DETERMINATION AND MOLECULAR CHARACTERIZATION OF WATERMELON MOSAIC VIRUS IN CUCURBIT PLANTS IN TEKIRDAG PROVINCE IN TURKIYE

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Abstract. A study was carried out to identify Watermelon mosaic virus (WMV) in cucurbit production areas in Tekirdag province of Turkiye. Infected plants were tested using Real-Time PCR and Reverse Transcriptase-PCR with oligonucleotides specific for the virus *CP* gene. Reverse Phylogenetic analysis of partial nucleotide sequences of the *CP* gene of 12 WMV isolates; TR-Tn2 (Genbank Acc. No. MT448602), TR-Tn5 (Genbank Acc. No. MT448603), TR-Tn8 (Genbank Acc. No. MT448604), TR-Tn9 (Genbank Acc. No. MT448605), TR-Tn10 (Genbank Acc. No. MT448606), TR-Tn18 (Genbank Acc. No. MT448607), TR-Tu75 (Genbank Acc. No. MT448612) and TR-Tu80 (Genbank Acc. No. MT448613); were showed 99.7-95% similarity to WMV ITA00-G (EU660590) from Italy, France isolate from France (NC_006262), TURK91 from Turkiye (EU660579), C05-464 from France (JF273459), CHI02-481 from Chile (EU660582), WMV-Pk from Pakistan (AB218280) and placed in WMV Group 1. Analysis of the 348 nucleotide of TR-Tf50 (MT448610), TR-Ta63 (MT448608), TR-Ta64 (MT448609) and TR-Ts71 (MT448611) isolates showed 98.2-92.2% similarity to WMV Watermelon isolate (AB369278) from South Korea, Ch99/69 (EF127832) and WMV-CHN (DQ399708) isolates from China, WMV TA-om3 (MN854651) from South Korea and S96-6 (AB353119) isolate from Japan and placed in WMV Group 3.
Keywords: *Real-Time PCR, RT-PCR, coat protein, phylogenetic analysis, cucumber*

Introduction

Viral infections reduced cucurbit production and caused important problems. Most of the viruses infecting cucurbits affect growth pattern of the plant, cause abnormal fruit formation in plants, and reduce the amount of marketable vegetable fruits or completely prevent fruit formation (Provvidenti, 1996; Kece and Kamberoglu, 2016). The most common viruses infecting cucurbits were reported as WMV, CaBYV and CMV (Yesil, 2021; Lopez-Martin et al., 2023; Mullholland et al., 2023; Rabadan et al., 2023). Watermelon mosaic virus (WMV, formerly Watermelon mosaic virus-2) is one of the most important aphid transmitted virus infecting cucurbits in the world (Desbiez and Lecoq, 2008; Juarez et al., 2013; Chickh-Ali et al., 2019; Desbiez et al., 2020; Radouane et al., 2021; Rabadan et al., 2023; Ben Mansour et al., 2023). WMV causing yellowing, mosaic and blisters on infected foliar parts of the plants, deformation and size reduction on fruits. The most common and efficient vectors of WMV reported as *Aphis craccivora*, *Aphis gossypii* and *Myzus persicae* transmit the virus in non-persistent manner (Lecoq and Desbiez, 2008). WMV reported as one of the most common virus and causing yield losses alone or as mixed infection compared to uninfected plants in the region (Koklu and Yılmaz, 2006). WMV isolates classified in three main groups based on the *P1*, *NIb* and

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N-terminal of *CP* as Group I (Classic, CL), Group 2 (G2) and Group 3 (Emergent, EM) (Desbiez et al., 2009; Glasa et al., 2011). G3 isolates contain '*KEKET*' motif instead of '*KEK*' motif at the position 3-5 in the *CP* by a two aminoacid insertion encoded by 6 additional nucleotides (Desbiez et al., 2007). This study included Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA), Real-Time Polymerase Chain Reaction (Real Time-PCR) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using specific primers to determine Watermelon mosaic virus (WMV) in cucurbit plants grown in Tekirdag province, Turkiye. The partial nucleotide sequences of the *NIb-CP* gene of 12 WMV isolates were identified and the obtained partial nucleotide sequences were compared to WMV sequences in GenBank.

Materials and methods

Collection of plant samples

Leaf samples of cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita moschata* Duch.), squash (*Cucurbita pepo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), melon (*Cucumis melo* L.) and gourd squash (*Lagenaria siceraria*) with yellowing, mosaic, crinkle, mottle, deformation and blister sypmtoms were collected from the production areas in Suleymanpasa, Ergene, Muratli, Saray, Malkara and Sarkoy counties of Tekirdag province in Thrace region of Turkiye during cucurbit growing period in 2017 and 2019 (*Fig. 1* and *Table 1*). Young leaf samples were collected from plants showing leaf curl, leaf deformation and mosaic symptoms on leaves, blistering, deformation and mosaic symptoms on fruits. Cucurbit plant samples with viral infection symptoms were collected and labeled to be used in DAS-ELISA and molecular studies, placed in ice boxes in transparent polyethylene bags, brought to the laboratory at a constant temperature of +4°C without disrupting the cold chain, and stored in the deep freezer at -20°C until use.

Figure 1. *Areas surveyed in Tekirdag province in Turkiye (Samples collection locations indicated by x)*

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Table 1. *Cucurbit samples collected in survey studies from Tekirdag province in 2017 and 2019*

Plant Species	Locations		Symptoms	Number of Samples	Date of Sampling
Melon	Suleymanpasa	Dedecik	Mosaic	9	08.08.2017
Melon		Kiniklar	Mosaic	7	09.08.2017
Pumpkin		Naip	Mosaic, Blistering	9	11.08.2017
Melon		Ferhadanli	Mosaic	8	14.08.2017
Squash		Evciler	Yellowing	7	16.08.2017
Squash		Barbaros	Yellowing	1	17.07.2019
Pumpkin		Naip-1	Mosaic, Blistering, Deformation in fruit	10	17.07.2019
Melon		Naip-1	Mosaic, Leaf curl, Blistering	9	17.07.2019
Watermelon		Naip-1	Mosaic	1	17.07.2019
Cucumber		Naip-2 (Greenhouse)	Mottle	10	17.07.2019
Pumpkin		Naip-3	Mosaic, Blistering	9	17.07.2019
Melon			Mosaic, Leaf curl, Blistering	3	17.07.2019
Squash		Ferhadanli-1	Mosaic	2	17.07.2019
Cucumber			Mottle	1	17.07.2019
Watermelon			Mosaic	3	17.07.2019
Melon			Mosaic, Leaf curl, Blistering	5	17.07.2019
Squash		Ferhadanli-2	Mosaic	3	17.07.2019
Melon			Mosaic, Leaf curl, Blistering	2	17.07.2019
Watermelon			Mosaic	1	17.07.2019
Pumpkin		Ferhadanli-3	Mosaic, Yellowing	1	17.07.2019
Melon		Tatarli	Mosaic	2	18.07.2019
Watermelon			Mosaic	2	18.07.2019
Pumpkin			Mosaic	2	18.07.2019
Squash			Mosaic	4	18.07.2019
Pumpkin	Malkara	Saglamtas	Mosaic, Blistering	1	18.07.2019
Pumpkin		Cinarlidere	Mosaic, Blistering	1	18.07.2019
Melon		Ahievran-1	Mosaic	4	18.07.2019
Melon		Ahievran-2	Mosaic	6	18.07.2019
Watermelon		Ahievran-3	Mosaic	1	18.07.2019
Cucumber		Ahievran-3	Mottle	2	18.07.2019
Melon	Sarkoy	Ulaman -1	Mosaic,	4	18.07.2019

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Plant Species	Locations		Symptoms	Number of Samples	Date of Sampling
Watermelon			Mosaic	2	18.07.2019
Squash			Mosaic, Mottle	2	18.07.2019
Cucumber		Ulaman-2	Mosaic, Mottle	1	18.07.2019
Watermelon			Mosaic	3	18.07.2019
Melon			Mosaic, Mottle	7	18.07.2019
Pumpkin			Mosaic, Mottle	1	18.07.2019
Cucumber		Yenikoy (Greenhouse)	Mottle	7	18.07.2019
Pumpkin		Ergene	Yellowing	2	19.07.2019
Melon		Saray	Yellowing	11	19.07.2019
Watermelon			Yellowing	4	19.07.2019
Gourd Squash		Muratli	Mosaic	2	19.07.2019
Melon			Mottle	5	19.07.2019
Watermelon			Mottle	1	19.07.2019
Pumpkin			Mottle	1	19.07.2019
Squash			Mottle	1	19.07.2019
Total Number Of Samples				**180**	

Mechanical inoculation studies

Cucumis sativus, *Cucumis melo* and *Cucurbita pepo* test plants were grown in pots using sterile peat, soil and perlite at the ratio of 1:1:1 in a climate room at 22-25ºC for 16 hours of daylight and 8 hours of night daily. Mechanical inoculation studies were carried out at the indicator plants had two leaves. 10 cucurbit plant leaf samples with virus infection symptoms were collected from Naip and Ferhadanli disctricts in Suleymanpasa county of Tekirdag province. Mechanical inoculation was made by crushing 1 g of selected leaf samples in 5 mL of 0.01 M Phosphate buffer (pH:7.0) containing 0.1% 2-mercaptoethanol in a sterile mortar at a ratio of 1:5 (w/v) as described by Yılmaz and Davis (1984). The leaf extracts were filtered to remove residues and inoculation carried out after indicator plant leaves abraded by carborundum powder and washed with sterile water.

Serological studies

The samples were serologically tested in the Plant Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Tekirdag, Turkiye. In serological studies, DAS-ELISA was conducted as described by Clark and Adams (1977). Antisera specific for Watermelon mosaic virus was used and absorption values at 405 nm wavelength were read in the Bio-Rad iMark Bench Microplate Reader. Absorbance values 2 times greater than negative samples were evaluated as positive.

RNA isolation and complementary DNA (cDNA) synthesis from plants

30 plant samples that gave positive results in DAS-ELISA were selected randomly and used for RNA isolation. Norgen Plant/Fungus Total RNA Purification Kit (Norgen

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Biotek, Canada) was used for RNA isolation from 50 mg leaf samples. The densities of the obtained RNAs were determined on a Thermo Nano Drop 2000/2000c Spectrophotometer at OD260 wavelength by adding 1 µL of the isolated sample RNA and diluted to a working concentration of 600 ng. cDNA was synthesized from the RNA samples whose concentrations were equalized using the Reverse Transcriptase enzyme for Real Time PCR and Reverse Transcriptase PCR studies. The study was carried out with the Xpert cDNA Synthesis Kit (Grisp Research, Portugal). 0.5 mL Eppendorf tubes were placed for each sample; dNTP mixture (10 mM each) 1 µL, Template RNA 1 µL, RNase free dsH_2O 14.5 µL and Oligo dT primer 1 µL was added. The entire mixture in Eppendorf was kept in the thermocycler device at 50ºC for 15 minutes. Then, cDNA was prepared by keeping it at 85ºC for 5 minutes.

Primers used in molecular studies

Oligonucleotides; primers WMV Forward 5'-GGCTTCTGAGCAAAGATG-3' and WMV Reverse 5'-CCCAYCAACTGTYGGAAG-3' (Desbiez et al., 2009) specific to the *NIb-CP* gene region of Watermelon mosaic virus in cucurbit plants that were found to be infected as a result of serological studies were obtained from Letgen Biotechnology (Izmir, Turkiye).

Real time PCR

The study was carried out on the Roche The Light Cycler® 480 Real Time PCR device. Roche (Germany) brand Universal 480 SYBR Green I Mastermix was used as the dye in Real Time PCR. In the study, dsH_2O 4.2 µL, Roche 480 SYBR Green I Mastermix 10 µL, Primer Forward 10 µM 0.4 µL, Primer Reverse 10 µM 0.4 µL, cDNA 5 µL a total of 20 µL Real Time PCR mixture was prepared for each sample. Real Time PCR studies were carried out with the cDNAs obtained by selecting 30 plant samples as a result of the survey areas and serological studies. In Real Time PCR studies, cDNA samples known to be infected with the disease-causing viral pathogen through serological tests were used as positive control, while 5 µL of nuclease-free water was added to 15 µL PCR mixture and used as negative control. 15 µL PCR mixture was added to each well on the plate and the volume was completed to 20 µL with 5 µL sample cDNA. In the PCR process, denaturation at 95ºC for 10 minutes, 40 cycles, denaturation at 95ºC for 10 seconds, annealing at 58ºC for 10 seconds, extension at 72ºC for 15 seconds, final extension at 95ºC for 10 seconds at 60ºC 1 minute, cooling 30 seconds end program at 40ºC was used.

Reverse transcriptase (RT) PCR analysis

cDNAs obtained from 30 selected plant samples and primers specific to *NIb-CP* gene region of Watermelon mosaic virus were used 90 in total for each cDNA sample. For the sample, dsH_2O 5.7 µL, $MgCl_2$ 2 µL, 10X Taq Buffer 5 µL, dNTP Mixture 2 µL, Primer Forward 2 µL, Primer Reverse 2 µL, Taq Polymerase 0.3 µL and DNA 1.0 µL for a total of 20.0 µL PCR mixtures were prepared. The PCR mixture was optimized for RT-PCR conditions created as a result of the modification of the protocol specified by Sambrook et al. (1989). PCR was runned as denaturation at 95ºC for 10 minutes, 40 cycles as denaturation at 95ºC for 10 seconds, annealing (variable according to the primer) at 58ºC for 10 seconds, extension at 72ºC for 15 seconds, final extension at 95ºC for 10 seconds, 1 minute at 60ºC, cooling 30 seconds at 40ºC end program.

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Gel electrophoresis

1% or 2% agarose gels were prepared depending on the expected product size as a result of Reverse Transcriptase-PCR amplifications. Agarose gel prepared using 30 mL gel: Agarose 0.37 g, 5xTBE 7.4 mL, dsH_2O 29.6 mL, Buffer (1%): 5xTBE 60 mL, dsH_2O 240 mL and Ethidium Bromide 4 µL were used. 5x Tris/Borate/EDTA (TBE) buffer was prepared by adding 20 mL/L of Tris-Base (pH:8) 54 g/L, Boric Acid 27.5 g/L and 0.5 M EDTA (pH:8) used in gel preparation (Sambrook et al., 1989). PCR products prepared from 5 µL PCR product DNA, 3 µL loading dye and 7 µL dsH_2O mixture were loaded into each well on the gel, and a 100 bp ladder (molecular weight marker) was loaded into the first well on the gel. The samples were run from negative (-) load to positive (+) load at 100 Volt/cm direct current.

Phylogenetic analysis

Sequence analysis studies were carried out by Letgen Biotechnology company in Switzerland, in order to determine the partial nucleotide sequences of the *CP* gene regions of the selected virus isolates through serological tests and real time PCR studies. The Reverse Transcriptase-PCR products were subjected to bidirectional sequencing using Sanger method. Mega.6 was used to compare the sequenced nucleotides of the virus isolates to WMV isolates registered in GenBank (Tamura et al., 2013).

Results

Results of mechanical inoculation

Mosaic and yellowing symptoms were observed in indicator plants after mechanical inoculation tests from squash plant leaf samples collected from field in Naip district of Suleymanpasa county in 2017. Leaf deformation and blisters were observed on the indicator plants after mechanical inoculation made from the melon samples collected from field from Ferhadanli district of Suleymanpasa county in 2017.

DAS-ELISA results

In the survey studies conducted in Tekirdag province, DAS-ELISA tests were carried out in order to determine Watermelon mosaic virus in cucurbits leaf samples. Totally 180 plant samples were serologicaly tested by DAS-ELISA and 4.44% of the samples (16 of 180) were determined infected by Watermelon mosaic virus. Positive reactions were detected in 8 samples collected from Naip district in Suleymanpasa county. Only pumpkin and cucumber plant samples were found infected with WMV.

Real time PCR results

Within the scope of the evaluations, 30 cucurbit cDNA samples were measured with primers specific to the *CP* gene of WMV, Watermelon mosaic virus isolates that showed high absorption values in serological tests used as positive controls. Watermelon mosaic virus infections were determined quantitatively by Real Time PCR in cucurbit plant samples where infection could not be detected due to low absorption values in serological tests. The melting curve graph was evaluated in the comparison of the amplification products of the cucurbit plant samples tested as a result of Real Time PCR (*Fig. 2*).

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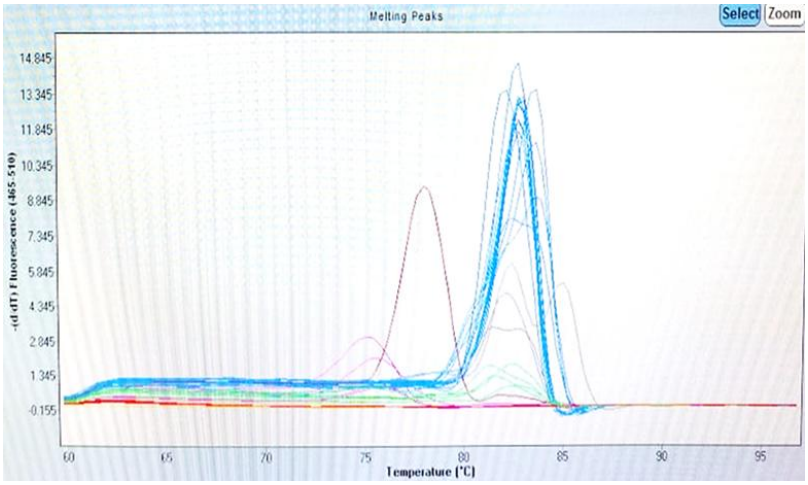

Figure 2. *Real Time PCR melting curve analysis curves; Blue: WMV positive cDNA samples, Red: Negative Control*

Watermelon mosaic virus was detected in 16 cucurbit plant samples by partial amplification using primers specific to the *NIb-CP* gene. High density of Watermelon mosaic virus infections were observed in cucurbit plants samples from Suleymanpasa, Sarkoy and Malkara counties (*Fig. 3*). Furthermore, WMV infections detected in pumpkin and cucumber samples from Naip district of Suleymanpasa county. Virus infections detected from, melon samples from Ferhadanli in Suleymanpasa county, pumpkin and melon plant samples from Tatarli district of Suleymanpasa county, pumpkin samples from Saglamtas and Cinarlidere districts in Malkara county, and squash and melon samples from Ulaman district in Sarkoy county. Additionally, Watermelon mosaic virus infections observed in pumpkin samples collected from Naip district of Suleymanpasa in 2017.

Figure 3. *Mosaic, discoloration and dark green blistering symptoms in pumpkin leaves collected from Ferhadanli and Naip districts in Suleymanpasa county*

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Results of reverse transcriptase PCR

In Reverse Transcriptase PCR studies, WMV infection was detected in 12 plant samples collected from Suleymanpasa county, in 2 plant samples collected from Malkara county and in 2 plant samples from Sarkoy county (*Figs. 4 and 5*).

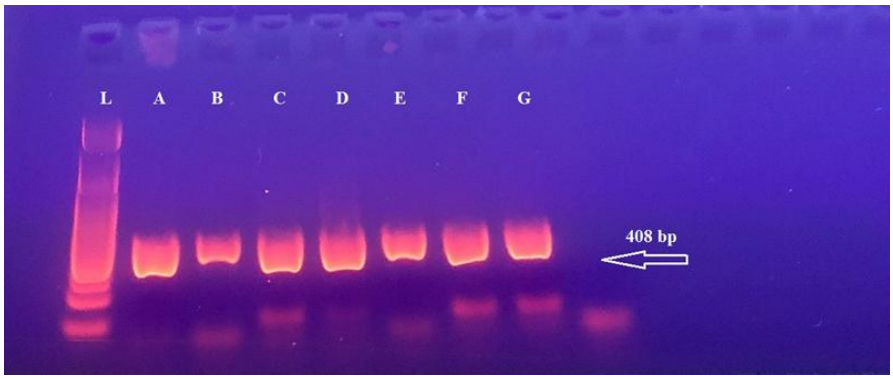

Figure 4. *Appearances on 2% agarose gel of RT PCR amplifications performed using oligonucleotides specific to the NIb-CP genome of WMV (A:TR-Tn2, B:TR-Tn5, C:TR-Tn8, D:TR-Tn9, E:TR-Tn10, F:TR-Tf50, G:TR-Ta63, L: 100 bp Ladder)*

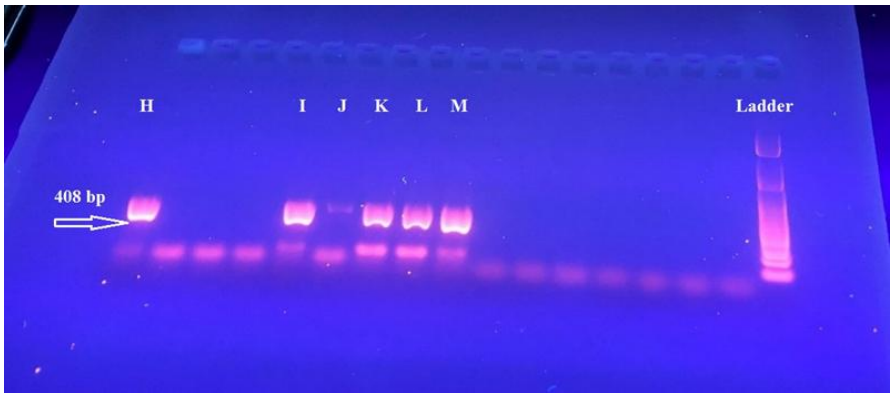

Figure 5. *Amplified nucleotide bands on 2% agarose gel of RT PCR amplifications performed using oligonucleotides specific to the NIb-CP genome of WMV (H:TR-Tn18, I:TR-Ta64, J:TR-Ts71, K:TR-Tc72, L: TR-Tu75, M: TR-Tu80, L: 100 bp Ladder)*

Phylogenetic analysis

The partial nucleotide sequences of the *NIb-CP* gene of 12 WMV isolates isolated from cuccurbit plants in Tekirdag province were determined by Real Time PCR and Reverse Transcriptase PCR, and bidirectionally sequenced using the Sanger method. Partial nucleotide sequences obtained as 342 and 348 nucleotides for WMV isolates from cucurbits collected in Tekirdag province. WMV TR-Tn2 (GenBank accession no. MT448602), WMV TR-Tn5 (GenBank accession no. MT448603), WMV TR-Tn8 (GenBank accession no. MT448604), WMV TR-Tn9 (GenBank accession no. MT448605), WMV TR-Tn18 (MT448607) and WMV TR-Tn10 (MT448606) isolated from pumpkin, WMV TR-Tu75 (MT448612) isolated from melon and WMV TR-Tu80 (MT448613) isolated from squash showed 100-98.2% similarity and with high similarity

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to WMV Group 1 members and evaluated in this group. WMV TR-Tf50 (GenBank accession no. MT448610) and TR-Ta63 (GenBank accession no. MT448608) isolated from melon; TR-Ta64 (GenBank accession no. MT448609) and TR-Ts71 (GenBank accession no. MT448611) isolated from pumpkin, with 100-98.9% similarity, and showed high similarity to WMV Group 3 isolates and replaced in WMV Group 3 (*Fig. 6*).

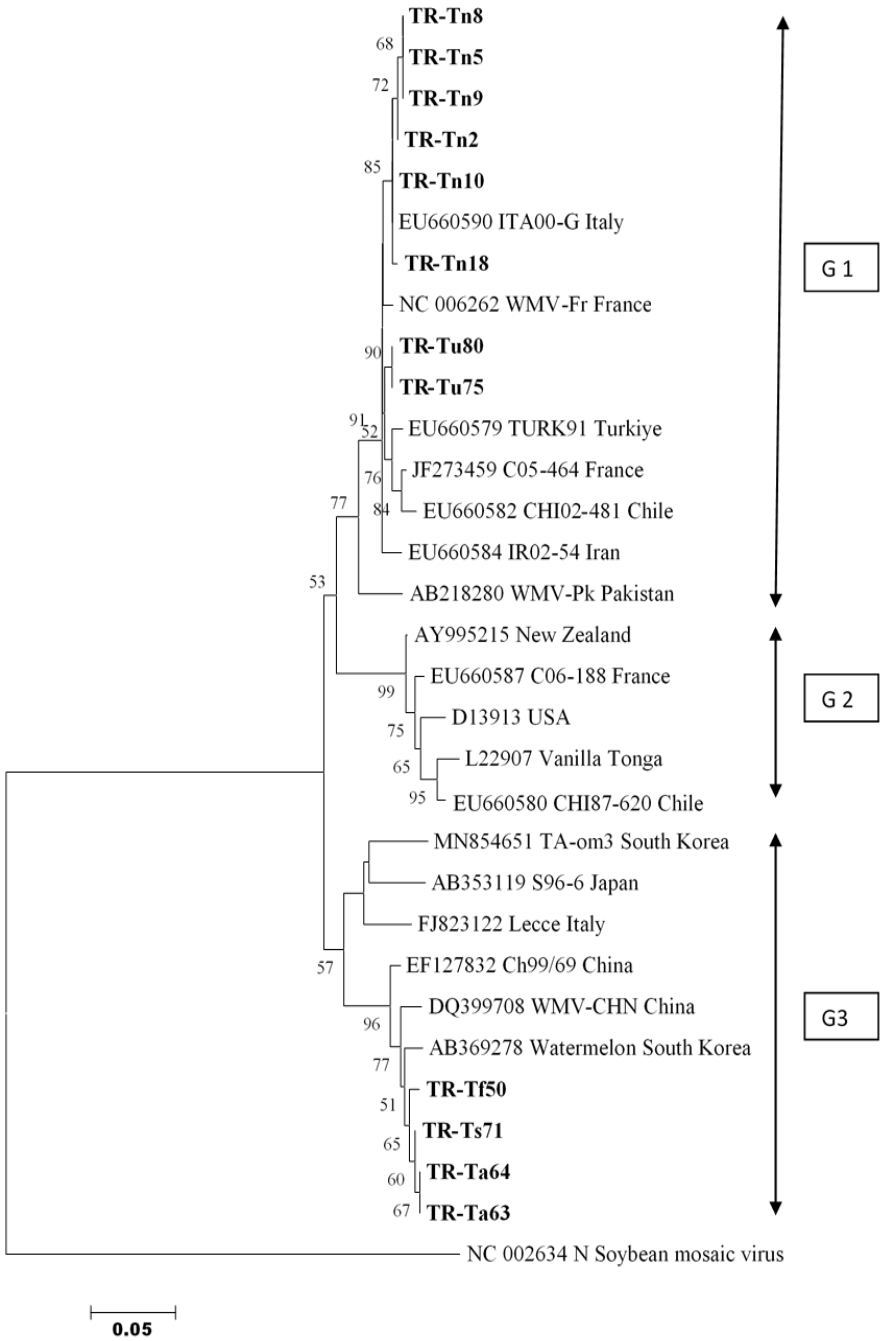

Figure 6. *Phylogenetic tree of the partial nucleotide sequences of the N-terminal of the NIb-CP of WMV isolates from Tekirdag with 18 isolates registered in GenBank obtained by Maximum Likelihood method based on the Tamura-Nei model. (Tamura et al. 2013, Mega.6). The tree is displayed with the Soybean mosaic virus as an outgroup. Bootstrap values (1000 bootstraps) above 70% are indicated for each node*

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342 nucleotide comparisons of the WMV TR-Tn2 (MT448602) isolate was found 99.7% similar to the WMV ITA00-G (EU660590, Desbiez and Lecoq, 2008) isolate from Italy and French isolate from France (NC_006262, Desbiez and Lecoq, 2004), 98.5% similar to isolate TURK91 (EU660579, Desbiez and Lecoq, 2008) from Turkey, 97.7% to isolate C05-464 from France (JF273459, Desbiez et al., 2011), 97.1% to the isolate CHI02-481 (EU660582, Desbiez and Lecoq, 2008) from Chile, and 95% to the isolate WMV-Pk (AB218280, Ali et al., 2006) from Pakistan (*Fig. 6*). 342 nt of WMV TR-Tn5 (MT448603) isolate comparison results 99.4% similarity to WMV ITA00-G (EU660590, Desbiez and Lecoq, 2008) isolate from Italy, 98.2% to French isolate (NC_006262, Desbiez and Lecoq, 2004) from France, 97.7% to isolate TURK91 (EU660579, Desbiez and Lecoq, 2008) from Turkey, 97.4% to isolate C05-464 from France (JF273459, Desbiez et al., 2011), 97.4% to isolate the CHI02-481 (EU660582, Desbiez and Lecoq, 2008) from Chile and 94.7% to isolate the WMV-Pk (AB218280, Ali et al., 2006) from Pakistan (*Fig. 6*). As a result of 342 nt of WMV TR-Tn8 (MT448604) and WMV TR-Tn9 (MT448605) isolates sequences showed 99.4% similarity to WMV ITA00-G (EU660590, Desbiez and Lecoq, 2008) isolate from Italy, 98.2% to the French isolate from France (NC_006262, Desbiez and Lecoq, 2004), 97.7% to the TURK91 isolate from Turkey (EU660579, Desbiez and Lecoq, 2008), 97.4% to C05-464 isolate from France (JF273459, Desbiez et al., 2011), 96.8% to the CHI02-481 (EU660582, Desbiez and Lecoq, 2008) isolate from Chile and 94.7% to the WMV-Pk (AB218280, Ali et al., 2006) isolate from Pakistan (*Fig. 6*).

342 nucleotides comparison of WMV TR-Tn10 (MT448606) isolate obtained from Naip district of Suleymanpasa county of Tekirdag province showed similarity as 99.7% to WMV ITA00-G (EU660590, Desbiez and Lecoq, 2008) isolate from Italy, 98.5% to French isolate from France (NC_006262, Desbiez and Lecoq, 2004), 98% with isolate TURK91 (EU660579) from Turkey, 97.7% with isolate C05-464 from France (JF273459, Desbiez et al., 2011), 97.1% to isolate CHI02-481 (EU660582, Desbiez and Lecoq, 2008) from Chile, 95.3% to isolate WMV-Pk (AB218280, Ali et al., 2006) from Pakistan and 90.1% to Vanilla isolate from Tonga (L22907, Wang et al., 1993) (*Fig. 6*). WMV TR-Tn18 (MT448607) collected from Naip district of Suleymanpasa county of Tekirdag province, showed 99.7% similarity to isolate WMV ITA00-G (EU660590, Desbiez and Lecoq, 2008) from Italy, 98.5% to French isolate from France (NC_006262, Desbiez and Lecoq, 2004), %98 to isolate TURK91 (EU660579, Desbiez and Lecoq, 2008), 97.7% to isolate C05-464 from France (JF273459, Desbiez et al., 2011), 97.1% to isolate CHI02-481 (EU660582, Desbiez and Lecoq, 2008) from Chile, 95% to isolate WMV-Pk (AB218280, Ali et al., 2006) from Pakistan and 90.4% to Vanilla isolate from Tonga (L22907, Wang et al., 1993) (*Fig. 6*). 342 nt comparisons, WMV TR-Tu75 (MT448612) and WMV TR-Tu80 (MT448613) isolates taken from the Ulaman district of Sarkoy county of Tekirdag province are 98.8% similar to the WMV ITA00-G isolate from Italy and the French isolate from France, 98.5% similar to TURK91 isolate 98.2% similar to C05-464 isolate from France 97.7% similar to CHI02-481 isolate from Chile 95.3% similar to WMV-Pk isolate from Pakistan and 90.1% similar to Vanilla isolate from Tonga (*Fig. 6*).

348 nucleotide comparison of WMV TR-Tf50 (MT448610) isolate taken from Ferhadanli district of Suleymanpasa county of Tekirdag province were 98% similar to Watermelon isolate from South Korea (AB369278, Choi et al., unpublished), 97.4% to isolates Ch99/69 (EF127832, Gu et al., unpublished) and WMV-CHN (DQ399708, Wu et al., unpublished) from China, 92.2% to isolates TA-om3 (MN854651, Kim et al.,

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unpublished) from South Korea and S96-6 (AB353119, Yamamoto and Fuji, 2008) from Japan, 91.2% to French isolate from France (NC_006262, Desbiez and Lecoq, 2004), 86.8% to the USA isolate from America (D13913, Quemada et al., 1990) and 86.3% to the Vanilla isolate from Tonga (L22907, Wang et al., 1993) (*Fig. 6*). 348 nt comparisons of WMV TR-Ta63 (MT448608) and WMV TR-Ta64 (MT448609) isolates taken from Tatarli district of Suleymanpasa county of Tekirdag province showed similarity as 98.2% to WMV isolate from South Korea (AB369278, Choi et al., unpublished), 97.7% to WMV-CHN (DQ399708, Wu et al., unpublished) isolate from South Korea, 93.1% to WMV TA-om3 (MN854651, Kim et al., unpublished) from South Korea, 92.5% to S96-6 from Japan (AB353119, Yamamoto and Fuji, 2008) isolate, 90.9% to the French isolate from France (NC_006262, Desbiez and Lecoq, 2004), 87.1% to the USA isolate from America (D13913, Quemada et al., 1990) and 86.5% to the isolate Vanilla from Tonga (L22907, Wang et al., 1993) (*Fig. 6*). 348 nt comparison of WMV TR-Ts71 (MT448611) isolate obtained from the Saglamtas county of Malkara district of Tekirdag province shows: match with 98.5% the Watermelon isolate (AB369278, Choi et al., unpublished) from South Korea, 98% to WMV-CHN isolate (DQ399708, Wu et al., unpublished) from South Korea, 93.4% to isolate WMV TA-om3 (MN854651, Kim et al., unpublished) from South Korea, 92.8% to S96-6 isolate from Japan (AB353119, Yamamoto and Fuji, 2008), to 91.2% the French isolate from France (NC_006262, Desbiez and Lecoq, 2004), 87.4% to USA isolate from America (D13913, Quemada et al., 1990), and 86.8% to Vanilla isolate from Tonga (L22907, Wang et al., 1993) (*Fig. 6*).

As a result of the comparison of predicted amino acid (aa) sequences of WMV isolates collected in Tekirdag province and sequence analysis, TR-Tn2 (MT448602), TR-Tn5 (MT448603), TR-Tn8 (MT448604), TR-Tn9 (MT448605), TR-Tn10 (MT448606), TR-Tn18 (MT448607), TR-Tu75 (MT448612) and TR-Tu80 (MT448613) located in WMV Group 1, but TR-Tf50 (MT448610), TR-Ta63 (MT448608), TR-Ta64 (MT448609) and TR-Ts71 (MT448611) formed a separate group and located in WMV Group 3.

Discussion

In this study, WMV infection was detected in 16 off 180 plant samples. WMV was determined as 8 in pumpkin plants, 3 in melon plants, 3 in cucumber plants and 2 in squash plants. WMV, which was the one of the three viruses with the highest prevalence in production areas among the virus diseases determined by DAS-ELISA tests performed by Altınay (2017) in plant samples collected from cucurbit plants in Tekirdag province, was selected for this study.

Plants having virus infection symptoms in the surveyed fields were more significiant in 2019 than 2017. One of the reasons for this is thought to be the low vector aphid population because of dry weather and high temperatures occurred during growing period in the region. The WMV-2 pathogen, which was most frequently detected in the melon and watermelon plant samples tested by Koklu and Yılmaz (2006) in their survey studies in the Thrace region, was identified as the most prominent viral infection agent in the region in this study. In the studies carried out by Nogay and Yorgancı (1985) with the aim of determining virus diseases in cucurbit production areas in the Marmara region, viral pathogens in cucurbit plants were investigated using physical, serological and electron microscopy. With the studies conducted, the researchers determined that Cucumber mosaic virus was more abundant than other cucurbit viruses in the Anatolian part of the Marmara region, while they reported that Watermelon mosaic virus was

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observed more frequently compared to other cucurbit viruses in the Thrace region, which was largely similar to the results found in this study.

Among the WMV isolates obtained in this study, evaluating 342 nt partial sequences of *NIb-CP* gene, TR-Tn5, TR-Tn8, TR-Tn9, TR-Tn10, TR-Tn18, TR-Tu75 and TR-Tu80 were grouped with WMV ITA00-G from Italy (EU660590, Desbiez and Lecoq, 2008), French isolate from France (NC_006262, Desbiez and Lecoq, 2004), TURK91 from Turkey (EU660579, Desbiez and Lecoq, 2008), C05-464 from France (JF273459, Desbiez et al., 2011), CHI02-481 from Chile (EU660582, Desbiez and Lecoq, 2008), WMV-Pk from Pakistan (AB218280, Ali et al., 2006) and located in WMV Group 1 (CL). Partial sequencing of 348 nucleotides of TR-Tf50, TR-Ta63, TR-Ta64 and TR-Ts71 isolates showed the 4 isolates having 6 more nucleotides coding *KEKET* instead of *KE* located in WMV Group 3 (EM) with Watermelon isolates from South Korea (AB369278, Choi et al., unpublished), Ch99/69 (EF127832, Gu et al., unpublished) and WMV-CHN (DQ399708, Wu et al., unpublished) from China and WMV TA-om3 (MN854651, Kim et al., unpublished) from South Korea and S96-6 from Japan (AB353119, Yamamoto and Fuji, 2008).

Kece and Kamberoglu (2016) conducted the RT-PCR study in watermelon growing areas in Adana, Mersin and Osmaniye provinces of Turkiye, resulted band formations of 408 bp in WMV-2. Within the scope of this study, band formations of approximately 408 bp were observed in WMV PCR products whose *CP* gene regions were detected using the same primers (Desbiez et al., 2009). Therefore, the results found by the researchers were similar to the results of this research conducted. In studies carried out worldwide, *Polyprotein P1* gene and *Coat Protein (CP)* have been identified as the most frequently used virus genomic regions in identifying Watermelon mosaic virus (Sanchez et al., 2007; Sidaros et al., 2009; Al-Saleh et al., 2010).

Conclusion

In this study, in the field observations made in the cucurbit production areas in the Tekirdag region, intense and similar mosaic, mottle, blistering and yellowing symptoms were detected on the leaves of cucurbit plants, which are thought to be of viral origin. There were no remarkable symptom differences in the plants examined, this suggest the possibility of mixed infections by other cucurbit viruses which were reported in previous studies (Koklu and Yılmaz, 2006; Altınay, 2017). Since the region where the study was conducted is a natural transition route between the European and Asian continents, other cucurbit viruses can also be found in the sampling areas.

The spread, interaction and genetic diversity of cucurbit viruses around the world should be revealed by identifying other cucurbit viruses at the molecular level with new studies to be carried out in cucurbit production areas in the Thrace Region, which is an important junction in Europe, Asia and the Mediterranean basin. As a result of the phylogenetic analysis of WMV isolates, some of them were located in group 1, which includes European isolates, while the other part was located in group 3, which includes Asian isolates. This confirms the existence of various genotypes in the region and emphasizes the importance of revealing effective control methods to prevent product loss in cucurbit plants and combating vectors, which are the most important ways of spreading viruses.

The isolates of Watermelon mosaic virus obtained from Tekirdag region were compared to isolates detected worldwide and the biodiversity of WMV in the region was

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revealed for the first time. The results obtained will be a reference for the next molecular studies, and will also be of indispensable importance for growing resistant commercial cucurbit plants.

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