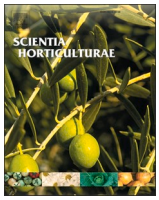
Scientia Horticulturae 322 (2023) 112440

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Occurrence of fig mosaic disease in Tuscany, Central Italy: Characterization of new fig mosaic virus isolates, and elucidation of physiochemical responses of infected common fig cv. Dottato

Athos Pedrelli, Alessandra Panattoni, Cristina Nali *, Lorenzo Cotrozzi

Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, Pisa 56124, Italy

ARTICLE INFO

Keywords:
Fig mosaic virus
Fig fleck associated virus
Leaf pigments
Organic acid
Photosynthesis
Phylogenetic analysis

ABSTRACT

Fig mosaic disease (FMD) is a major disease affecting fig trees, for which only fig mosaic virus (FMV) has been identified as etiological agent. In the present study, trees of common fig cv. Dottato, belonging to the old Tuscan germplasm, were investigated to pioneering (i) evaluate the presence of FMV in Tuscany (Central Italy), as well as other FMD-associated viruses previously reported in Italy, (ii) type and phylogenetically characterize the reported FMV isolates, and (iii) elucidate some physiological and biochemical responses of fig trees challenged by FMV. Although many studies on FMD have been carried out in Southern Italy, the present study represents the first identification of the disease in another Italian area. This work (i) showed that FMD is present also in Central Italy, also suggesting a wider diffusion than what has been reported so far; (ii) confirmed that the disease is caused by FMV, tested positive in both symptomatic and asymptomatic leaves (100 and 27% of tested samples, respectively); and also fig fleck-associated virus (FFKaV) was reported; (iii) identified three new FMV isolates (i.e., Dot-1, Dot-2 and Dot-3, deposited in GenBank), which resulted not close to other FMV Italian isolates; and (iv) pioneering elucidated that FMV impaired photosynthesis and organic acid biosynthesis in symptomatic leaves, but negative effects occurred also in asymptomatic ones in terms of photosynthetic and accessory pigments. More research should be carried out to improve our knowledge on FMD diffusion and FMV (and FFKaV) genetic features, as well on the effects of this regulated non quarantine pest on fig trees, also investigating its fruits representing an ancient source of food and health.

1. Introduction

Ficus (Moraceae family) is one of the largest genera of angiosperms with more than 800 species worldwide, including several edible ones with milky latex and aggregated drupes or achenes as fruits (Ayuso et al., 2022). The fig species of greatest commercial importance is *Ficus carica*, also known as 'common fig', which consists of numerous varieties with significant genetic diversity (Salhi-Hannachi et al., 2006). Native to the Middle East, common fig tree is one of the first plants that was domesticated (around 11,400 years ago) and is today grown worldwide because of its adaptability to different soil and environmental conditions, as well as for the esteemed nutritional and nutraceutical values of its fruits consumed in both dry and fresh form (Barolo et al., 2014). Most of the world's fig production occurs nowadays in the Mediterranean basin (FAOSTAT, 2022). Figs are commercially propagated by grafting or self-rooted cuttings, and these methods favor the dissemination of viral pathogens, including the viruses and viroids associated with fig mosaic disease (FMD; Preising et al. 2021).

Fig mosaic disease is the major disease affecting fig trees and it represents the main threat to global fig production (Toima et al., 2022). It is a virosis firstly reported in 1933 in USA (Condit and Horne, 1933), and nowadays spread worldwide in Africa (Elbeshehy and Elbeaino, 2011), Asia (Chirkov et al., 2021), Europe (Elbeaino et al., 2006), the Middle East (Alkowni et al., 2015), North, Central and South America (Tzanetakis et al., 2010), and Oceania (Elbeaino et al., 2022). The disease is characterized by variable symptomatology which is clearly visible from spring to the end of the growing season (Preising et al., 2021). Fig trees affected by FMD can show vigour reduction, and symptoms include chlorotic and yellowish spots, discoloration, deformation, and mosaic patterns on the leaves and fruits (Preising et al., 2021; Elbeaino, 2022).

Although such symptoms have been observed in fig trees for almost a

* Corresponding author.
E-mail address: cristina.nali@unipi.it (C. Nali).

https://doi.org/10.1016/j.scienta.2023.112440
Received 29 May 2023; Accepted 22 August 2023
Available online 31 August 2023

century, the etiological agents associated with FMD have been investigated only in the last 20 years (Elbeaino et al., 2006, 2007, 2009, 2010). Several viruses and viroids have been identified and found to be associated with FMD in various fig producing areas of the world (Preising et al., 2021; Chirkov et al., 2022). Most of these pathogens are long-distance transmitted via vegetative propagation of infected plant material, whereas only fig mosaic virus (FMV) and fig leaf mottle-associated virus 1 (FLMaV-1) can be transmitted in fields by the eriophyid mite (*Aceria ficus*; Credi et al., 2012) and fig wax scale (*Ceroplastes rusci*; Yorganci and Açıkgöz, 2019), respectively. Transmission by seeds is restricted only to fig latent virus-1 (FLV) and fig cryptic virus (FCV; Preising et al., 2021).

Among these viruses and viroids, FMV is the only one identified to be an etiological agent of FMD, showing its correlation with double-membrane bodies, a consistent feature of diseased figs in the cytoplasm of symptomatic leaf parenchyma cells, regardless of the variety and the country of origin of infected samples (Elbeaino et al., 2009). Fig mosaic virus is a (-)ssRNA virus and a member of the emaravirus genus (Fimoviridae family). The genome is multipartite, containing six RNA segments. Each RNA segment has a single ORF with a 5′ and 3′ end and is individually incapsulated in a separate virus capsid (Walia et al., 2014). Other than being identified as the only virus causing FMD, FMV was found naturally infecting *Cyclamen persicum*, which is the only non-fig host so far reported for FMV, adding more complexity to the management of this important fig virus (Elbeaino et al., 2018). Surveys indicated that FMV is one of the mostly found viruses infecting fig plants in the world, although percentage of FMV infected plants varies drastically among regions. Globally, approximately 2000 samples have been investigated for FMV and more than 30% tested FMV positive (Preising et al., 2021). Despite ambiguity remains in determining the correct FMD causal agent (Elebaino, 2022), European Union indicated FMV as regulated non quarantine pest to be avoided in vegetative materials and seeds (European Union, 2019).

Italy is among the major producers of figs in Southern Europe (11,297 tons year^{-1}; FAOSTAT, 2022), but (similarly to other countries worldwide) its fig production has drastically decreased in recent decades (−65%, compared to 1998; FAOSTAT, 2022), mainly due to the rapid ripening of the fruits. Furthermore, the fig production is still based on old accessions, grown locally, which are the result of the empirical selection made by farmers based on fig tree tolerance to several environmental constraints. These ecological characteristics of the fig tree make it a promising species and at the same time highlight critical issues that require applied research (Usai et al., 2021). In this sense, although many studies on FMD were carried out in Southern Italy (Elbeaino et al., 2006, 2007, 2009; Elbeaino et al., 2010, 2011a, 2011b), no research is available from other Italian regions. Furthermore, to the best of our knowledge, globally, only Zare et al. (2021) investigated the responses of fig trees challenged by FMD in Iran (i.e., the fourth fig producer worldwide; FAOSTAT, 2022).

To address these knowledge gaps, trees of common fig cv. Dottato, belonging to the old Tuscan germplasm, were here investigated, specifically to pioneering (i) evaluate the presence of the etiological agent of FMD in Tuscany, i.e., FMV, as well as other FMD-associated viruses previously reported in Italy, i.e., FCV, fig fleck-associated virus (FFKaV), and FLMaV-1 (Preising et al., 2021), (ii) type and phylogenetically characterize the reported FMV isolates, and (iii) elucidate the physiochemical responses of fig trees challenged by FMV. We anticipate that outcomes here reported will be useful for the challenging contrast to the detrimental FMD.

2. Materials and methods

2.1. Plant material and experimental design

Experimental activities were carried out in 2022, in a three-year-old *Ficus carica* cv. Dottato orchard located in Capalbio (Grosseto), Tuscany, Central Italy (42°27′15″ N, 11°25′21″ E, 165 m a.s.l.). The orchard had an east-west orientation, a weed barrier landscape fabric covering the land under rows, and a drip irrigation system. The climate of the experimental area is Mediterranean [Hot-summer mediterranean climate (Csa), Köppen and Geiger classification; Beck et al., 2018], with annual mean, minimum and maximum temperatures of 15.5, 7.3 (January) and 24.9 °C (August), respectively, and an annual rainfall of around 750 mm. The soil type is sandy loam (18% clay, 17% silt, 65% sand). Average soil pH, organic matter, and phosphorous and potassium contents were 6, 2%, 17 mg kg^{-1} and 790 mg kg^{-1}, respectively.

Fifteen trees were selected for size uniformity, and branch samples were collected in February. In July, leaves were firstly measured in field (from 10.00 am to 1.00 pm, under clear sky conditions), and then sampled. One branch sample per tree was collected, whereas, as visible leaf symptoms occurred in summer, two leaves per tree were measured/collected, one symptomatic and one asymptomatic (all leaves were in the middle part of canopy, fully expanded and sun exposed). All collected samples were kept refrigerated until quickly reaching the Plant Pathology Lab at the Department of Agriculture, Food and Environment of the University of Pisa. Here, branch and leaf samples for molecular diagnosis were immediately handled for total nucleic acid (TNA) extraction, whereas leaf samples for biochemical analyses were stored at −80 °C.

2.2. Extraction of TNA and retrotranscription

TNA was recovered from 500 mg of cambial scrapings of branches or of vein tissues and petioles of leaves, according to the method reported by Pedrelli et al. (2021). Briefly, tissues were crushed in liquid nitrogen and 5 ml cetyltrimethylammonium bromide 2% (w/v) buffer was added. After incubation at 65 °C for 15 min, one volume of chloroform:isoamyl alcohol (24:1, v/v) was added, and TNA precipitated with one volume of isopropanol. Pellet was then washed with 70% ethanol (v/v), air-dried, and dissolved in 80 µl of RNase/DNase free water. cDNA synthesis was finally performed using M-MMLV reverse transcriptase (GeneSpin s.r.l., Milan, Italy), according to the manufacturer instructions, and kept at −20 °C until it was utilized.

2.3. Virus detection

The detection of FMV was carried out by using an end point polymerase chain reaction (End point PCR) protocol which amplifies 302 bp DNA fragment from viral RNA-dependent RNA polymerase (RdRp; Elbeaino et al., 2009). The assay was performed in a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA), and amplified products were observed on 1.2% (w/v) agarose gel electrophoresis. The detection of FCV, FFKaV, and FLMaV-1 was achieved by using quantitative polymerase chain reaction (qPCR) protocol amplifying RdRp, coat protein (CP), and heat shock protein 70 (HSP70) of 108, 112 and 140 bp fragments respectively (Alsaheli et al., 2021). The assays were performed in a Rotor-Gene Q Thermocycler (Qiagen, Venlo, The Netherlands), and the results observed with the instrument software. The End point PCR and qPCR were conducted in 25 and 20 µl reaction volume containing DreamTaq Green (Thermo Fisher Scientific, Waltham, MA, USA) and ITaq (Bio-Rad, Hercules, CA, USA), respectively. Positive, negative, and no-template controls for each experiment were included.

2.4. Virus sequencing and in silico assays

The FMV amplicons were directly sequenced by Sanger DNA method (Eurofins genomics, Ebersberg, Germany), *in silico* analysed using Bioedit (Hall, 1999), and compared in BLASTn (www.ncbi.nlm.ni.gov). RDP4 program (v.4.101) using 3Seq, Bootscan, Chimaera, GENECONV, MaxChi, RDP, and SiScan algorithms (Martin et al., 2015) was used to evaluate the recombinant events. The alignments were performed to observe the presence of non-synonymous (dN) and synonymous (dS)

single nucleotide polymorphisms (SNPs), and to construct the phylogenetic trees by the Maximum Likelihood (ML) method (Jukes-Cantor model) with 1000 bootstrap replicates in MEGA X (Kumar et al., 2018). An *ad hoc* database (Supplementary table S1) was established including FMV isolates retrieved in GenBank and containing RdRp genomic region. The isolates selected were engaged in the phylogenetic analysis and *European mountain ash ringspot-associated virus* isolate (EMARAV; NC_0131105) was used as out-group.

2.5. Leaf gas exchange and chlorophyll content

Leaf net carbon dioxide (CO_2) assimilation (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were determined by using a Li-6800 portable photosynthesis system equipped with a 6800–01A LED light source (Li-Cor, Lincoln, NE, USA), operating at 400 ppm CO_2 concentration, and saturating light conditions (1700 μmol photons m^{-2} s^{-1}).

A SPAD 502 (Konika Minolta, Chiyoda, Tokyo, Japan) was used to determine leaf greenness as an estimate of chlorophyll content (Chl_{SPAD}). Three measurements per leaf were collected (right, left and upper part of leaf), and the mean of these measurements was recorded.

2.6. Leaf water status

Water status parameters were determined on the same leaves used for gas exchange and Chl_{SPAD} at midday according to Stanton and Mickelbart (2014). A leaf portion was used to determine relative water content (RWC), which was calculated as (FW-DW)/(TW-DW) × 100, where FW is the fresh weight, TW is the turgid weight after rehydrating samples for 24 h, and DW is the dry weight after oven-drying leaves at 60 °C until constant weight. To determine leaf osmotic potential (Ψ_π), another leaf portion was placed in a mesh inserted into a microcentrifuge tube, immersed in liquid nitrogen, and then stored at −20 °C until processing. Osmolality was determined with a Wescor 5500 vapor pressure osmometer (Wescor, Logan, UT, USA), and Ψ_π was converted from osmolality using the Van't Hoff equation (Stanton and Mickelbart, 2014).

2.7. Leaf pigment contents

Leaf pigments were determined by ultra-high performance liquid chromatography (UHPLC) by using a Dionex UltiMate 3000 system equipped with an Acclaim 120 C18 column (5 μm particle size, 4.6 mm internal diameter × 150 mm length) maintained into a Dionex TCC-100 column oven at 30 °C, and a Dionex UVD 170 U detector (Thermo Scientific, Waltham, MA, USA; Zhang et al., 2018). Leaf material (50 mg FW) was homogenized in 1 ml of 100% HPLC-grade methanol and incubated overnight at 4 °C in the dark. The supernatants were filtered through 0.2 μm Minisart® SRT 15 aseptic filters. The pigments were eluted using 100% solvent A (acetonitrile/methanol, 75/25, v/v) for the first 14 min to elute xanthophylls (neoxanthin, Neo; violaxanthin, Vio; antheraxanthin, Ant; lutein, Lut; zeaxanthin, Zea; in order of elution), followed by a 1.5 min linear gradient to 100% solvent B (methanol/ethylacetate, 68/32, v/v), which was pumped for 14.5 min to elute chlorophyll *b* (Chl *b*) and chlorophyll *a* (Chl *a*), α-carotene (α-car), and β-carotene (β-car), followed by 2 min linear gradient to 100% solvent A. The flow rate was 1 ml min^{-1}. Chlorophylls, xanthophylls and carotenes were detected by their absorbance at 445 nm, while tocopherols (α, β, γ, δ – isomers) at 295 nm. To quantify the pigment content, known amounts (0.003–0.5 mg ml^{-1}) of pure standards (Sigma-Aldrich, St. Louis, MO, USA) were injected into the UHPLC system, and an equation correlating the peak area to pigment concentration was formulated. Chromatographic data were processed and recorded by Chromeleon Chromatography Management System software, version 7.2.10–2019 (Thermo Scientific). The sum of all compounds from the specific group identified in the study was calculated as follows: total chlorophyll content (Chl_{TOT}) was calculated as Chl *a* + Chl *b*, total carotenoid content (Car_{TOT}) was calculated as Neo + Vio + Ant + Lut + Zea + α-car+β-car, while the xanthophyll cycle pigment content (VAZ) was calculated as Vaz + Ant + Zea. The de-epoxidation state (DEPS) was calculated as (Ant + Zea)/VAZ.

2.8. Sugar and organic acid contents

Soluble sugars (i.e., D-glucose, D-fructose and sucrose) were measured using a K-SUFRG commercial kit (Megazyme, Wicklow, Ireland) following the manufacturer's protocol. After extraction with ethanol 80% (v/v), D-glucose, D-fructose and sucrose were determined with a spectrophotometer (UV-1900 UV–vis, Shimadzu, Kyoto, Japan) at 340 nm. Organic acids (i.e., citric, malic, succinic and quinic acids) were determined according to Eyéghé-Bickong et al. (2012), with minor modifications. After extraction with 100% HPLC-demineralized water, organic acids were measured by the same UHPLC reported above equipped with a pre-column Repromer H (9 μm particle size, 8 mm internal diameter × 20 mm length), and a Repromer H column (9 μm particle size, 8 mm internal diameter × 300 mm length) by using 9 mM sulphuric acid as eluent and a flow rate of 1 ml min^{-1}. Organic acids were detected by their absorbance at 210 nm with the same detector reported above. To quantify their content, known amounts (0.003–0.5 mg ml^{-1}) of pure standards (Sigma-Aldrich, St. Louis, MO, USA) were injected into the UHPLC system, and an equation correlating the peak area to organic acid concentration was formulated.

2.9. Statistical analysis

The Shapiro-Wilk test was used to evaluate the normal distribution of leaf physiological and biochemical parameters. The statistical differences of these parameters among FMV negative (FMV-; i.e., controls), asymptomatic FMV positive (As/FMV+) and symptomatic FMV positive (Sy/FMV+) leaves were investigated by a one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test. Statistically analysis was performed in JMP 13.0 (SAS Institute, Cary, NC, USA), and significant differences were considered for $P \leq 0.05$.

3. Results

3.1. Virus detection

No symptoms were reported in branches collected in winter, whereas leaf deformation, vein clearing, chlorotic blotching and mosaic symptoms occurred in summer on several leaves (ca. 25%, randomly distributed across the canopy) of all the investigated trees.

The molecular diagnosis of branches showed only the presence of FFKaV, which was detected in 11 trees (73%). FFKaV was also reported in leaf samples collected in summer (even in 13 symptomatic and 14 asymptomatic ones), but here also FMV was found in all the investigated trees: all symptomatic samples tested positive to FMV, but it was also reported in four asymptomatic ones (27%). FCV and FLMaV-1 were never detected in both branch and leaf samples.

3.2. Virus sequencing and in silico assays

FMV amplicons sequencing allowed to obtain 19 sequences (RdRp genomic region), from which emerged three new variants, named Dot-1, Dot-2 and Dot-3, and deposited in GenBank (www.ncbi.nlm.ni.gov; Table 1). The Dot-2 variant was found in 13 samples (i.e., 68% of tested positive ones), and displayed 96.72% similarity with isolate FMV-HF41 (KU198369). Dot-1 and Dot-3 were only found in five and one samples, respectively, and displayed 92.15 and 92.56% similarity with isolate Tun-Tg122 (LN908805).

The number of SNPs among the three variants was 71. Maximum changes were found in Dot-1 and Dot-2 (24 nt) with 5 dN each, followed

Table 1
Symptomatology, fig mosaic virus (FMV) diagnosis by polymerase chain reaction assay, sequence variants, accession number, and homology isolates on GenBank database of FMV variants identified in leaves of common fig cv. Dottato in Tuscany. [a] = isolate Tun-Tg122, LN908805; [b] = isolate FMV-HF41, KU198369; Sy = symptomatic; As = asymptomatic; + = Positive; - =Negative.

Plant	Symptomatology	FMV	Sequence variant	Accession number	Homology (%) NCBI
1	Sy	+	Dot-1	OQ291242	92.15[a]
	As	–		—	
2	Sy	+	Dot-1	OQ291242	92.15[a]
	As	–		—	
3	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	
4	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	
5	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	
6	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	—
7	Sy	+	Dot-2	OQ291243	96.72[b]
	As	+	Dot-2	OQ291243	96.72[b]
8	Sy	+	Dot-2	OQ291243	96.72[b]
	As	+	Dot-2	OQ291243	96.72[b]
9	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	—
10	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	—
11	Sy	+	Dot-1	OQ291242	92.15[a]
	As	–		—	—
12	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	—
13	Sy	+	Dot-2	OQ291243	96.72[b]
	As	+	Dot 3	OQ291244	92.56[b]
14	Sy	+	Dot-2	OQ291243	96.72[b]
	As	–		—	—
15	Sy	+	Dot-1	OQ291242	92.15[a]
	As	+	Dot-1	OQ291242	92.15[a]

by Dot-3 (23 nt) with 3 dN. The identity levels of nucleotide and deduced amino acid sequences ranged from 88.08 to 89.97% and from 95.06 to 96.30%, respectively (Table 2). The genetic variability (π) was 0.108±0.018.

The recombinant analysis conducted on the FMV sequences associated with those retrieved from GenBank (Supplementary Table S1) showed no putative recombinant events in the FMV isolates. The phylogenetic analysis showed low bootstrap values. However, three major groups were observed, with the first group further splittable in two subgroups (Fig. 1). All three new variants (i.e., Dot-1, Dot-2, Dot-3) resulted included in subgroup 1, but Dot-1 and Dot-3 were close to isolates Tun-By145 (LN908806) and Tun-Tg122 (LN908805), while Dot-2 was close to isolates FMV-Cro-19S (KT312843) and FMV-HF41 (KU198369).

3.3. Physiochemical responses to FMV

In Sy/FMV+ leaves, A and g_s decreased of more than 35%, compared to FMV- (Fig. 2), while C_i did not change, as well as RWC and Ψ_π (ANOVA: $P>0.05$). Chl_{SPAD} decreased in As/FMV+ leaves, and even more in Sy/FMV+ ones (−8 and −28%, respectively; Fig. 3A). In Sy/FMV+ leaves, reductions of Lut and VAZ contents were also observed (−32 and −26%, respectively), while Vio decreased only in As/FMV+ samples (−20%; Fig. 3B-D). No other significant differences were observed in terms of leaf pigment contents (i.e., Ant, Neo, Zea, Chl a, Chl b, α-car, β-car, α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherols) and derived parameters (i.e., DEPS, Chl_{TOT}, Car_{TOT}; ANOVA: $P>0.05$).

No significant effects were also reported on D-glucose, D-fructose and sucrose contents (ANOVA: $P>0.05$), whereas both quinic and succinic acid contents decreased in Sy/FMV+ leaves (−62 and −53%, respectively; Fig. 4), while no significant effects were reported on citric and malic acids (ANOVA: $P>0.05$).

Table 2
Identity matrix between fig mosaic virus nucleotide (lower left) and deduced amino acid (upper right) sequences recovered in leaves of common fig cv. Dottato in Tuscany.

	Dot-1	Dot-2	Dot-3
Dot-1		96.30	95.06
Dot-2	89.50		96.30
Dot-3	88.08	89.97	

4. Discussion

4.1. FMD is present also in Tuscany (Central Italy), and it was confirmed to be associated with FMV, even if also FFKaV was reported

Although FMD was largely investigated and reported in Southern Italy in the last decades (Elbeaino, 2022), no data were available from other Italian regions. Thus, to the best of our knowledge, the present study represents the first investigation of FMD in Tuscany (Central Italy), a region in which fig cultivation is important. Although no symptoms were reported in branches collected in winter, typical FMD leaf symptoms occurred in summer, in all the investigated trees, according to Delić et al. (2021).

Actually, the molecular diagnosis of branches collected in winter showed only the presence of FFKaV. This virus was previously reported in some Mediterranean areas, including Southern Italy (Elbeaino et al., 2011a; Yahyaoui et al., 2017), but it is important to stress that, based on the available information, it is not possible to establish a cause-effect relationship between FFKaV and the FMD symptomatology (Elbeaino et al., 2011a). FFKaV was also reported in leaf samples collected in summer, but here also FMV (i.e., the FMD causal agent) was found in all the investigated trees, supporting the abovementioned observations of visible symptoms. It is interesting to note that all symptomatic samples tested positive to FMV, but it was also reported in four asymptomatic

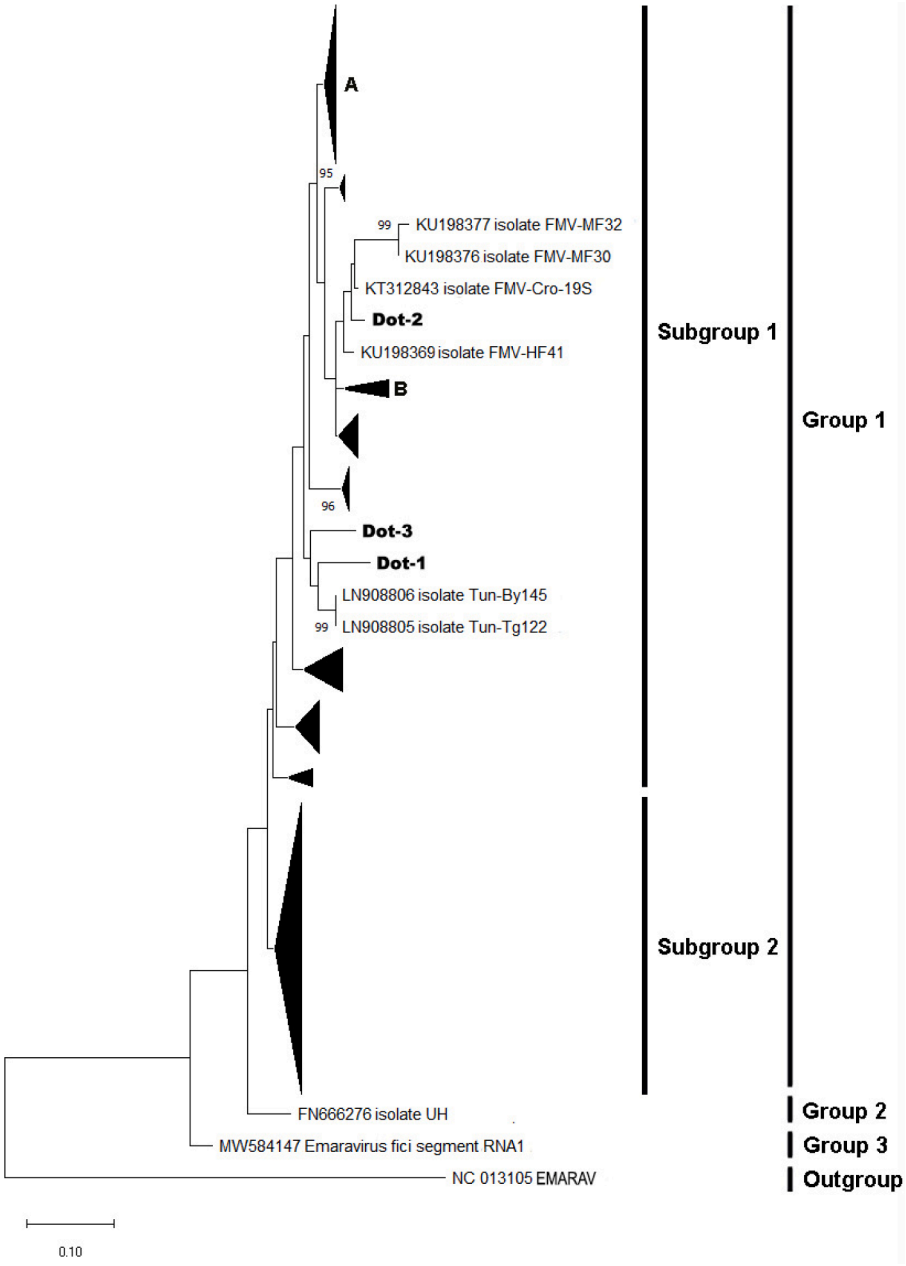


Fig. 1. Phylogenetic tree of fig mosaic virus (FMV) reconstructed from partial RdRp genomic region. The trees were generated by Maximum Likelihood (ML) using the Jukes-Cantor model of evolution for nucleotide. The significance of each branch was evaluated by constructing 1000 trees in bootstrap analysis. Only bootstrap values > 70% are shown. The scale represents a distance of 0.10 substitutions per site. The isolates sequenced in this study are in bold. Condensed portion A and B hold Italian isolates from other studies (LS997746, LS997749, NC 029,562, and LT978305, LS997747, respectively). European mountain ash ringspot-associated virus isolate (EMARAV; NC_0131105) was used as the outgroup.

ones. This difference in FMV infection rate between tissues with contrasting symptomatology was previously reported by Ishikawa et al. (2012). Differently, FFKaV tested positive in more than 93% of leaf samples, regardless their symptomatology, according to Ale-Agha and Rakhshandehroo (2013), which found FFKaV infected leaves in Iran (but only in asymptomatic ones), as well as to Yahyaoui et al. (2017), which reported the lower presence of FMV than FFKaV (as well as their mixed infection) in autochthonous fig trees in Sicily (Southern Italy). Finally, the absence of FCV and FLMaV-1 in both branch and leaf samples results in contrast with previous Italian investigations which reported these viruses in 45 and 14% of tested samples, respectively (Elbeaino et al., 2007; 2011b).

Overall, these outcomes seem to confirm that FMV is the aetiological agent of FMD, being it previously associated to the observed symptomatology, also linked with the characteristic microscopic one in leaves (Elbeaino et al., 2009). Furthermore, since this study is the first investigation of FMD at higher latitudes than Southern Italy, the immediate report of the disease suggests its much wider diffusion than what has been reported so far. This evidence also imposes more attention for contrasting the diffusion of virus infected vegetative material (which likely was the force of a so long distance spread of FMV and FFKaV), as well as for larger monitoring of this detrimental fig disease, keeping in mind that also asymptomatic leaves can be FMV infected.

4.2. Three new FMV isolates were identified, phylogenetically not close to other FMV Italian isolates

Among the obtained 19 sequences, emerged the three new variants Dot-1, Dot-2 and Dot-3. The most occurring variant was Dot-2, which displayed high similarity with isolate FMV-HF41 (KU198369) recovered in Bosnia and Herzegovina on a fig tree. Dot-1 and Dot-3 showed high similarity with isolate Tun-Tg122 (LN908805) recovered on a fig tree in Tunisia.

The identity levels of nucleotide and deduced amino acid sequences was in accordance with Alfaro-Fernandez et al. (2013), while the genetic variability was in accordance with those emerging from the database

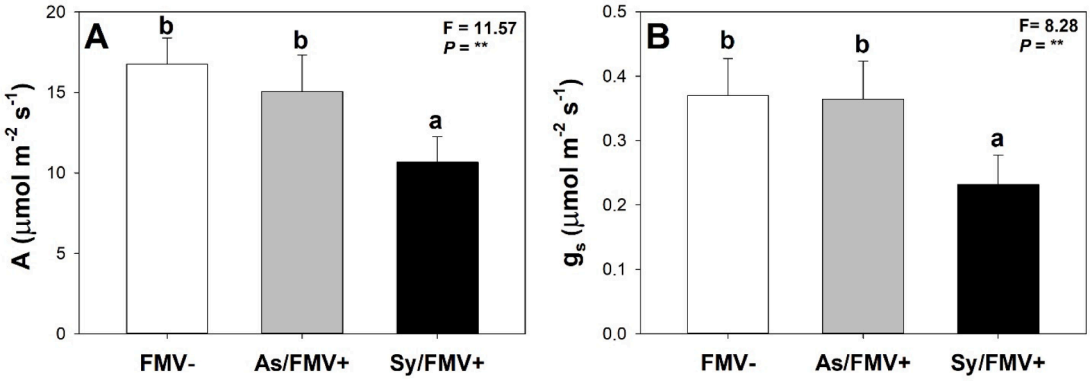


Fig. 2. **(A)** Net carbon dioxide assimilation rate and **(B)** stomatal conductance (g_s) in fig mosaic virus (FMV) negative (FMV-, *white*), asymptomatic FMV positive (As/FMV+, *gray*) and symptomatic FMV positive (Sy/FMV+, *black*) leaves of common fig cv. Dottato. Data are shown as mean ± standard deviation. F-values and *P*-levels from a one-way ANOVA are shown in the top-right corner of panels. According to Tukey's *post-hoc* test, different letters indicate significant differences among means ($P \leq 0.05$).

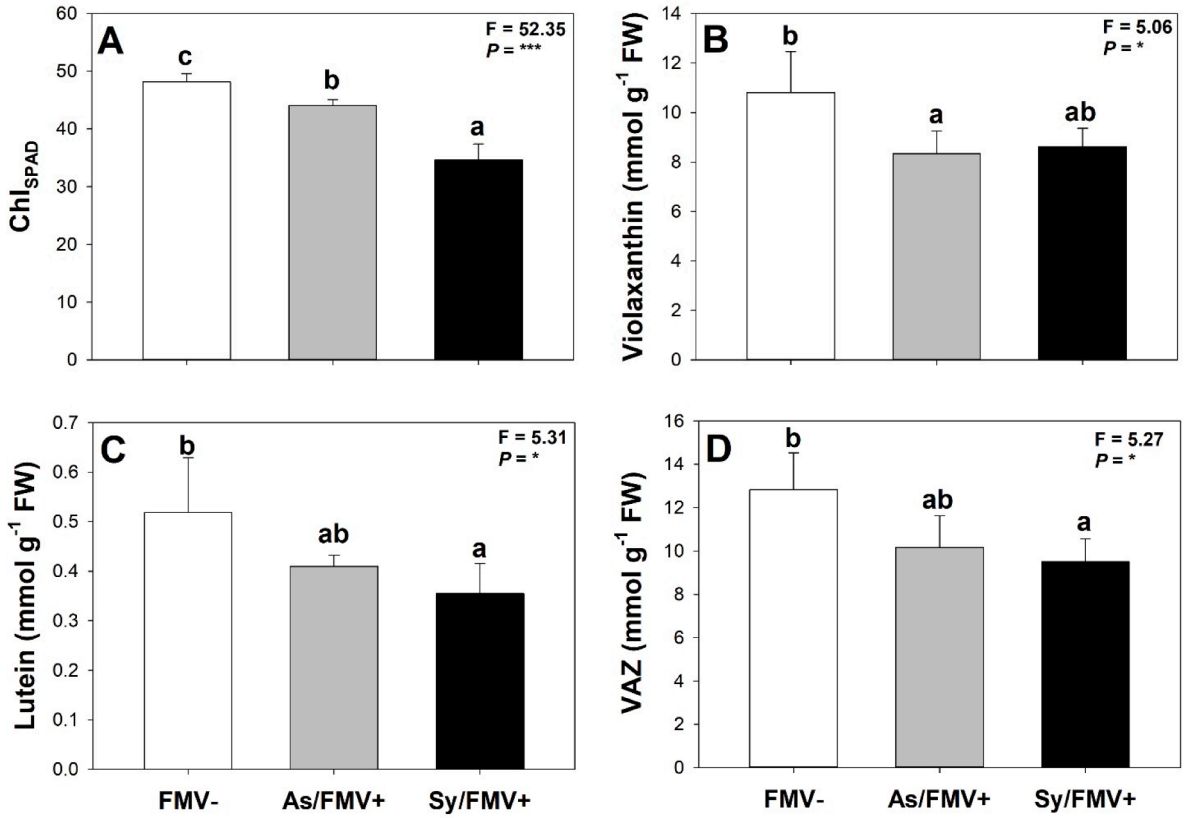


Fig. 3. **(A)** Chlorophyll (estimated as SPAD index; Chl_{SPAD}), **(B)** violaxanthin, **(C)** lutein and **(D)** violaxanthin + antheraxanthin + zeaxhantin (VAZ) contents in fig mosaic virus (FMV) negative (FMV-, *white*), asymptomatic FMV positive (As/FMV+, *gray*) and symptomatic FMV positive (Sy/FMV+, *black*) leaves of common fig cv. Dottato. Data are shown as mean ± standard deviation. F-values and *P*-levels from a one-way ANOVA are shown in the top-right corner of panels. According to Tukey's *post-hoc* test, different letters indicate significant differences among means ($P \leq 0.05$). FW: fresh weight.

here *ad hoc* created (Supplementary Table S1) taking into account both Mediterranean (0.125±0.016; Algeria, Bosnia and Herzegovina, Croatia, France, Greece, Italy, Lebanon, Montenegro, Palestine, Tunisia, and Turkey), and worldwide isolates (0.117±0.014; Argentina, Canada, Costa Rica, Iran, Iraq, Japan, Mexico, and Russia). These values are also in accordance with those reported by Walia et al. (2014).

Although the three new variants (i.e., Dot-1, Dot-2, Dot-3) resulted included in subgroup 1 by the phylogenetic analysis, Dot-1 and Dot-3 were close to isolates Tun-By145 (LN908806) and Tun-Tg122 (LN908805) recovered on fig trees in Tunisia, while Dot-2 was close to isolates FMV-Cro-19S (KT312843) and FMV-HF41 (KU198369) found on fig trees in Croatia and in Bosnia and Herzegovina, respectively. Interestingly, the three new variants resulted very close to their closest counterparts at GenBank, whereas less close to other FMV Italian isolates, although they were also included in subgroup 1.

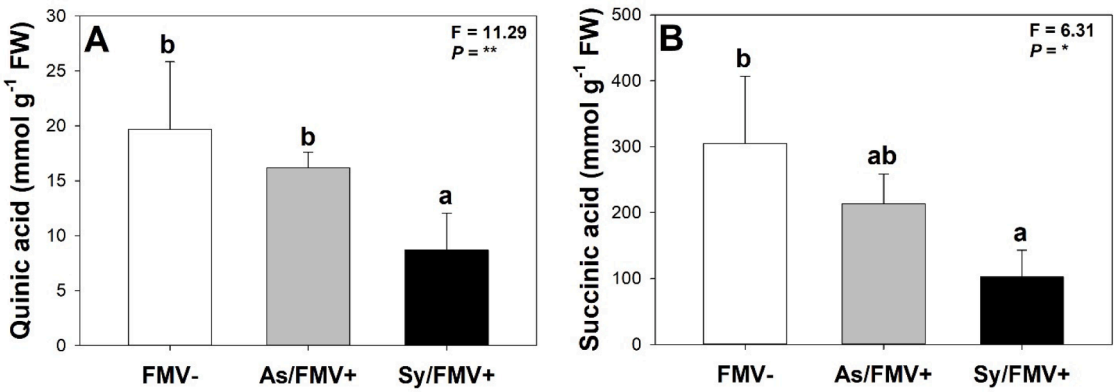


Fig. 4. **(A)** Quinic and **(B)** succinic acid contents in fig mosaic virus (FMV) negative (FMV-, *white*), asymptomatic FMV positive (As/FMV+, *gray*) and symptomatic FMV positive (Sy/FMV+, *black*) leaves of common fig cv. Dottato. Data are shown as mean ± standard deviation. F-values and *P*-levels from a one-way ANOVA are shown in the top-right corner of panels. According to Tukey's *post-hoc* test, different letters indicate significant differences among means ($P \leq 0.05$).

4.3. FMV impaired photosynthesis and organic acid biosynthesis in symptomatic leaves, but negative effects occurred also in asymptomatic ones in terms of photosynthetic and accessory pigments

Compared to the large number of diagnostic and molecular characterization studies on phytoviroses, less investigations have been carried out on the effects of viral pathogens on plant physiology and function. To the best of our knowledge, only Zare et al. (2021) investigated such fig tree/FMD interactions, in Iran. The present study pioneering evidenced the capability of FMV of decreasing fig CO_2 assimilation rate due to both stomatal limitations and mesophyll impairments, while no negative effects were observed at water status level. Mesophyll impairments seem in accordance with previous studies reporting the FMV-associated microscopic alterations in terms of occurrence of double-membrane bodies (Appiano et al., 1995; Çağlayan et al., 2009); whereas unchanged water content was previously reported in symptomatic and asymptomatic kale leaves infected by turnip mosaic virus (TuMV; Sevik and Canzis, 2021). However, these photosynthetic impairments occurred only in positive and symptomatic leaves (A and g_s, while C_i did not accumulate), suggesting that these negative effects did not occur in the first phases of leaf FMV infection, accordingly to the lack of visible symptoms.

Actually, an FMV-induced reduction of chlorophyll content was observed also in infected asymptomatic leaves, even if at a lower rate than in symptomatic ones, partially according with Zare et al. (2021). This outcome suggests that also asymptomatic leaves started to be impaired by FMV, which likely still had to reach the detrimental condition observed in symptomatic ones. The degradation of photosynthetic (i.e., chlorophylls) and accessory pigments (i.e., carotenoids) was already reported in plants infected by viruses (e.g., Espinoza et al. 2021), as well as to other environmental stressors (e.g., Cotrozzi et al., 2018; Pellegrini et al., 2018), signifying that the chloroplast ultrastructure and pigments were impaired. Indeed, also Vio content resulted reduced in infected asymptomatic samples, while no significant effects were reported in symptomatic ones, curiously. This divergence might be due to a specific xanthophyll cycle regulation for signaling and/or defense against oxidative stress in the first phase of FMV infection (Latowski et al., 2011). Overall, these results confirm the importance of monitoring also asymptomatic samples. No other significant differences were observed in terms of leaf pigment contents, except for lutein and VAZ that decreased only in infected symptomatic leaves, indicating that FMV impaired the capability of fig leaves to cope with oxidative stress by carotenoid accumulation and xanthophyll cycle regulation (Latowski et al., 2011; Jahns and Holzwarth, 2012). Actually, virus-induced loss of plant capability to activate such strategies against oxidative stress was previously reported (e.g., Plum pox virus in peach; Hernandez et al., 2004).

Abovementioned photosynthetic impairments did not result in a decrease of sugars. Although a high variability was reported among samples (likely due to the fact that the experimental activities were run under field conditions), the lack of significant effects on glucose, fructose and sucrose contents, was not in accordance with Zare et al. (2021), which instead showed sugar accumulation in infected symptomatic leaves. Conversely, the reduction of both quinic and succinic acid contents observed only in symptomatic leaves confirmed that most detrimental effects occurred in the later phases of FMV infection, accordingly to photosynthetic alterations reported above. Actually, contrasting results were previously reported in terms of virus effects on both sugar and organic acid contents in leaves (e.g., Llave, 2016; López-Gresa et al., 2012; Kogovšek et al., 2016), thus further investigations are encouraged, also in fruit tissue.

5. Conclusions

Although many studies on FMD were carried out in Southern Italy, the present study represents the first identification of the disease in another Italian area. Investigating trees of common fig cv. Dottato, belonging to the old Tuscan germplasm, this work (i) showed that FMD is present also in Central Italy, also suggesting a much wider diffusion than what has been reported so far; (ii) confirmed that the disease is caused by FMV, tested positive in both symptomatic and asymptomatic leaves (also FFKaV was reported); (iii) identified three new FMV isolates (i.e., Dot-1, Dot-2 and Dot-3), which resulted not close to other FMV Italian isolates; and (iv) pioneering elucidated that FMV impaired photosynthesis and organic acid biosynthesis in symptomatic leaves, but negative effects occurred also in asymptomatic ones in terms of photosynthetic and accessory pigments. More research should be carried out to improve our knowledge on FMD diffusion and FMV (and FFKaV) genetic features, as well on the effects of this regulated non quarantine pest on fig trees, also investigating its fruits representing an ancient source of food and health.

Data availability

All the experimental data are available and accessible via the main text and/or the supplementary information.

CRediT authorship contribution statement

Athos Pedrelli: Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Alessandra Panattoni:** Conceptualization, Methodology, Investigation, Data curation, Writing –

review & editing. **Cristina Nali:** Resources, Writing – review & editing, Supervision, Project administration. **Lorenzo Cotrozzi:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was carried out within the AMARCORT project funded by PSR 2014–2020 of Tuscany Region. Authors are grateful to Luciano Pagano for assistance in data collection and analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112440.

References

Ale-Agha, G.N., Rakhshandehroo, F., 2013. Detection and molecular variability of fig fleck-associated virus and fig cryptic virus in Iran. J. Phytopathol. 162 (7–8), 417–425. https://doi.org/10.1111/jph.12204.

Alfaro-Fernández, A., Hernández-Llopis, D., Font, M.I., 2013. Fig viruses in Mainland Spain. J. Phytopathol. 162 (5), 332–337. https://doi.org/10.1111/jph.12187.

Alkowni, R., Chiumenti, M., Minafra, A., Martelli, G.P., 2015. A survey for fig-infecting viruses in Palestine. J. Plant Pathol. 97, 383–386. https://doi.org/10.4454/JPP.V97I2.034.

Alsaheli, Z., Abdallah, A., Incerti, O., Shalaby, A., Youssef, S., Digiaro, M., Elbeaino, T., 2021. Development of singleplex and multiplex real-time (Taqman®) RT-PCR assays for the detection of viruses associated with fig mosaic disease. J. Virol. Methods 293, 114145. https://doi.org/10.1016/j.jviromet.2021.114145.

Appiano, A., Conti, M., Zini, N., 1995. Cytopathological study of the double-membrane bodies occurring in fig plants affected by fig mosaic disease. Acta Hortic. 386, 585–592. https://doi.org/10.17660/actahortic.1995.386.86.

Ayuso, M., Carpena, M., Taofiq, O., Albuquerque, T.G., Simal-Gandara, J., Oliveira, M.B. P.P., Prieto, M.A., Ferreira, I.C.F.R., Barros, L., 2022. Fig "*Ficus carica* L." and its by-products: a decade evidence of their health-promoting benefits towards the development of novel food formulations. Trends Food Sci. Technol. 127, 1–13. https://doi.org/10.1016/j.tifs.2022.06.010.

Barolo, M.I., Ruiz Mostacero, N., López, S.N., 2014. *Ficus carica* L. (Moraceae): an ancient source of food and health. Food Chem. 164, 119–127. https://doi.org/10.1016/j.foodchem.2014.04.112.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1km resolution. Sci. Data 5 (5), 180214. https://doi.org/10.1038/sdata.2018.214.

Çağlayan, K., Medina, V., Gazel, M., Serçe, Ç.U., Serrano, L., Achon, A., Soylu, S., Çalişkan, O., Gümüş, M., 2009. Putative agents of fig mosaic disease in Turkey. Turk. J. Agric. For. 33 (5), 469–476. https://doi.org/10.3906/tar-0807-20.

Chirkov, S., Sheveleva, A., Tsygankova, S., Sharko, F., Mitrofanova, I., 2022. Characterization of divergent Grapevine badnavirus 1 isolates found on different fig species (*Ficus* spp.). Plants 11 (19), 2532. https://doi.org/10.3390/plants11192532.

Chirkov, S., Tsygankova, S., Rastorguev, S., Mitrofanova, I., Chelombit, S., Boulygina, E., Slobodova, N., Sharko, F., 2021. First report of fig mosaic virus on fig in Russia. Plant Dis. 105 (8) https://doi.org/10.1094/pdis-11-20-2463-pdn.

Condit, J., Horne, W.T., 1933. A mosaic of the fig in California. Phytopathology 823, 887–896.

Cotrozzi, L., Remorini, D., Pellegrini, E., Guidi, L., Nali, C., Lorenzini, G., Massai, R., Landi, M., 2018. Living in a Mediterranean city in 2050: broadleaf or evergreen "citizens"? Environ. Sci. Pollut. Res. 25 (9), 8161–8173. https://doi.org/10.1007/s11356-017-9316-7.

Credi, R., Terlizzi, F., Beber, R., Poggi Pollini, C., Ratti, C., 2012. Transmission trials of the fig mosaic disease agent. Petria 22 (3), 123–458.

Delić, D., Elbeaino, T., Lolić, B., Đurić, G., 2021. Fig viruses in Bosnia and Herzegovina. Acta Hortic. 1308, 319–324. https://doi.org/10.17660/actahortic.2021.1308.45.

Elbeaino, T., 2022. Fig Pathogens: viruses, viroids, and phytoplasmas. In: Advances in Fig Research and Sustainable Production, pp. 279–292. https://doi.org/10.1079/9781789242492.0016.

Elbeaino, T., Digiaro, M., Martelli, G.P., 2011a. Complete sequence of fig fleck-associated virus, a novel member of the family Tymoviridae. Virus Res. 161 (2), 198–202. https://doi.org/10.1016/j.virusres.2011.07.022.

Elbeaino, T., Digiaro, M., Alabdullah, A., De Stradis, A., Minafra, A., Mielke, N., Castellano, M.A., Martelli, G.P., 2009. A multipartite single-stranded negative-sense RNA virus is the putative agent of fig mosaic disease. J. Gen. Virol. 90 (5), 1281–1288. https://doi.org/10.1099/vir.0.008649-0.

Elbeaino, T., Digiaro, M., De Stradis, A., Martelli, G.P., 2006. Partial characterisation of a closterovirus associated with a chlorotic mottling of fig. J. Plant Pathol. 88 (2), 187–192. https://www.jstor.org/stable/41998310.

Elbeaino, T., Digiaro, M., De Stradis, A., Martelli, G.P., 2007. Identification of a second member of the family closteroviridae in mosaic-diseased figs. J. Plant Pathol. 89 (1), 119–124. https://www.jstor.org/stable/41998365.

Elbeaino, T., Digiaro, M., Heinoun, K., Stradis, A.D., Martelli, G.P., 2010. Fig mild mottle-associated virus, a novel closterovirus infecting fig. J. Plant Pathol. 92 (1), 165–172. https://doi.org/10.4454/jpp.v92i1.26.

Elbeaino, T., Kubaa, R.A., Digiaro, M., Minafra, A., Martelli, G.P., 2011b. 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 (3), 415–421. https://doi.org/10.1007/s11262-011-0581-0.

Elbeaino, T., Marais, A., Faure, C., Trioano, E., Candresse, T., Parrella, G., 2018. High-throughput sequencing reveals Cyclamen persicum Mill. as a natural host for fig mosaic virus. Viruses 10 (12), 684. https://doi.org/10.3390/v10120684.

Elbeaino, T., Nahdi, S., Digiaro, M., Alabdullah, A., Martelli, G.P., 2009b. Detection of FLMAV-1 and FLMAV-2 in the Mediterranean region and study on sequence variation of the hsp70 gene. J. Plant Pathol. 91 (2), 425–431. https://doi.org/10.4454/jpp.v91i2.973.

Elbeshehy, E.K.F., Elbeaino, T., 2011. Viruses infecting figs in egypt. Phytopathol. Mediterr. 50 (2), 327–332. https://www.jstor.org/stable/26458706.

Espinoza, C., Bascou, B., Calvayrac, C., Bertrand, C., 2021. Deciphering *Prunus* responses to PPV infection: a way toward the use of metabolomics approach for the diagnostic of sharka disease. Metabolites 11 (7), 465. https://doi.org/10.3390/metabo11070465.

European Union, 2019. Regulations-commission implementing regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing commission regulation (EC) No 690/2008 and amending commission implementing regulation (EU) 2018/2019. Off. J. Eur. Union 1–279. L319/1 1.

Eyéghé-Bickong, H.A., Alexandersson, E.O., Gouws, L.M., Young, P.R., Vivier, M.A., 2012. Optimisation of an HPLC method for the simultaneous quantification of the major sugars and organic acids in grapevine berries. J. Chromatogr. B 885-886, 43–49. https://doi.org/10.1016/j.jchromb.2011.12.011.

FAOSTAT, Statistical Database (2022). Food and Agriculture Organization of the United Nations, Rome. https://www.fao.org/faostat/en/.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hernandez, J.A., Rubio, M., Olmos, E., Ros-Barcelo, A., Martinez-Gomez, P., 2004. Oxidative stress induced by long-term Plum pox virus infection in peach (*Prunus persica*). Physiol. Plant. 122 (4), 486–495. https://doi.org/10.1111/j.1399-3054.2004.00431.x.

Ishikawa, K., Maejima, K., Nagashima, S., Sawamura, N., Takinami, Y., Komatsu, K., Hashimoto, M., Yamaji, Y., Yamamoto, J., Namba, S., 2012. First report of fig mosaic virus infecting common fig (*Ficus carica*) in Japan. J. Gen. Plant Pathol. 78 (2), 136–139. https://doi.org/10.1007/s10327-012-0359-9.

Jahns, P., Holzwarth, A.R., 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophys. Acta BBA Bioenerg. 1817 (1), 182–193. https://doi.org/10.1016/j.bbabio.2011.04.012.

Kogovšek, P., Pompe-Novak, M., Petek, M., Fragner, L., Weckwerth, W., Gruden, K., 2016. Primary metabolism, phenylpropanoids and antioxidant pathways are regulated in potato as a response to Potato virus Y infection. PLoS One 11 (1), e0146135. https://doi.org/10.1371/journal.pone.0146135.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.

Latowski, D., Kuczyńska, P., Strzałka, K., 2011. Xanthophyll cycle – a mechanism protecting plants against oxidative stress. Redox Report 16 (2), 78–90. https://doi.org/10.1179/174329211x13020951739938.

Llave, C., 2016. Dynamic cross-talk between host primary metabolism and viruses during infections in plants. Curr. Opin. Virol. 19, 50–55. https://doi.org/10.1016/j.coviro.2016.06.013.

López-Gresa, M.P., Lisón, P., Kim, H.K., Choi, Y.H., Verpoorte, R., Rodrigo, I., Conejero, V., Bellés, J.M., 2012. Metabolic fingerprinting of Tomato mosaic virus infected Solanum lycopersicum. J. Plant Physiol. 169 (16), 1586–1596. https://doi.org/10.1016/j.jplph.2012.05.021.

Martin, D.P., Murrell, B., Golden, M., Khoosal, A., Muhire, B., 2015. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol. 1, vev003. https://doi.org/10.1093/ve/vev003.

Pellegrini, E., Campanella, A., Cotrozzi, L., Tonelli, M., Nali, C., Lorenini, G., 2018. Ozone primes changes in phytochemical parameters in the medicinal herb *Hypericum perforatum* (St. John's wort). Ind. Crops Prod. 126, 118–128. https://doi.org/10.1016/j.indcrop.2018.10.002.

Pedrelli, A., Panattoni, A., Materazzi, A., 2021. Occurrence of Grapevine Pinot gris virus in Chianti vineyards. Agrochim. Spec. Issue 65, 81–87. https://doi.org/10.12871/00021857202201.

Preising, S., Borges, D.F., de Queiroz Ambrósio, M.M., da Silva, W.L., 2021. A fig deal: a global look at fig mosaic disease and its putative associates. Plant Dis. 105 (4), 727–738. https://doi.org/10.1094/pdis-06-20-1352-fe.

Salhi-Hannachi, A., Chatti, K., Saddoud, O., Mars, M., Rhouma, A., Marrakchi, M., Trifi, M., 2006. Genetic diversity of different Tunisian fig (*Ficus carica* L.) collections revealed by RAPD fingerprints. Hereditas 143 (2006), 15–22. https://doi.org/10.1111/j.2005.0018-0661.01904.x.

Şevik, M.A., Cansiz, N., 2021. The impact of Turnip mosaic virus on physiological and morphological parameters of kale plants. Gümüşhane Üniv. Fen Bilim. Enst. Derg. 11 (3), 919–924. https://doi.org/10.17714/gumusfenbil.885232.

Stanton, K.M., Mickelbart, M.V., 2014. Maintenance of water uptake and reduced water loss contribute to water stress tolerance of *Spiraea alba* Du Roi and *Spiraea tomentosa* L. Hortic. Res. 1, 14033. https://doi.org/10.1038/hortres.2014.33.

Toima, N.I., El-Banna, O.-H.M., Sayed, A.M., Youssef, S.A., Shalaby, A.A., 2022. Incidence, molecular detection, and partial nucleotide sequencing of some viruses causing fig mosaic disease (FMD) on fig plants in Egypt. Int. J. Microbiol. 2022, 1–9. https://doi.org/10.1155/2022/2093655.

Tzanetakis, I.E., Laney, A.G., Keller, K.E., Martin, R.R., 2010. New viruses found in fig exhibiting mosaic symptoms. Jul. Kühn Arch. 427, 79. https://ojs.openagrar.de/index.php/JKA/article/view/410.

Usai, G., Giordani, T., Castellacci, M., Vangelisti, A., Mascagni, F., Ventimiglia, M., Simoni, S., Natali, L., Cavallini, A., 2021. Genomics and breeding of the fig tree, an ancient crop with promising perspectives. Agrochim. Spec. Issue 65, 99–106. https://doi.org/10.12871/000218572022012.

Walia, J.J., Willemsen, A., Elci, E., Caglayan, K., Falk, B.W., Rubio, L., 2014. Genetic variation and possible mechanisms driving the evolution of worldwide fig mosaic virus isolates. Phytopathology 104 (1), 108–114. https://doi.org/10.1094/phyto-05-13-0145-r.

Yahyaoui, E., Frasheri, D., Germanà, M.A., Burruano, S., D'Onghia, A.M., Elbeaino, T., 2017. Viruses infecting different Mediterranean genotypes of *Ficus carica* and their distribution in different plant organs. Acta Hortic. 1173, 273–278. https://doi.org/10.17660/actahortic.2017.1173.47.

Yorganci, S., Açıkgöz, S., 2019. Transmission of fig leaf mottle-associated virus 1 by *Ceroplastes rusci*. J. Plant Pathol. 101 (4), 1199–1201. https://doi.org/10.1007/s42161-019-00331-5.

Zare, H., Zamanifar, A., Yassaie, M., 2021. Effect of fig mosaic disease on carbohydrates metabolism and pigments content of fig leaves. Acta Hortic. 1315, 439–444. https://doi.org/10.17660/actahortic.2021.1315.65.

Zhang, L., Hoshika, Y., Carrari, E., Cotrozzi, L., Pellegrini, E., Paoletti, E., 2018. Effects of nitrogen and phosphorous imbalance on photosynthetic traits of poplar Oxford clone under ozone pollution. J. Plant Res. 131, 915–924. https://doi.org/10.1007/s10265-018-1071-4.