Eur J Plant Pathol (2019) 153:359–369
https://doi.org/10.1007/s10658-018-1562-0

Prevalence and molecular diversity of the main viruses infecting cucurbit and solanaceous crops in Azerbaijan

Cécile Desbiez · Eric Verdin · Benoît Moury · Hervé Lecoq · Pauline Millot · Catherine Wipf-Scheibel · Samra Mirzayeva · Nargiz Sultanova · Gulnara Balakishiyeva · Alamdar Mammadov · Ahmed Kheyr-Pour · Irada Huseynova

Accepted: 27 July 2018 / Published online: 4 August 2018

Abstract Two surveys were conducted in September 2014 and July 2015 in important vegetable-growing areas in Azerbaijan. Cucurbit and solanaceous plants exhibiting symptoms of mosaic, yellowing, leaf curl or necrosis were collected and tested serologically and molecularly for the presence of the major viruses infecting these crops. For cucurbits, the most common viruses in both sampling sets were aphid-transmitted ones, including potyviruses (watermelon mosaic virus, WMV, zucchini yellow mosaic virus, ZYMV), cucumoviruses (cucumber mosaic virus, CMV) and poleroviruses (cucurbit aphid-borne yellows virus, CABYV). Eggplant mottled dwarf (EMDV) was also detected for the first time in Azerbaijan on cucumber at a low prevalence. In solanaceous crops, CMV was the most common virus detected, followed by potato virus Y (PVY). Tomato spotted wilt virus (TSWV), alfalfa mosaic virus (AMV) and tobamoviruses (tomato mosaic virus (TMV), pepper mild mottle virus (PMMoV)) were also detected in 2015. The begomovirus tomato yellow leaf curl virus (TYLCV) was present on tomato only in the Absheron area, where it had a high prevalence and induced important losses. TYLCV-like symptoms on tomato in other areas of Azerbaijan were due to phytoplasma diseases.

Keywords Epidemiology · Genetic diversity · Cucurbitaceae · Tomato · Pepper

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10658-018-1562-0) contains supplementary material, which is available to authorized users.

C. Desbiez · E. Verdin · B. Moury · H. Lecoq · P. Millot · C. Wipf-Scheibel
INRA, UR407, Unité de Pathologie Végétale, 84140 Montfavet, France

S. Mirzayeva · N. Sultanova · G. Balakishiyeva · A. Mammadov · I. Huseynova (✉)
Azerbaijan National Academy of Sciences, Institute of Molecular Biology and Biotechnology, 1073 Baku, AZ, Azerbaijan
e-mail: nargizsultanova@mail.ru

A. Kheyr-Pour
Institut des Sciences du Végétal, CNRS, 91198 Gif sur Yvette, France

Introduction

Virus and phytoplasma diseases are particularly damaging in vegetable crops, as they affect not only the yield but also the visual and organoleptic quality of the products, particularly important for these crops. The great variety of soil and climatic conditions of Azerbaijan supports a very rich diversity of plant genetic resources for vegetable growing. Vegetable cultivation is considered one of the major economically important sectors in agriculture and accounts for 61% of the crop production (Sadikhova 2005). About 32 species of vegetables are widely cultivated (Sadikhova 2005). Leading vegetables in the country are the solanaceous crops tomato, pepper and eggplant, as well as cucumber, cabbage, string bean and onion. Presently, cucurbit and solanaceae crops are mainly produced in Ganja-Kazakh, Guba-Khachmaz, Lenkoran-Astara regions and Absheron peninsula. Over the past decade, tomato yellow leaf curl virus (TYLCV),

cucumber mosaic virus (CMV), pepper mild mottle virus (PMMoV), tomato mosaic virus (ToMV), tobacco mosaic virus (TMV), tomato spotted wilt virus (TSWV) on tomato and/or pepper crops, as well as CMV, zucchini yellow mosaic virus (ZYMV), squash mosaic virus (SqMV) in cucurbits have been reported in different regions of Azerbaijan (Huseynova et al. 2016; Huseynova et al. 2017; Verdin et al. 2018). Among the phytoplasmas widely distributed in Azerbaijan, '*Candidatus* Phytoplasma solani' is the most common one, causing diseases on many cultivated plants, including tomato, pepper, eggplant, cherry, cherry plum, common medlar trees and grapevine (Balakishiyeva et al. 2010; Balakishiyeva et al. 2016).

The aim of the present study was to investigate the prevalence and genetic diversity of the main plant viruses infecting cucurbit and solanaceous crops growing in Azerbaijan, in order to characterize the major agronomic problems and estimate if the highly damaging whitefly-transmitted viruses and/or emerging strains of known viruses now emerging in the Mediterranean Basin (Lecoq and Desbiez 2012) also threaten vegetable production in Azerbaijan.

Materials and methods

Surveys and plant sampling

Two surveys were performed in Azerbaijan in September 2014 and July 2015. The sample plants were mostly cucurbits (cucumber, melon, watermelon and squash) and Solanaceae (tomato, pepper, eggplant). In 2014, three important production regions were surveyed: Absheron, Ganja and Guba areas, in experimental stations or private farms. Sampling was performed at the end of the growing season, when the prevalence of viral diseases is usually highest since viral infection has been building up throughout the season. Many of the cucurbit plants showed symptoms of mosaic, vein banding, yellowing of the older leaves, and occasionally fruit deformation and discoloration, suggestive of virus infection. Many tomato plants, particularly in the Absheron region, showed symptoms of yellowing and upward leaf curl, besides common mosaics and necrosis. Pepper plants showed diverse symptoms of mosaic and yellowing, whereas eggplant plants looked healthy and symptomless except for one plant showing mosaics. Few aphids were observed on the plants, but whiteflies were frequently observed on the leaves of cucurbit and tomato plants in the three surveyed areas. A total of 171 samples was collected, 96 from cucurbits and 75 from tomato, pepper and eggplant (only one sample).

In 2015, the same crops were surveyed in four regions: Absheron, Khachmaz, Shamkir and Lankaran areas, in experimental stations or private farms: 66 cucurbit, 91 tomato, 84 pepper and 10 eggplant plants. Most of the crops were in open fields, but some tomato and cucumber greenhouses were also sampled in the Shamkir area. The plants were at different physiological stages, from recently planted to harvesting stage and end of production. Almost no aphids were observed, however some whiteflies, which were monitored on the leaves were present in greenhouse conditions but not in the open field. The proportions of plants showing symptoms were usually low. Virus-like symptoms on cucurbits included mosaic, leaf crispation, yellowing of the older leaves, and occasionally fruit deformation and discoloration. On pepper, most symptomatic plants showed leaf mosaic or yellowing and stunting, sometimes associated with upward leaf curling. Tomato plants exhibited mostly stunting with leaf curling, leaf yellowing and flower virescence suggestive of phytoplasma infection.

Twenty nine cucurbit samples from a survey performed in 2003 in the Lankaran, Guba and Baku areas, and previously tested serologically and molecularly (Lecoq and Desbiez, unpublished) were also added to the analyses.

Serological tests

All cucurbit samples were tested serologically in DAS-ELISA, using antisera obtained at the Plant Pathology Unit of INRA in Avignon, for 16 viruses frequent in cucurbits worldwide and/or present in countries neighboring Azerbaijan: watermelon mosaic virus (WMV, potyvirus), zucchini yellow mosaic virus (ZYMV, potyvirus), cucumber mosaic virus (CMV, cucumovirus), cucurbit aphid-borne yellows virus (CABYV, polerovirus), papaya ringspot virus (PRSV, potyvirus), Moroccan watermelon mosaic virus (MWMV, potyvirus), cucumber vein yellowing virus (CVYV, ipomovirus), cucurbit yellow stunting disorder virus (CYSDV, crinivirus), beet pseudo-yellows virus (BPYV, crinivirus), melon necrotic spot virus (MNSV, carmovirus), squash mosaic virus (SqMV, comovirus), squash leaf curl virus (SLCV,

begomovirus), watermelon chlorotic stunt virus (WmCSV, begomovirus), Ourmia melon virus (OuMV, ourmiavirus), eggplant mottle dwarf virus (EMDV, rhabdovirus) and cucumber green mottle mosaic virus (CGMMV, tobamovirus).

The Solanaceae samples were tested serologically for 6 viruses frequent in these plants worldwide and/or present in countries neighboring Azerbaijan: alfalfa mosaic virus (AMV, alfamovirus), CMV, EMDV, potato virus Y (PVY, potyvirus), tomato mosaic virus (ToMV, tobamovirus), and tomato spotted wilt virus (TSWV, tospovirus). In 2014, they were also tested for impatiens necrotic spot virus (INSV, tospovirus) and parietaria mottle virus (PMoV, ilarvirus). The samples collected in 2015 were not tested for these two viruses, but they were tested for pepper mild mottle virus (PMMoV, tobamovirus), as well as with CABYV antiserum, which cross reacts in ELISA with poleroviruses infecting pepper crops (beet western yellows virus, pepper vein yellows virus) (Buzkan et al. 2013).

Molecular tests

Molecular diagnostic tests by reverse transcription-polymerase chain reaction (RT-PCR) were performed for viruses for which no antiserum was available. RNA was extracted from all cucurbit samples using TRI-reagent® following the manufacturer's recommendations, and tested by RT-PCR for the presence of cucumber chlorotic yellows virus (CCYV, crinivirus), recently detected in Iran, using primers CCYV-CP-5′ and CCYV-CP-3′ (Mohammed et al. 2014).

Plants that appeared positive in ELISA for EMDV were tested by RT-PCR with primers EMDV-3500-5′ (5'-GCATTTGAGTTYTTCTATGAGGG-3′) and EMDV-4656-3′ (5'-CCTGCTTGATTGACTATCTC-3′), and the PCR products were sent for direct sequencing to Genoscreen (Lille, France).

RNA was also extracted from all tomato samples using TRI-reagent®, and tested by RT-PCR for tomato chlorosis virus (ToCV, crinivirus) and tomato infectious chlorosis virus (TICV, crinivirus), with the primers and protocols defined by Jacquemond et al. (2009). DNA was also extracted from the tomato samples before testing by PCR for the presence of begomoviruses with primers Gem-CP-V-5 and Gem-CP-C-3 (Wyatt and Brown 1996). For the solanaceous samples from 2015, phytoplasma detection was also performed by PCR with the universal primers for phytoplasmas R16mF2/R16mR1 and R16F2n/R16R2 (Gundersen and Lee 1996), and the amplified fragments were sent for direct sequencing to Genoscreen.

Diversity analyses

RT-PCR were performed on RNA from all WMV samples with primers W-VVIAM-5′ and WMV-3′ (Lecoq and Desbiez 2012) in order to amplify a 900 nucleotide fragment corresponding to the C-terminal part of the RNA-dependent RNA polymerase (RdRp, NIb protein) and the variable N-terminal part of the coat protein (CP). In order to characterize further the WMV isolates present in Azerbaijan, partial sequences from the HC-Pro coding region in the 5′ part of the genome were obtained after RT-PCR amplification with primers W-milHC-5 5'-TTGCCATGACACAGTGGTGG-3′ and W-finHC-3 5'-CTTCCCCACCAACCCTGTA-3′ for 29 WMV-infected samples from the different sampling sites. Partial sequences of the P3 to CI coding regions were also obtained for 10 samples from 2014 and 3 samples from 2015 after amplification with primers WMV-Xba-5′ 5'-TATGAATGCTCAGTCACACC-3′ and WMV-debCI-3′ 5'-GTYAAATTYGAGGAYTGGTGG-3′.

For ZYMV, a 600-nt fragment corresponding to the NIb-CP coding region was amplified with primers ZYMV-CP-5′ and ZYMV-CP-3′ (Lecoq and Desbiez 2012). For CABYV and other putative poleroviruses, a fragment encompassing part of the RNA-dependent RNA polymerase (RdRp), and intergenic region (IR) and part of the overlapping CP and MP (movement protein) coding region was amplified by RT-PCR with polerovirus-polyvalent primers Pol-G-F and Pol-G-R (Knierim et al. 2010) for 19 isolates from 2014 and 7 isolates from 2015.

For CMV, RT-PCR amplifications were performed for 18 isolates from the different sampled areas using primers specific of each genomic component: G1–2.F (5'-GCTCAGACACGTTCCCC-3′) and G1–2.R (5'-ACAGTCGGACATTCATTAAG-3′), G2-I.F (5'-GGCTGCTTTAATGTTAGGCG 3′) and G2-I.R (5'-GGATGGACAACCCGTTCACC-3′), G3-CP.F (5'-CTCAGCGGCTACGTCTGACG-3′) and G3-CP.R-(5'-TCCTCGGACTCACTGCGCGC-3′) (Ben Tamarzizt et al. 2013).

The amplified fragments were sent for direct sequencing to Genoscreen.

The sequences were aligned using ClustalW included in MEGA 6.0 (Tamura et al. 2013), with manual

corrections when needed. Reference sequences from GenBank were added to the analyses. The best nucleotide substitution model was selected with MEGA. Recombination analyses were performed with RDP4.0, using 6 different recombination detection methods.

Distance and maximum-likelihood trees were built with MEGA using the previously selected model for nucleotide substitution correction. A bootstrap resampling ($n = 500$ bootstraps) was performed for each analysis.

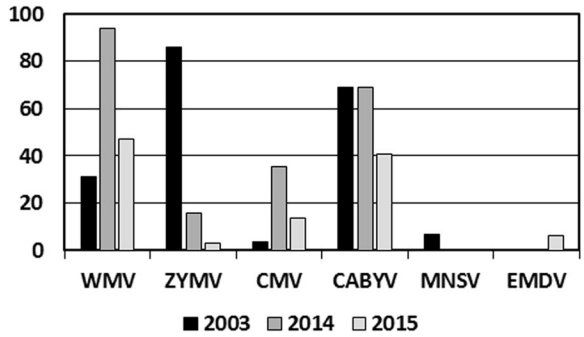


Fig. 1 Prevalence (%) of different viruses on cucurbits in Azerbaijan in the 2003, 2014 and 2015 surveys

Results

In cucurbits, WMV was the most common virus in the 2014–2015 surveys, infecting 90 out of the 96 samples (94%) in 2014 and 31 out of 66 samples (47%) in 2015 (Table 1).

CABYV was also frequent (69 and 41% of the samples in 2014 and 2015 respectively), followed by CMV (35.4 and 13.6%) and ZYMV (15.6 and 3%). All plants were negative in ELISA or RT-PCR against MWMV, PRSV, MNSV, BPYV, CCYV, CYSDV, CVYV, SqMV, SLCV, WmCSV, OuMV and CGMMV.

Three isolates from 2014 appeared slightly positive in ELISA for EMDV, but this was not confirmed by RT-PCR using EMDV-specific primers, and no characteristic bullet-shaped rhabdovirus particle was observed in transmission electron microscopy on these samples (data not shown). In contrary, in 2015, four cucumber samples, originating from all the sampled areas except Lankaran and exhibiting symptoms of leaf crispation and downward curling, were positive in ELISA for EMDV. Observations in transmission electron microscopy confirmed the presence of bullet-shaped particles. RT-PCR amplification with EMDV-specific primers yielded fragments of the expected size (GenBank accession MG964325) that exhibited 77% sequence identity (90% amino-acid identity) with EMDV sequences from Greece, Cyprus and Iran. To our knowledge, this is the first description of EMDV in Azerbaijan. In the survey from 2003 in the Khachmaz and Lankaran regions, ZYMV was the most common virus (86% of the samples) followed by CABYV (69%), WMV (31%), MNSV (6.9%) and CMV (3.4%) (Fig. 1).

Mechanical inoculations were performed on susceptible cucurbits in a quarantine greenhouse for 30 samples from 2014 and 9 samples from 2015 infected by WMV, ZYMV, CMV or EMDV. The viruses initially present in the original samples were recovered from most of the inoculated plants, as confirmed by DAS-ELISA, and symptom type and severity in the inoculated plants were consistent with those expected from ELISA. These results suggest that no important mechanically-transmissible virus had been missed in the study.

Based on ELISA results, mixed infections of two to four viruses in the same plant were rather common, particularly between WMV and CABYV (62/96 (64.6%) and 15/66 (22.7%) samples respectively in 2014 and 2015) or WMV and CMV (30/96 (31%) and 4/66 (6.1%)).

Table 1 Prevalence (number of infected plants) of viruses on cucurbits in the different sampling areas in Azerbaijan in 2014 and 2015

	Number of samples		WMV		ZYMV		CMV		CABYV		EMDV	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Absheron	42	21	40	16	1	1	7	4	20	10	0	1
Ganja	36	26	35	14	2	1	10	4	29	10	0	2
Quba	18	11	15	1	12	0	17	1	17	6	0	1
Lankaran	0	8	0	0	0	0	0	0	0	1	0	0
Total	96	66	90	31	15	2	34	9	66	27	0	4

Table 2 Prevalence of viruses on tomato in the different sampling areas in Azerbaijan in 2014 and 2015

	Number of samples		CMV		PVY		TMV		TSWV		TYLCV	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Absheron	13	16	5	10	0	7	0	0	0	0	11	9
Ganja	20	43	20	1	7	0	0	0	0	0	0	0
Quba	13	10	8	16	5	1	0	1	0	1	0	0
Lankaran	0	22	0	2	0	0	0	0	0	0	0	0
Total	46	91	33	29	12	8	0	1	0	1	11	9

In solanaceous plants, CMV was the most common virus detected in tomato (33/46 (72%) and 29/91 (32%) in 2014 and 2015 respectively) (Table 2), pepper (12/28 (41%) and 16/84 (19%) (Table 3) and eggplant (1/1 in 2014, 3/10 in 2015). PVY was also detected but at a lower frequency (12/46 and 5/28 (26%–18%) in 2014 and 8/91 vs. 1/84 (8.8%-1,2%) in 2015) on tomato and pepper respectively. In 2014, no other viruses were found by ELISA, whereas in 2015 PMMoV (5/84) and AMV (3/84) were observed at a low prevalence on pepper, AMV was present in eggplant (4/10), while TMV (1/91) and TSWV (1/91) were detected on tomato.

Mechanical inoculations were performed on susceptible plants (*Nicotiana benthamiana, Nicotiana tabacum* cv. *xanthi, Capsicum annuum, Solanum melongena, Solanum lycopersicum, Vigna unguiculata, Chenopodium quinoa*) with 20 samples from 2014 originating from the three prospected regions. The infected plant species and symptom types observed on this host range were consistent with those expected from ELISA results. No other atypical symptoms were observed, suggesting that no important mechanically transmissible virus had been missed.

In addition to viruses, phytoplasma were very frequently detected in 2015, in pepper (57.1%) and tomato (19.8%) crops and in eggplant (three plants out of 10). After sequencing of the PCR products obtained from 14 pepper, tomato or eggplant samples, the phytoplasma corresponded to '*Candidatus* Phytoplasma solani' (the causal agent of the stolbur-related disease) in every case.

Molecular tests did not yield any positive results for CCYV in cucurbits, nor TICV and ToCV in tomato (data not shown).

PCR with begomovirus-specific primers followed by sequence analysis performed on tomato samples revealed the presence of TYLCV in tomato plants exclusively localized in the region of Baku (11/12 positive samples in 2014 (Verdin et al. 2018) and 9/16 in 2015). These data were correlated with the symptoms observed: stunting aspect of the plant and strong leaf curl (Supplementary Fig. 1).

Molecular diversity of cucurbit viruses in Azerbaijan

Partial NIb-CP coding sequences were obtained for 85 WMV samples in 2014 and 30 samples in 2015

Table 3 Prevalence of viruses on pepper in the different sampling areas in Azerbaijan in 2014 and 2015

	Number of samples		CMV		PVY		PMMoV		AMV	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Absheron	12	10	2	5	5	1	nt	0	0	2
Ganja	7	17	2	5	0	0	nt	0	0	0
Quba	9	34	7	5	0	0	nt	2	0	0
Lankaran	0	23	0	1	0	0	nt	3	0	1
Total	28	84	11	16	5	1	0	5	0	3

(Genbank accessions MG964114-MG964228). All isolates displayed a low molecular variability, with more than 98% nucleotide sequence identity between samples. They were closely related to WMV isolates from the survey performed in Azerbaijan in 2003, and to isolates from Iran (Fig. 2). They all belonged to the same molecular group named "Group1" or "CL" (classic) present in several countries of Europe, Asia and Africa (Lecoq and Desbiez 2012). Sequences were obtained in the HC-coding region for 29 isolates from 2014 (accessions MG964296-MG964324). Contrary to the CP-coding region, all isolates belonged to molecular group "G2" rather than "G1". This indicates that, like most isolates considered as "G1" based on CP sequences (Desbiez and Lecoq 2008), they are indeed recombinant between groups G2 and G1. To further characterize the recombination breakpoint, 13 sequences (accessions MG964283-MG964295) were obtained in the P3 to CI coding region that is considered as a recombination hotspot for WMV (Desbiez and Lecoq 2008). These sequences revealed that the 13 isolates had the same recombination breakpoint located in the C-terminal part of the P3-coding region (data not shown).

As well as for WMV, the molecular diversity among 40 ZYMV isolates from Azerbaijan (GenBank MG964050-MG964089) was limited, with more than 98% nucleotide identity among isolates. ZYMV isolates collected in 2014 and 2015 were closely related or even identical in the sequenced fragment to isolates collected in 2003 (Fig. 3). Both in 2003, 2014 and 2015, some of the isolates were characterized by a unique "signature", i.e. one extra amino-acid in the CP compared to all worldwide isolates (data not shown). All ZYMV isolates collected in 2014 and 2015 belonged to the same molecular cluster known as group A1 that is the most common worldwide and contains isolates from all continents (Lecoq and Desbiez 2012). In 2003, group A1 but also A2 had been detected in Azerbaijan (Fig. 3).

For CMV, fragments of the three RNAs were amplified for 18 isolates from 2014 (GenBank MG964229-MG964282). Sequences from RNAs 1 and 2 were highly homogeneous, and for these 2 RNAs the isolates clustered in the worldwide IA group of CMV. Surprisingly, for RNA 3, while eight isolates clustered in group IA, the 10 remaining isolates belonged to group IB, without correlation with their host plant or sampling place in Azerbaijan (Supplementary Fig. S2). The fact that the three RNAs belong to different molecular groups indicates that the isolates are pseudo-recombinants or reassortants that can be described as "IA-IA-IB" according to the nature of their three RNAs.

Seventeen and seven polerovirus sequences were obtained in 2014 and 2015 respectively after amplification of cucurbit samples with primers Pol-G-F and Pol-G-R. All sequences (GenBank MG964090-MG964113) corresponded to CABYV only, and not to the other cucurbit-infecting poleroviruses including pepo aphid-borne yellows virus (PABYV) that is present in Africa and has been recently detected in Europe (Greece) (Knierim et al. 2014; Desbiez et al. 2016; Lotos et al. 2016). All Azerbaijan isolates were closely related molecularly to each other and they shared at least 98% nucleotide sequence identity in the sequenced fragment. In the RdRp-intergenic region (IR)-CP fragment used for phylogenetic analysis, the isolates from Azerbaijan appeared to constitute a distinct group, different both from isolates found in Europe (Spain, France) or Eastern Asia (China, Philippines, Thailand) (Supplementary Fig. S3).

Discussion

Except for TYLCV on tomato in the Absheron region, the most common and important viruses present in cucurbits, tomato, pepper and eggplant during the 2014–2015 surveys in Azerbaijan were aphid-transmitted ones, mostly potyviruses and cucumoviruses. During the surveys, very few aphids were observed in the crops but they had been noticed earlier by the growers, whereas whiteflies were present. The presence of large populations of whiteflies was considered as a new situation by the growers, suggesting that an invasive *Bemisia* biotype, maybe Med or MEAM (formerly Q and B) although this was not tested, has been introduced recently. In cucurbits, the most common viruses were WMV, CABYV, CMV and ZYMV, which are also among the most common worldwide when they are not superseded by whitefly-transmitted emerging viruses (Lecoq et al. 1998; Lecoq and Desbiez 2012). Some viruses were not detected in all surveys, like MNSV that had been observed in 2003 at a low prevalence but not in the 2014–2015, or like AMV, TSWV and TMV that were observed in tomato and pepper in 2015 but not in 2014. This may simply be due to random sampling effects. The prevalence of ZYMV was much higher in the 2003 survey than in 2014–2015, what could suggest an increase in

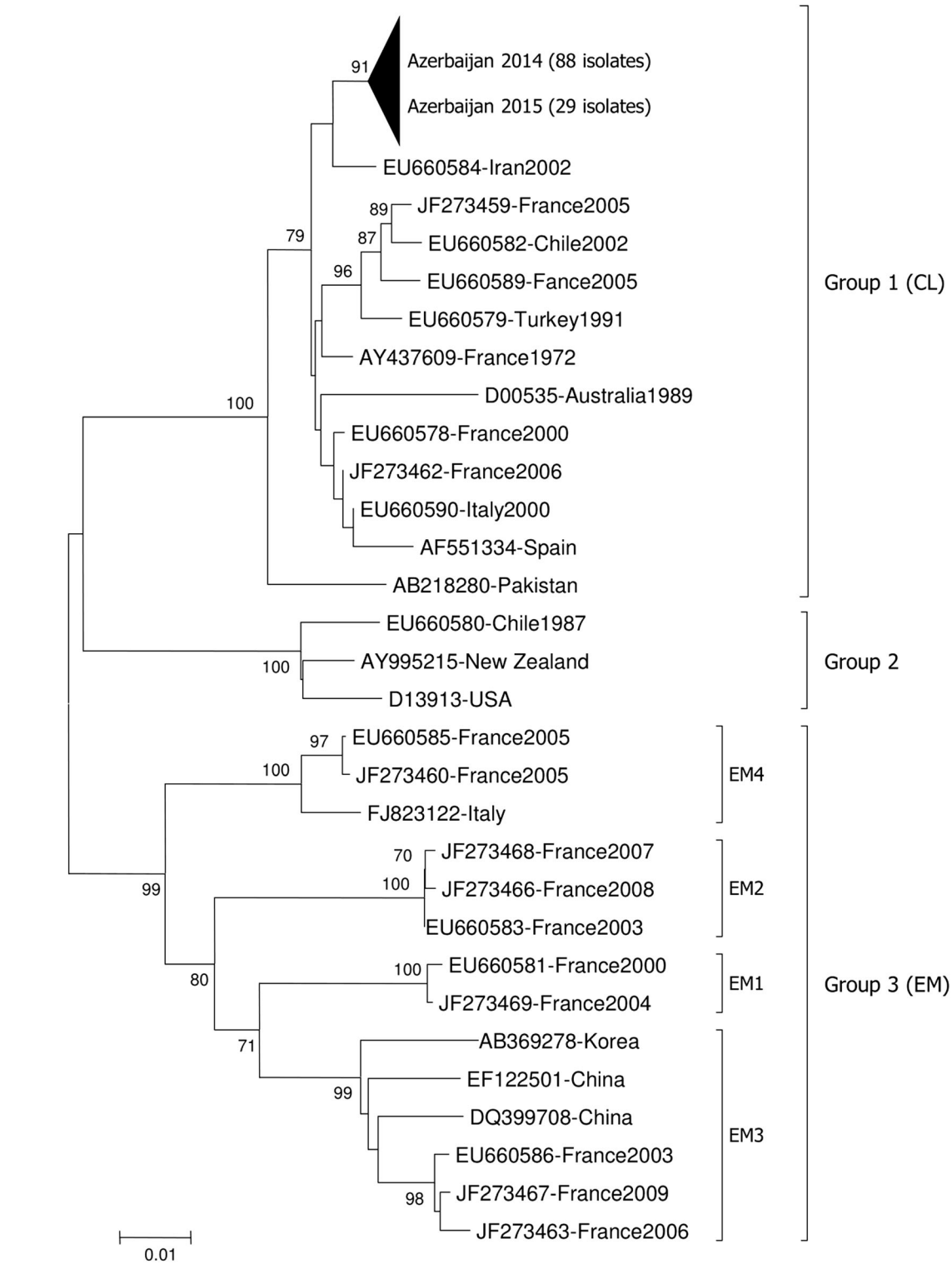
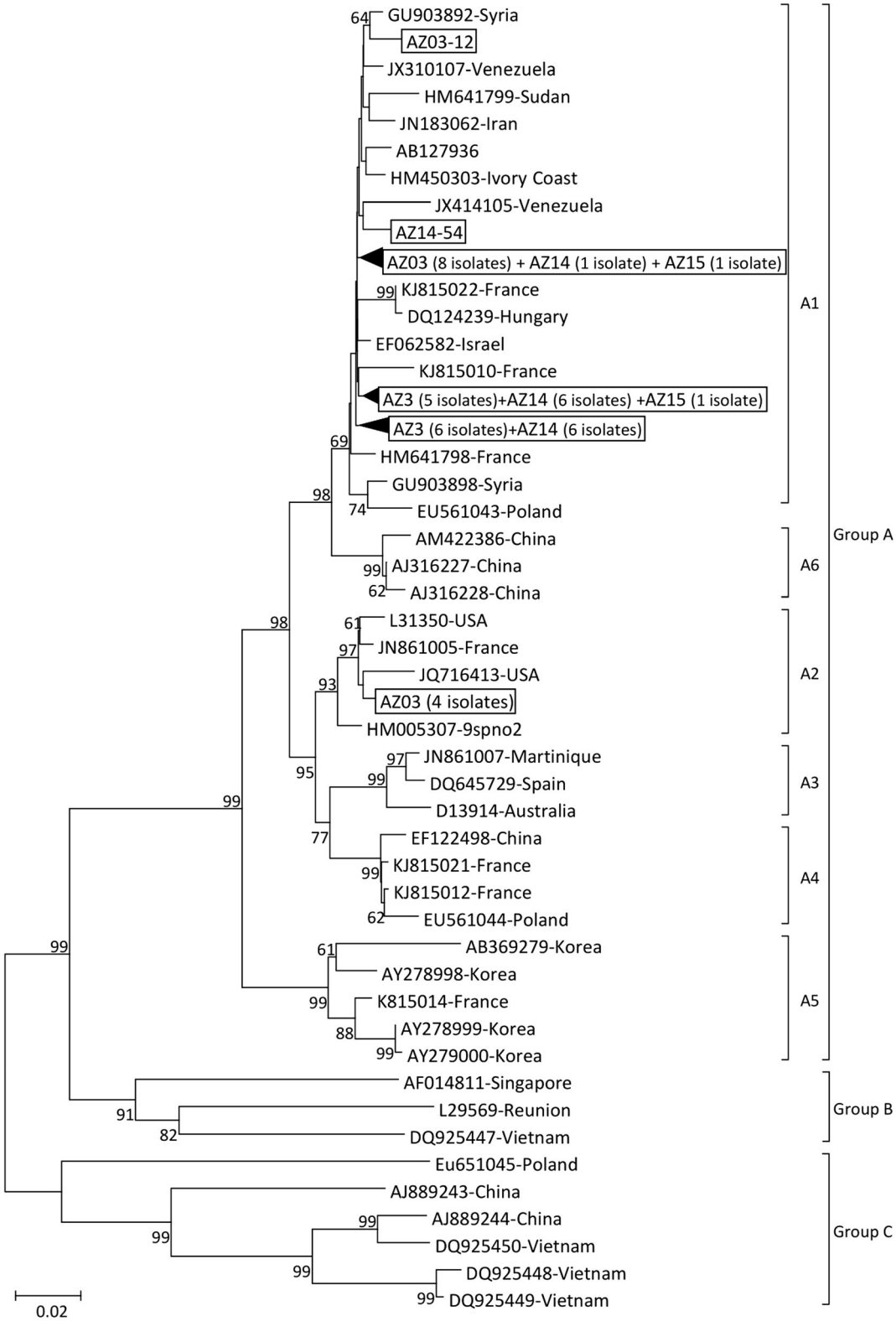


Fig. 2 Distance tree based on a fragment of the NIb-CP coding region of watermelon mosaic virus (WMV). The scale bar represents a genetic distance of 0.01. Bootstrap values above 70% (500 bootstraps) are indicated for each node


GU903892-Syria
AZ03-12
JX310107-Venezuela
HM641799-Sudan
JN183062-Iran
AB127936
HM450303-Ivory Coast
JX414105-Venezuela
AZ14-54
AZ03 (8 isolates) + AZ14 (1 isolate) + AZ15 (1 isolate)
KJ815022-France
DQ124239-Hungary
EF062582-Israel
KJ815010-France
AZ3 (5 isolates)+AZ14 (6 isolates) +AZ15 (1 isolate)
AZ3 (6 isolates)+AZ14 (6 isolates)
HM641798-France
GU903898-Syria
EU561043-Poland
AM422386-China
AJ316227-China
AJ316228-China
L31350-USA
JN861005-France
JQ716413-USA
AZ03 (4 isolates)
HM005307-9spno2
JN861007-Martinique
DQ645729-Spain
D13914-Australia
EF122498-China
KJ815021-France
KJ815012-France
EU561044-Poland
AB369279-Korea
AY278998-Korea
K815014-France
AY278999-Korea
AY279000-Korea
AF014811-Singapore
L29569-Reunion
DQ925447-Vietnam
Eu651045-Poland
AJ889243-China
AJ889244-China
DQ925450-Vietnam
DQ925448-Vietnam
DQ925449-Vietnam
0.02
A1
A6
A2
A3
A4
A5
Group A
Group B
Group C

◀ **Fig. 3** Distance tree based on a fragment of the NIb-CP coding region of zucchini yellow mosaic virus (ZYMV). Isolates from the 2003 and 2014–2015 surveys in Azerbaijan are boxed. The scale bar represents a genetic distance of 0.01. Bootstrap values above 60% (500 bootstraps) are indicated for each node

the relative frequency of WMV and CMV and a decrease of ZYMV. There may be a sampling bias related to the necessarily limited number of sites and plants surveyed, but ZYMV can also display very irregular epidemics, with important inter-annual fluctuations of prevalence (Lecoq et al. 2009).

Among the cucurbit-infecting potyviruses WMV and ZYMV, the molecular diversity of Azeri samples was very low in 2014–2015 and all isolates belonged to the most common molecular groups of their respective virus worldwide, "G1" for WMV and "A1" for ZYMV, even though ZYMV group "A2" had been observed in 2003. WMV isolates from 2014 to 2015 were closely related to those collected in Azerbaijan in 2003, and to isolates from Iran. As in many countries, WMV isolates from Azerbaijan are indeed recombinants between groups G2 in the 5′ part of the genome and G1 in the 3′ part (Desbiez and Lecoq 2008). Isolates from Iran collected before 2008 belonged mostly to group G1 in the CP-coding region although one isolate from group G2 was observed (Sharifi et al. 2008). One isolate from Iran was found to have a G2/G1 recombination breakpoint located in the HC-Pro coding region (Desbiez and Lecoq 2008), but according to the 29 partial HC-Pro sequences obtained in this study this was not the case for the Azerbaijan isolates. For 13 Azerbaijan isolates, the recombination breakpoint was located in the P3-CI coding region that was shown to be a recombination hotspot for G2/G1 recombinants (Desbiez and Lecoq 2008). This recombination breakpoint is different from those previously described for isolates from France, Italy, Turkey, Iran, Pakistan and Chile (Desbiez and Lecoq 2008) but appears identical to that of some Tunisian isolates (Yakoubi, Desbiez and Lecoq, unpublished). These results show that despite their molecular similarity in the CP coding region with isolates from Iran or other origins, the AZ14 isolates have no direct relation with these since they have a different recombination pattern and thus a different evolutionary history.

In France and probably in other European and Mediterranean countries, strains from Group 1 that had been present for more than 40 years are now being replaced by "emerging" strains from molecular group 3 (G3 or "EM"), probably originating from South-Eastern Asia (Desbiez et al. 2009; Lecoq and Desbiez 2012). According to the molecular characteristics of the isolates from this survey, EM strains have not yet been introduced in Azerbaijan or at least have a very low prevalence so far in the areas surveyed.

Groups A1 and A2 of ZYMV are common in France and other European and Mediterranean countries, but as in the case of WMV, emerging strains belonging to other molecular groups (named A4 and A5) are now also present (Lecoq et al. 2009; Lecoq and Desbiez 2012). These emerging strains were not found in this survey. These data indicate that contrary to many European and Mediterranean countries, there has been no major change in ZYMV populations in Azerbaijan in the last decade.

For CMV, sequences from RNAs 1 and 2 were highly homogeneous, and clustered in the worldwide IA group of CMV that is the most common in Europe and the Mediterranean Basin (Jacquemond 2012). However, for RNA 3, 10/18 isolates belonged to group IB. Subgroup IB originates from Eastern Asia, but several introductions have taken place in Europe and the Mediterranean Basin in the last decade(s) (Jacquemond 2012). The isolates appear as pseudo-recombinants or reassortants that can be described as "IA-IA-IB" according to the nature of their 3 RNAs. Pseudo-recombination has long been considered as a minor phenomenon in CMV even if its evolutionary consequences could theoretically be important (Escriu et al. 2007), but different reassortants have now been observed worldwide (Ohshima et al. 2016). A large proportion of CMV isolates infecting pepper in Tunisia has been shown recently to consist of peudo-recombinants, mostly IB-IA-IB and IB-IA-IA (Ben Tamarzizt et al. 2013). Isolates belonging to group IB for their RNA3 have also been observed since 2011 on tomato and cucurbits in Iran (Nematollahi et al. 2012; Farzadfar et al. 2013). They are closely related molecularly to the isolates from Azerbaijan and from Turkey, suggesting that they correspond to the same introduction/reassortment event of IB group in Western Asia that was estimated to have happened about 80 years ago (Ohshima et al. 2016).

The lack of whitefly-transmitted viruses, including some that are present in Iran (CVYV, CCYV, CYSDV, WmCSV, TLCPaV) (Keshavarz et al. 2014; Bananej et al. 2014; Esmaeili et al. 2015) in spite of high whitefly infestations suggests that these viruses have not yet been introduced in Azerbaijan. It is important to avoid introduction of these very damaging viruses and to control

whitefly populations, all the more since highly damaging whitefly-transmitted viruses are present in tomato crops.

In solanaceous crops, the only whitefly-transmitted virus detected was TYLCV, in the Absheron area only (Verdin et al. 2018). TYLCV-like symptoms of leaf curling and yellowing in the Ganja and Guba regions were related to phytoplasma infections. As in cucurbits, the major viruses present were mostly aphid-transmitted ones, even if TSWV and tobamoviruses were observed at a low prevalence. Several important viruses that are now emerging and/or very common in tomato and pepper crops in the Mediterranean Basin (particularly poleroviruses and criniviruses) (Buzkan et al. 2013; Moury and Verdin 2012; Hanssen and Lapidot 2012) were not detected in Azerbaijan during this survey. It is thus particularly important to avoid introducing them through exchanges of plant material.

The high prevalence of agronomical important aphid-transmitted viruses highlights the need for aphid control measures, appropriate cultural practices including weeding to remove virus and vector reservoirs and space management to avoid planting young crops close to old infected ones, and use of resistant varieties when they are available.

Acknowledgments This work was supported by the Science Development Foundation under the President of the Republic of Azerbaijan (EİF-2014-9(24)-KETPL-14/11/3-M-10) and by Presidium of Azerbaijan National Academy of Sciences (decision № 7/3 dated on 14.03.2018). We thank Isabelle Bornard at the Microscopy Platform of INRA-Montfavet for the electron microscope observations, the Experimental Infrastructure team in Montfavet for their help in the greenhouse experiments, and Dr. Michel Pitrat for his participation in the 2003 survey.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Balakishiyeva, G., Danet, J. L., Qurbanov, M., Mamedov, A., Kheyr-Pour, A., & Foissac, X. (2010). First report of phytoplasma infections in several temperate fruit trees and vegetable crops in Azerbaijan. *Journal of Plant Pathology, 92*, 115.

Balakishiyeva, G., Mamedov, A., Foissac, X., Huseynova, I., & Aliyev, J. (2016). First report of grapevine 'bois noir' in Azerbaijan. *Plant Disease, 100*, 2522.

Bananej, K., Kianfar, N., Winter, S., & Menzel, W. (2014). The status of cucumber vein yellowing virus in Iran. *Phytopathologia Mediterranea, 53*, 269–276.

Ben Tamarzizt, H., Montarry, J., Girardot, G., Fakhfakh, H., Tepfer, M., & Jacquemond, M. (2013). Cucumber mosaic virus populations in Tunisian pepper crops are mainly composed of virus reassortants with resistance-breaking properties. *Plant Pathology, 62*, 1415–1428.

Buzkan, N., Arpaci, B., Simon, V., Fakhfakh, H., & Moury, B. (2013). High prevalence of poleroviruses in field-grown pepper in Turkey and Tunisia. *Archives of Virology, 158*, 881–885.

Desbiez, C., & Lecoq, H. (2008). Evidence for multiple intraspecific recombinants in natural populations of watermelon mosaic virus (WMV, Potyvirus). *Archives of Virology, 153*, 1749–1754.

Desbiez, C., Joannon, B., Wipf-Scheibel, C., Chandeysson, C., & Lecoq, H. (2009). Emergence of new strains of watermelon mosaic virus in South-Eastern France: evidence for limited spread but rapid local population shift. *Virus Research, 141*, 201–208.

Desbiez, C., Millot, P., Wipf-Scheibel, C., Blancard, D., Chesneau, T., & Lecoq, H. (2016). First report of Pepo aphid-borne yellows virus in cucurbits in Tanzania and Mayotte. *New Disease Reports, 33*, 20.

Escriu, F., Fraile, A., & Garcia-Arenal, F. (2007). Constraints to genetic exchange support gene coadaptation in a tripartite RNA virus. *PLoS Pathogens, 3*, e8. https://doi.org/10.1371/journal.ppat.0030008.

Esmaeili, M., Heydarnejad, J., Massumi, H., & Varsani, A. (2015). Analysis of watermelon chlorotic stunt virus and tomato leaf curl Palampur virus mixed and pseudo-recombination infections. *Virus Genes, 51*, 408–416.

Farzadfar, S., Pourrahim, R., & Arafati, N. (2013). Molecular identification of cucumber mosaic virus subgroup 1B isolates in South Iran. *Journal of Plant Pathology, 95*, 423–428.

Gundersen, D. E., & Lee, I. M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea, 35*, 144–151.

Hanssen, I. M., & Lapidot, M. (2012). Major tomato viruses in the Mediterranean Basin. *Advances in Virus Research, 84*, 31–66.

Huseynova, I. M., Sultanova, N. F., Mirzoyeva, S. T., & Aliyev, J. A. (2016). Serological and molecular detection of virus infections of tomato (*Solanum lycopersicum* L.) plants in Azerbaijan. *Reports of ANAS, 3*, 73–78.

Huseynova, I. M., Mirzayeva, S. M., Sultanova, N. F., Aliyeva, D. R., Mustafayev, N. S., & Aliyev, J. A. (2017). Virus-induced changes in photosynthetic parameters and peroxidase isoenzyme contents in tomato (*Solanum lycopersicum* L.) plants. *Photosynthetica, 55*, 1–10.

Jacquemond, M. (2012). Cucumber mosaic virus. *Advance in Virus Research, 84*, 439–504.

Jacquemond, M., Verdin, E., Dalmon, A., Guilbaud, L., & Gognalons, P. (2009). Serological and molecular detection of tomato chlorosis virus and tomato infectious chlorosis virus in tomato. *Plant Pathology, 58*, 210–220.

Keshavarz, T., Shams-Bakhsh, M., Izadpanah, K., & Malboobi, M. A. (2014). Occurrence and genome analysis of cucurbit chlorotic yellows virus in Iran. *Journal of Phytopathogy, 162*, 523–526.

Knierim, D., Deng, T. C., Tsai, W. S., Green, S. K., & Kenyon, L. (2010). Molecular identification of three distinct Polerovirus species and a recombinant cucurbit aphid-borne yellows virus strain infecting cucurbit crops in Taiwan. *Plant Pathology, 59*, 991–1002.

Knierim, D., Tsai, W. S., Maiss, E., & Kenyon, L. (2014). Molecular diversity of poleroviruses infecting cucurbit crops in four countries reveals the presence of members of six distinct species. *Archives of Virology, 159*, 1459–1465.

Lecoq, H., & Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. *Advances in Virus Research, 84*, 67–126.

Lecoq, H., Wisler, G., & Pitrat, M. (1998). Cucurbit viruses: The classics and the emerging. In J. D. McCreight (Ed.), *Cucurbitaceae '98. Evaluation and enhancement of cucurbit germplasm* (pp. 126–142). Alexandria: ASHS Press.

Lecoq, H., Wipf-Scheibel, C., Chandeysson, C., Le Van, A., Fabre, F., & Desbiez, C. (2009). Molecular epidemiology of zucchini yellow mosaic virus in France: an historical overview. *Virus Research, 141*, 190–200.

Lotos, L., Maliogka, V. I., & Katis, N. I. (2016). New poleroviruses associated with yellowing symptoms in different vegetable crops in Greece. *Archives of Virology, 161*, 431–436.

Mohammed, H. S., Zicca, S., Manglli, A., Mohamed, M. E., El Siddig, M. A., Tomassoli, L., & El Hussein, A. A. (2014). Identification and phylogenetic analysis of common pumpkin viruses in Sudan. *Journal of Plant Pathology, 96*, 77–84.

Moury, B., & Verdin, E. (2012). Viruses of pepper crops in the Mediterranean Basin: a remarkable stasis. *Advances in Virus Research, 84*, 127–162.

Nematollahi, S., Bashir, N. S., Rakhshandehroo, F., & Zamanizadeh, H. R. (2012). Phylogenetic analysis of new isolates of cucumber mosaic virus from Iran on the basis of different genomic regions. *Plant Pathology Journal, 28*, 381–389.

Ohshima, K., Matsumoto, K., Yasaka, R., Nishiyama, M., Soejima, K., Korkmaz, S., Ho, S. Y. W., Gibbs, A. J., & Takeshita, M. (2016). Temporal analysis of reassortment and molecular evolution of cucumber mosaic virus: Extra clues from its segmented genome. *Virology, 487*, 188–197.

Sadikhova, L. (2005). Research and development of the vegetable system in azerbaijan. In: Increasing Market –oriented vegetable production in Central Asia and the Caucasus through collaborative research and development. Workshop Proceedings, 25-27 April 2005, Tashkent, Uzbekistan. AVRDC – The World Vegetable Center, Shanhua, Taiwan, p.29–36.

Sharifi, M., Massumi, H., Heydarnejad, J., Hosseini Pour, A., Shaabanian, M., & Rahimian, H. (2008). Analysis of the biological and molecular variability of Watermelon mosaic virus isolates from Iran. *Virus Genes, 37*, 304–313.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*, 2725–2729.

Verdin, E., Desbiez, C., Wipf-Scheibel, C., Gognalons, P., Kheyr-Pour, A., Gronenborn, B., Mirzayeva, S., Sultanova, N., Mammadov, A., & Huseynova, I. (2018). First report of tomato yellow leaf curl virus infecting tomato in Azerbaijan. *Journal of Plant Pathology, 100*, 335. https://doi.org/10.1007/s42161-018-0050-x.

Wyatt, S. D., & Brown, J. K. (1996). Detection of subgroup III geminiviruses isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology, 86*, 1288–1293.