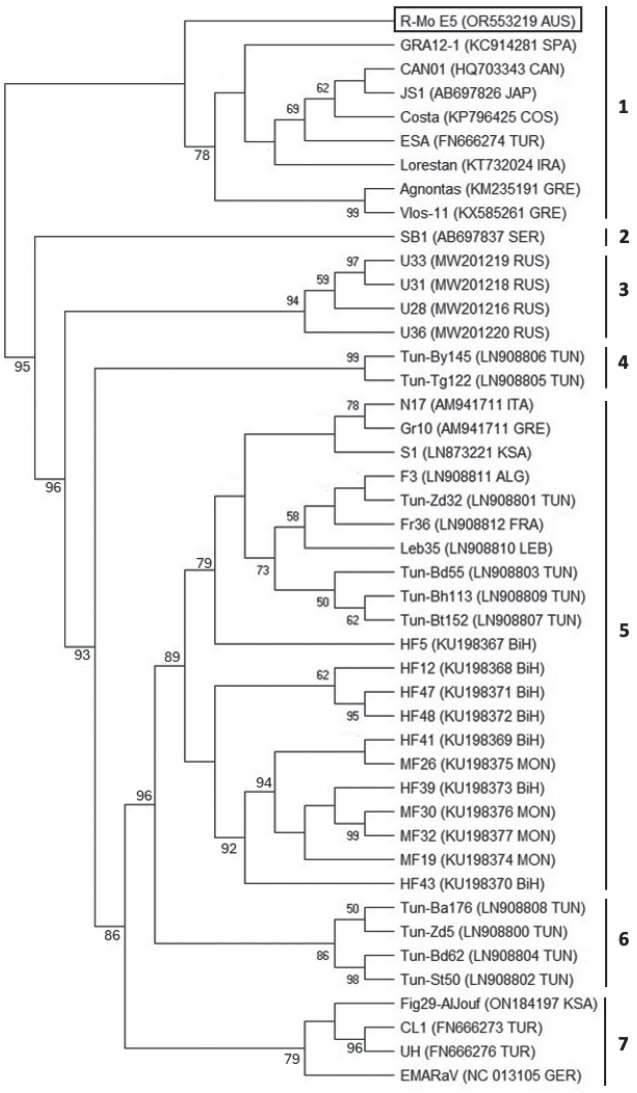

Figure 2. Phylogenetic tree generated from the nucleotide sequence alignment of partial RNA-dependent RNA polymerase genes (RNA1) of FMV isolates from Austria (boxed), and from virus sequences reported in GenBank, using the NJPLOT method implemented in 'Geneious Prime' program. GenBank accession numbers of the sequences used are reported in parentheses. European mountain ash ringspot-associated emaravirus (EMARaV) was used as the outgroup species. Bootstrap values >50% are shown at branch points (1000 replicates). Branch lengths represent bootstrap values. Bar represents 0.01 changes per site. Bootstrap values less than 50% were dropped. The numerals 1 to 7 are number of clades.

sequenced. This yielded three different sequences in the cultivars 'Negronne' (Acc. no. OR553220), 'Pastilière' (Acc. no. OR553221), 'Rivers Brown Turkey' (Acc. no. OR553222) that shared 99.2 to 99.7% identity. In the Blastn analysis they showed 99.7 to 100% identity with isolate Podg2015 (Acc. no. MG584625) from Montenegro, and isolate HF19 (Acc. no. KY473907) from Bosnia and Herzegovina.

The four fig genotypes were infected with FLMaV-1, and sequencing of PCR amplicons of the genotypes 'Pastilière' and 'Rivers Brown Turkey' yielded two sequences (Acc. nos. OR553223 and OR553224) that shared 91.4% nucleotides identity. Blastn analysis showed the greatest identity (respectively, 95.1 and 95.4%) with isolates MF39 (Acc. no. KU198387) and HF43 (Acc. no. KU198382) from Bosnia and Herzegovina.

FMV was detected in the four fig genotypes, and the isolates shared 100% nucleotide identity with each other (Acc. no. OR553219). Blastn analysis showed the greatest nucleotide identity (respectively, 91 and 92%) with isolates Agnontas (Acc. no. KM235191) from Greece and GRA12-1 (Acc. no. KC914281) from Spain.

PCR results showed that FMMaV was present in the fig genotypes 'River Brown Turkey' and 'Laimer'. Pairwise sequence comparisons of PCR amplicons showed 90.6% of identity, whereas with those of from GenBank they showed greatest identities (respectively, 95.7 and 92.5%) with isolate MAZ-1 (Acc. no. MG242131) from Iran, and isolate Cas-12.2 (Acc. no. KC914283) from Spain.

FFkaV was found in two fig genotypes, and Blast analysis of the two sequences (Acc. nos. OR553225 and OR553226) showed 90.5 and 89.7% nucleotide identities with isolate N17 (Acc. no. NC_015229) from Italy; and there was 91.2% identity between them.

Mosaic-disease on figs

All the fig accessions examined in two different seasons over 2 years showed consistent MD leaf symptoms induced by FMV. These mainly consisted of vein clearing and mottling, ringspots, leaf deformation and mosaic (Figure 1). The symptoms observed and the infections found in the three commercial fig accessions tested confirmed the etiology of FMV with MD. Repeated testing did not detect FMV in the fourth assessed fig accession.

Phylogenetic analysis of FMV

Due to the importance of FMV as a fig pathogen, a phylogenetic tree was constructed based on the FMV sequences obtained and those retrieved from the GenBank from different origins. FMV isolates have been designated in seven clades based on their geographical origins, with some exceptions. The Austrian isolate was allocated to clade 1, together with homologues from Spain, Greece and Turkey, whereas clade 2 groups virus isolates from Serbia and clade 3 groups those from Rus-

Table 2. Results of PCR and RT-PCR assays, indicating presence (+) or absence (-) of eight fig viruses in four fig genotypes.

Fig genotype	FMV	FLMaV-1	FLMaV-2	FMMaV	FLV-1	FCV-1	FFkaV	FBV
'Pastilière' (plants 1, 2 and 3)	+	+	-	-	-	-	+	+
'River Brown Turkey' (plants 1 and 2)	+	+	-	+	-	-	-	+
'Negronne' (plants 1 and 2)	+	+	-	-	-	-	-	+
'Laimer' (plant 1)	-	+	-	+	-	-	+	+

sia. Clade 4 includes isolates from the Mediterranean basin (Algeria, France, Greece, Italy, Lebanon, Tunisia), and clade 5 includes isolates from the Balkan area (Montenegro and Bosnia & Herzegovina). Clades 6 and 7, similarly grouped with Mediterranean isolates, with some exceptions.

CONCLUSIONS

The present study is the first to record the presence of five viruses (FLMaV-1, FMMaV, FMV, FBV-1 and FFkaV) in different FMD-associated fig accessions in Austria. FLMaV-2, FLV-1 and FCV-1 were not not be detected in the host plants assessed. The presence of FMV in three of four genotypes is not surprising given the cosmopolitan nature of this virus. The phylogenetic analysis conducted on the set of FMV isolates reported in GenBank showed distinct distribution of FMV isolates according to their geographical origins, with some exceptions. These exceptions are probably due to infections by FMV isolates different from those naturally indigenous to a specific area, thus breaking the rule of geographic origin of FMV. The presence of FBV-1 in all the assessed fig accessions represent a major challenge in attempts to produce virus-free fig planting material. The results obtained in this study could be useful for further monitoring and diagnosis of fig viruses in Austrian plantations. Further investigations of these viruses, in different plots, varieties, and locations, are ongoing in Austria, to support a future certification programme for fig in this country.

AUTHOR CONTRIBUTIONS

ML and EB designed the experiment, carried out the analyses and wrote the paper. TE analyzed the virus sequences and assisted drafting of the manuscript. AK and NK assisted with cultivars descriptions, and reviewed the text. FF assisted with the sampling and the analyses of the experiments.

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