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Araştırma Makalesi

In Silico and Molecular Analysis of Some Mosaic Diseases on Cucurbit Plants in Iğdır Province, Türkiye

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ABSTRACT

Cucumber mosaic virus (CMV) and watermelon mosaic virus (WMV) are plant viruses with positive single-stranded RNA genome that infect cucurbits and cause economic losses. Both viruses cause especially mosaic pattern and deformities in leaves, in cucurbit plants. The leaves of 23 melon and 28 watermelon plants showing such symptoms were sampled from different regions of Iğdır province. Samples with and without symptoms were tested by the Reverse Transcription Polymerase Chain Reaction (RT-PCR) using coat protein gene (CP)-specific primers. Polymerization tests amplified DNA fragments of the expected size for CMV and WMV. Some fragments with positive results were purified, bacterial cloned, nucleotide sequences revealed and registered in the GeneBank (NCBI). Sequence analyzes showed that it contained 593 bp and 822 bp for CMV and WMV, corresponding to the partial CP gene. Phylogenetic relationships with isolates from different geographical regions and plant material were investigated for both viruses. The generated phylogenetic tree confirmed that CMV-Iğdır isolate was in Group I and subgroup B, and WMV-Iğdır isolates were in different groups. In addition, coat proteins of virus isolates were characterized by in silico tools. In the current study, the presence WMV of and CMV in watermelon and melon was determined for the first time in Iğdır province, and group/subgroup assignments of CMV were revealed.

Key words: Cucumber mosaic virus, In silico analysis, Molecular characterization, RT-PCR, Watermelon mosaic virus

Iğdir İli Kabakgil Bitkilerinde Bazı Mozaik Hastalıklarının In Siliko ve Moleküler Analizi

ÖZ

Cucumber mosaic virus (CMV) ve watermelon mosaic virus (WMV) kabakgilleri infekte eden ve ekonomik kayıplara neden pozitif tek iplikli RNA genomuna sahip bitki virüslerdir. Her iki virüs kabakgil bitkilerinde özellikle yapraklarda mozaik deseni ve şekil bozukluklarına yol açmaktadır. Iğdır ilinin farklı bölgelerinden bu tür belirtiler gösteren 23 kavun ve 28 karpuz bitkisinin yaprakları Iğdır ilinin farklı bölgelerinden örneklenmiştir. Simptomlu ve simptomsuz örnekler Reverse Transkripsiyon Polimeraz Zircir Reaksiyonu (RT-PCR) ile kılıf protein genine (CP) spesifik primerler kullanılarak testlenmiştir. Polimerizasyon testleri, CMV ve WMV için beklenen boyutta DNA fragmentleri amplifiye etmiştir. Pozitif sonuçlu bazı fragmentler saflaştırılmış, bakteriyel klonlamaları gerçekleştirilmiş, nükleotit dizileri ortaya çıkarılmış ve gen bankasına (NCBI) kaydedilmiştir. Dizi analizleri, CMV ve WMV'nin kısmi kılıf protein dizilerine karşılık gelen 593 bp ve 822 bp nükleotit içerdiğini göstermiştir. Her iki virüs için farklı coğrafi bölgelerden sağlanan izolatların nükleotit dizilerine dayalı olarak filogenetik ilişkileri ortaya konmuştur. Oluşturulan filogenetik ağaç CMV Iğdır izolatının Grup I ve alt grup B'de olduğunu ve WMV-Iğdır izolatlarının ise farklı gruplarda yer aldığını doğrulamıştır. Ayrıca virus izolatlarının kılıf proteinleri in silico araçlar ile karakterize edilmiştir. Bu çalışmayla

Iğdır ilinde moleküler olarak ilk defa kavunda ve karpuzda CMV ile WMV varlığı saptanmış ve CMV'nin grup/altgrup atamaları gerçekleştirilmiştir.

Anahtar Kelimeler: Hıyar mozaik virüsü, In silico analiz, Karpuz mozaik virüsü, Moleküler karakterizasyon, RT-PCR

INTRODUCTION

Cucurbits are an important vegetable group cultivated worldwide (Robinson and Decker-Walters, 1999). The Cucurbitaceae family comprises of approximately 118 genera and 825 species spread throughout the world's tropical and subtropical regions. (Jeffrey 1990). In this family, melon (*Cucumis melo*), zucchini (*Cucurbita pepo*), cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*) are the main crop groups. It is well known that these vegetables potentially have nutritional and medicinal values (FAOSTAT, 2007). Cucurbits are one of the main hosts of many pathogenic microorganisms and pests, including viruses. As most cultivated plant crops, cucurbits are frequently subject to viral infections. Cucumber mosaic virus (CMV), squash mosaic virus (SqMV), zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV) and papaya ringspot virus (PRSV), tobacco mosaic virus (TMV) are specific cucurbit viruses. All pumpkin plants and weeds, including melons, cucumbers, squash, summer and winter squash, can be infected by all mosaic viruses (Radouane et al., 2021; Karanfil, 2022).

According to the literature, more than 30 viruses infecting cucurbits and limiting cucurbit production have been reported. Over time, new virus strains emerge due to suitable climatic conditions and adaptation to the host, and this number is increasing (Köklü and Yilmaz, 2006; Lecoq and Desbiez, 2012; Pozzi et al., 2020; Karanfil et al., 2023). The symptom severity and geographic distribution of many viral diseases in cucurbits are variable. Common symptoms produced by cucurbit viruses include mosaic and curling of leaves, rosette, necrosis, mottling, and yellowing. Apart from this, the aesthetics of the product may deteriorate, the fruit size may decrease and the market value may decrease. (Blancard et al., 1994; Juárez and., 2019).

CMV (Bromoviridae, Cucumovirus) was first discovered in the USA and has since been reported in many countries (Luis-Arteaga et al., 1998; Yuki et al., 2000; Massumi et al., 2007; Nouri et al., 2014; Yeşil, 2019; Akdura and Culal-Kilic, 2022). The virion contains three single-stranded RNAs (positive-sense) with a diameter of 29 nm. The virus, which primarily infects melons and pumpkins, is diagnosed by serological and DNA-based methods. (Adams et al., 2011; Sabry, 2011; Karanfil et al., 2016; Karanfil ve Korkmaz, 2017; Srivastava et al., 2019; Karanfil and Korkmaz, 2021). The virus is vector (about 60 species) inherited and incapable of progeny transmission. It may also be seed-transitive in cucurbits growing from infected seed. (Mauck et al., 2015). Currently, by analyzing CP genes and 5' non-reading regions, CMV isolates have been classified into two subgroups: Grup I (subgrup A, subgrup B) ve Grup II (Roossinck et al. 1999). While nucleotide similarity in subgroups is more than 90%, intergroup similarity rates have been reported to be 69-77% (Palukaitis et al., 1992).

WMV (Potyviridae, Potyvirus), first reported in Israel in 1963, has a flexible and filiform morphology. The main host of cucurbit plants, the virus is also infectious to approximately 170 plant species, including legumes, orchids and weeds. (Cohen and Nitzany, 1963, Desbiez et al, 2009; Wang and Li, 2017; Aguiar et al., 2018; Randa-Zelyüt et al., 2022). WMV or WMV-2 has the potential to induce mosaic, vein banding, blisters, deformation, and reduction in leaf size in cucurbits. The virus causes color irregularities and anomalies in the fruits of some infected cucurbit varieties, and it has also been reported that some WMV isolates cause necrosis in watermelon fruit (Crescenzi et al., 2001). WMV isolates have been studied since 1979 in two groups, WMV-1 and WMV-2, based on their serological characteristics. Recently, WMV-1 is referred to as the W strain of PRSV (papaya ring spot virus), while WMV-2 is known as the watermelon mosaic virus (WMV) (Purcifull and Hiebert, 1979). On the other hand, a serologically distinct isolate was discovered in South Africa and identified as moroccan watermelon mosaic virus (MWMV) (Lecoq and Desbiez, 2008).

The virus is presently present in numerous agricultural production regions and is regarded as one of the most prevalent and severe viruses of cucurbit crops (Loebenstein and Lecoq, 2012; Radouane et al., 2020). In diverse hosts and agroecosystems, WMV infection has been detected in numerous investigations conducted in Türkiye. However, there is no previous study on viral diseases of cucurbits in Iğdır province. Therefore, the purpose of this study is to detect the presence of WMV and CMV in commercially grown cucurbit fields using DNA-based techniques, to examine the phylogenetic relationships based on the viral coat protein gene, and to characterize the coat protein using in silico tools.

MATERIAL and METHODS

Sampling, Total Nucleic Acid Extraction (TNA), Complementary DNA Synthesis (cDNA)

In 2021, field research was conducted in the three districts of the province of Iğdır. In a total of 51 samples, 23 melon plants and 28 watermelon plants exhibiting severe virus and virus-like symptoms were collected. Also, 2 asymptomatic plants were included in the study to serve as negative control. Silica-based method was used for TNA extraction of all collected samples (Foissac et al., 2001). The obtained RNAs were converted to cDNAs using a random hexamer primer (5'- d (NNNNNN) – 3') according to the method adopted by Usta et al (2020). All primer sets used in the study were synthesized by the relevant company and diluted using nuclear free water in accordance with the company's recommendations.

Molecular detection of viral infections in cucurbits

Using partial coat protein gene specific primer pairs and reverse transcription polymerase chain reaction (RT-PCR), the presence of CMV and WMV in the synthesized cDNAs was determined (Table 1).

Table 1. Primer sets used in this study to determine the presence of CMV and WMV infections

Primers	Sequences (5' → 3')	References	Amplicons
WMV Forward	GAATCAGTGTCTCTGCAATCAGG	Sharifi et al., 2008	822 bp
WMV Reverse	ATTCACGTCCCTTGCAGTGTG		
CMV Forward	GCCACCAAAAATAGACCG	Usta et al., 2020	593 bp
CMV Reverse	ATCTGCTGGCGTGGATTTCT		

For both amplifications, 25 µl of standard reaction mix was prepared, containing 4 µl cDNA, 15.6 µl nuclease-free water, 1.5 µl 25 mM $MgCl_2$, 0.5 µl 10 mM dNTPs, 0.5 µl 20 pmol forward and reverse primers, 2.5 µl 10X Taq buffer, 0.4 µL Taq DNA polymerase (5 U μL^{-1}). The following thermocycling conditions were established: 3 min at 94 °C (first denaturation), then 35 cycles of 1 min at 94 °C for WMV (30 s for CMV) (denaturation), 60 s at 60 °C for WMV (30 s at 52 °C for CMV) (annealing), 60 s at 72 °C for WMV (extension) (45 s for CMV), and 72 °C for 5 minutes (final extension). DNA fragments of the coat protein gene of the target viruses and DNA size markers (1 kb) were separated in a 1.5% agarose gel containing EtBr (0.1%) at 85 volts for 50 minutes and visualized on the gel imaging device (Syngene™ UV Transilluminator 2020LM).

Sequencing, BLAST, and phylogenetic relationships

For the molecular characterization of isolates, prokaryotic cloning was performed on randomly selected PCR-positive isolates. Subsequently, visible DNA fragments were extracted from gel (GeneJET Gel Extraction Kit, Thermo Scientific, USA), subjected to a direct T-A cloning system (Promega, USA), and recombinant plasmids were transmitted to the *Escherichia coli* JM 109 strain via electrotransformation. Plasmids isolated from bacteria with GeneJET Plasmid Miniprep Kit (Thermo Scientific, USA) were sequenced utilizing the next generation sequencing (NGS) technique (Sentebiolab, Ankara, Türkiye). The partial CP gene sequences of two WMV and one CMV isolates have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/). To investigate the species specificity of the raw sequence data of 3 different isolates, BLAST analysis was performed separately in the NCBI online program. In addition, sequence data were analyzed in the Mega 7 software program and made suitable for similarity and phylogenetic analysis. For this purpose, the nucleotide sequences of partial CP genes of 3 isolates were compared with Sequence Demarcation Tool (Version 1.2), and determined the similarity rates at the nucleotide level with other the world isolates. The phylogenetic relationships of the Iğdır-cucurbit virus isolates were determined with 1000 repetitive bootstrap analysis by using the Neighbor-joining algorithm by using Mega7 software. To increase the accuracy of the phylogenetic tree, FJ376388 (*Soybean mosaic virus*) and DQ821116 (*Tobacco etch virus*) isolates are assigned as out-source for WMV and CMV, respectively. Using the coat protein sequences of Iğdır isolates, the physical and chemical properties of proteins such as amino acid content, charged residue and molecular weight were calculated via ProtParam online server. The coat proteins of the virus isolates detected in the study were estimated as 3D models in silico using the Phyre server. (Kelley and Sternberg, 2009). 3D structures visualized with Pymol software (DeLano, 2002). Model quality for 3D structure of proteins was evaluated by Ramachandran plot analysis using structure assessment in Expasy server.

RESULTS and DISCUSSION

Symptomatology and infection rate

During the survey studies carried out in three districts of Iğdır province, viral infection suspected 51 cucurbit samples were collected and tested using RT-PCR assays. 593 bp and 822 bp of specific DNA amplicons were obtained for CMV and WMV agents, respectively (Fig. 1).

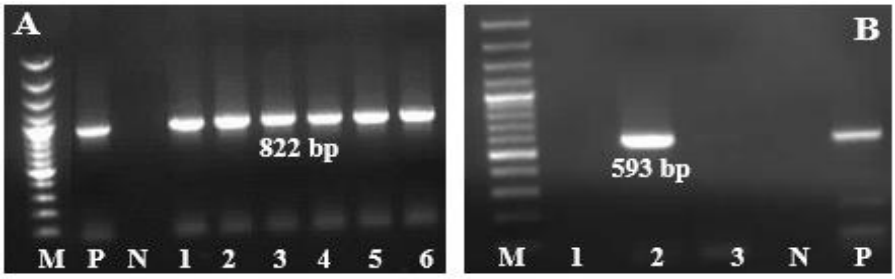

Figure 1. Agarose gel images indicating the presence of mosaic diseases in melon and watermelon samples collected under field conditions. A and B refer to DNA fragments amplified using primer sets specific for Watermelon mosaic potyvirus and Cucumber mosaic cucumovirus, respectively. N: negative control, P: positive control, M: molecular marker

CMV and WMV are the main virus diseases of cucurbit crops. In this mini-survey conducted with cucurbits in Iğdır province, the overall infection rate is detected 35% for WMV and CMV Mosaic disease positivity in melon and watermelon plants was calculated as 39.13% and 32.14%, respectively. Plant-based infection numbers are given in Table 2. In this study, the number of virus-suspected samples was high, but the presence of both viruses was at a low rate. This is likely due to the presence of mosaic disease agents such as squash mosaic virus (SqMV), zucchini yellow mosaic virus (ZYMV), or the presence of other cucurbit viruses in the Potyviridae, Tombusviridae, Luteoviridae, Virgaviridae families infecting cucurbits (Radouane et al., 2021).

Table 2. Melon and watermelon plants collected from different districts of Iğdır province and the number of infected samples

Locations	Number of cucurbits		CMV positivity		WMV positivity	
	Melon	Watermelon	Melon	Watermelon	Melon	Watermelon
Center	10	12	5	2	2	3
Aralık	5	8	-	1	1	2
Tuzluca	8	8	1	1	-	-
Total	**23**	**28**	**6**	**4**	**3**	**5**

Mosaic disease of cucurbits is well studied worldwide. The symptoms associated with this disease are almost the same. In many cases, symptoms such as wrinkling, irregularity, and shoestrings in leaves, patchy patterns on leaves, dwarfism, vein banding, and yellowing raise the suspicion of viral infection (Lecoq and Desbiez, 2012; Hamza et al., 2022), which is consistent with what was observed in this study in cucurbit-grown areas in this study. In Iran, 100 plant samples including green bean, cucumber, eggplant, squash, tomato and watermelon plants were molecularly tested against different viral pathogens of cucubits. From high to low, infection rates were determined as CMV, ZYMV, WMV and Cucumber green mottle mosaic virus (CGMMV). In addition, CMV and WMV infections have been reported in watermelon, cucumber and zucchini plants, as well as the presence of both viruses in tomatoes and green beans plants (Mohammadi et al., 2016). Yeşil (2019) tested squash plants showing simptoms such as mosaic, curling, mottling, filiformism, dwarfing, and leaf and fruit anomalies using DAS-ELISA in 2014. Tests confirmed single and mixed viral infection of ZYMV, WMV-2, CMV, Papaya ringspot virus-watermelon strain (PRSV-W) and SqMV. Pérez-de-Castro et al. (2020) were conducted surveys for viral diseases of melon, squash and watermelon in the main production areas of commercial and organic farming in Spain. RT-PCR tests in three symptomatic species of cucurbits showed that WMV was the most frequently detected among the eight plant virus agents tested

The prevalence of both viruses in different host and agricultural areas in is extensively mapped in the report published by Güller and Usta (2020). In Diyarbakir and Mardin provinces of , 160 cucurbit samples (melon, watermelon, zucchini, and cucumber) were analyzed against different cucurbit viruses by DAS-ELISA. Kızmaz et al. (2016), in their study determined CMV and WMV with high infection rates. Melon and watermelon plants are important hosts for CMV amd MMV. WMV has been reported to be an asymptomatic

host for many weeds. This increases the ability to survive the winter in favor of the virus. Besides weeds such as Capsella bursa-pastoris, Senecio vulgaris, and Lamium amplexicaule, some winter crops such as spinach can also be virus reservoirs (Lecoq, 1992a). In this study, it was observed that the presence of CMV was more common in melon plants. Compared to other cucurbits, a higher preference for CMV has been reported in melon or squash (Fabre et al., 2010). CMV spreads rapidly each year, especially in melon crops, and usually reaches full infection level 4-6 weeks after planting. In zucchini, however, CMV outbreaks are usually slow and the final infection rate rarely exceeds 10%. This is probably related to reduced plant susceptibility in the adult stage to limit the second spread (Lecoq, 1992b). Like WMV, CMV can select as hosts many winter crops that serve as virus reservoirs between two cucurbit crops, as well as weeds that can persist through the winter (Quiot et al., 1983; Sacristan et al., 2004).

GenBank submission and sequence homologies of obtained isolates

In the present study, CMV and WMV-specific DNA fragments were purified, bacterially cloned, and sequenced. Sequence analyzes revealed that the nucleotide sequences of the partial CP gene for CMV and WMV contained 593 and 822 nucleotides, respectively. Sequences of two isolates of WMV (from melon and watermelon) and one isolate of CMV (from melon) were registered in the GenBank under the accession numbers MZ130405, MZ055421, and MW962980, and denominated as Igdir 7, Igdir 6, and Igdir 2, respectively. According to nucleotide sequences of the CMV and WMV CP gene obtained, nucleotide similarities were determined using the BLASTn pathway in NCBI site. The Iğdır 7 and Igdır 6-WMV isolates (MZ130405 and MZ055421) shared sequence consensus between 97-100%, with other WMV isolates. Based on the Sequence Demarcation Tool (Version 1.2) program, both isolates showed 94% sequence homology among themselves (Fig. 3). According to nucleotite sequence analysis, the Iğdır-CMV isolate (Igdır 2, MW962980) showed nucleotide similarity between 87-100%, with the other CMV isolates (Fig. 2).

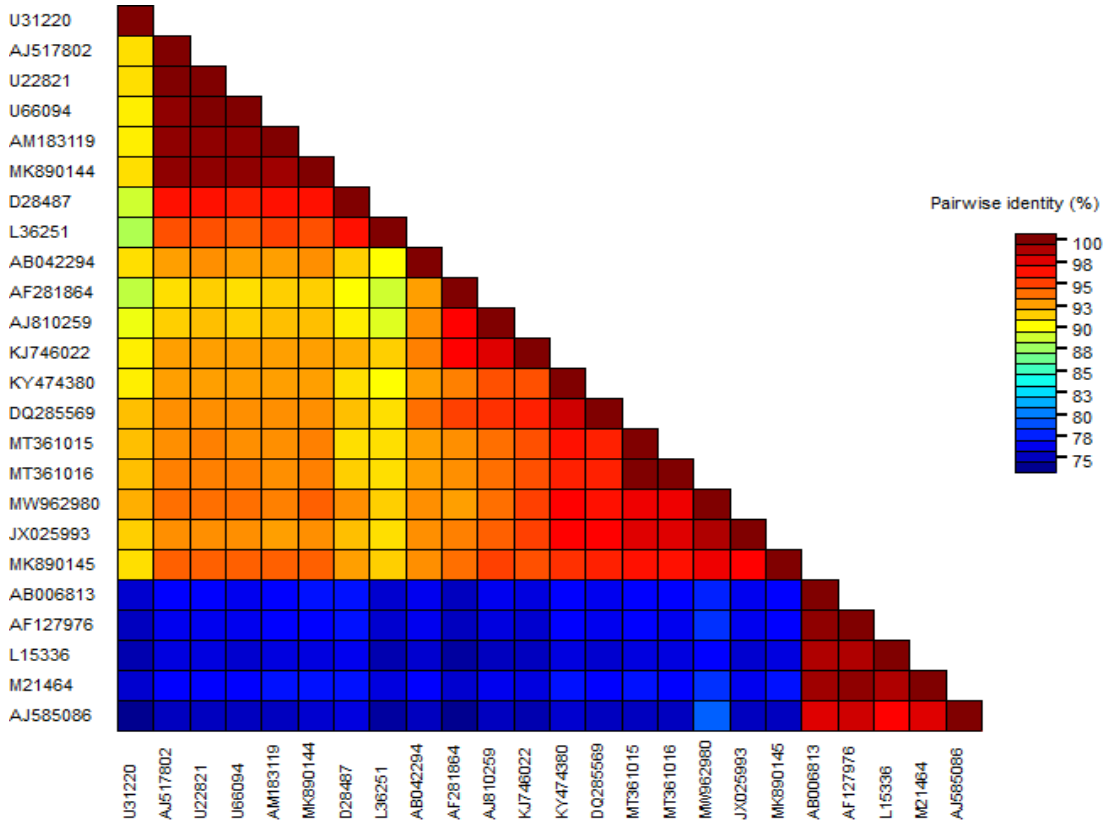

Figure 2. Nucleotide sequence identity of CMV isolates created using different gene isolates in this study

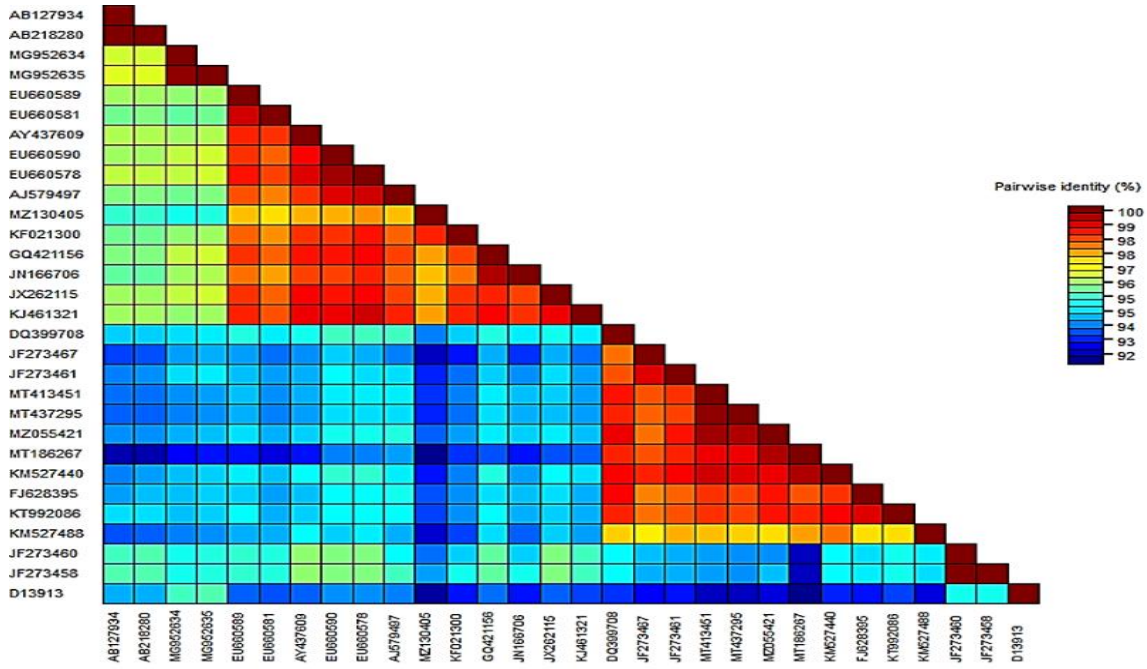

Figure 3. Nucleotide sequence identity of WMV isolates created using different gene isolates in this study

Phylogenetic tree and evolutionary inference of CMV isolate

Based on viral coat protein sequences, phylogenetic relationships were investigated for CMV viruses using other isolates detected in the world. Iğdır 2-CMV isolate was combined with a total of 22 isolates, including Groups I (A and B) and II isolates from different genetic sources, except for the outgroup (DQ821116) (Table 3).

Table 3. Table showing data on Turkish and other CMV isolates used in molecular phylogeny analysis

No	Accession no	Origin	Strain names	Host
1	MT361015 (CMV)	Türkiye	Bingol W2	*Cucumis melo*
2	MT361016 (CMV)	Türkiye	Bingol W6	*Cucumis melo*
3	MW962980 (CMV)	Türkiye	Igdir 2	*Cucumis melo*
4	JX025993 (CMV)	Iran	Khn1	*Solanum lycopersicum*
5	KY474380 (CMV)	Türkiye	CWP17	-
6	DQ285569 (CMV)	India	-	*Piper longum*
7	MK890145 (CMV)	Türkiye	Adiyaman TR 128	*Nicotiana tabacum*
8	KJ746022 (CMV)	China	YB6	*Nicotiana tabacum*
9	AF281864 (CMV)	India	-	*Datura innoxia*
10	AJ810259 (CMV)	Germany	KS44	-
11	AB042294 (CMV)	Japan	IA-3a	-
12	U31220 (CMV)	USA	Pak	-
13	D28487 (CMV)	Japan	FT	*Solanum lycopersicum*
14	L36251 (CMV)	-	Kor	-
15	AM183119 (CMV)	Spain	Ri-8	*Solanum lycopersicum*
16	AJ517802 (CMV)	Hungary	Rs	*Raphanus sativus*
17	MK890144 (CMV)	Türkiye	Adiyaman TR 93	*Nicotiana tabacum*
18	U22821 (CMV)	Australia	Ny	-
19	U66094 (CMV)	Israel	Sny	*Cucurbita pepo*
20	M21464 (CMV)	-	Q	-
21	AJ585086 (CMV)	India	Indian isolate	Lilium
22	AF127976 (CMV)	USA	LS	-
23	AB006813 (CMV)	Japan	m2	-
24	L15336 (CMV)	-	trk7	-
25	DQ821116 (TEV)	USA	Bodles-2	*Capsicum chinense* var. *Jacq*

The phylogenetic tree, consisting mainly of 3 groups, confirmed that the Iğdır-CMV melon isolate (MW962980) is in Group I and subgroup B (blue box in Fig. 4), which covers mostly Asian isolates, including isolates from different plants from Indonesia, Thailand, India, China, Türkiye, and Iran. This output is in line with the report of Roossinck (2002), who stated that associated that all world isolates with CMV IA and II subgroups, Asian isolates with subgroup IB. Although there were 4 Turkish isolates of CMV (MK890145, KY474380, MT361016, and MT361015) in Subgroup IB, this isolate showed primarily closer intraspecific affinity with the tomato CMV isolate from Iran (JX025993) (Fig. 3). This could possibly be due to the international exchange of plant material, as Iğdır and Iran are close border neighbors.

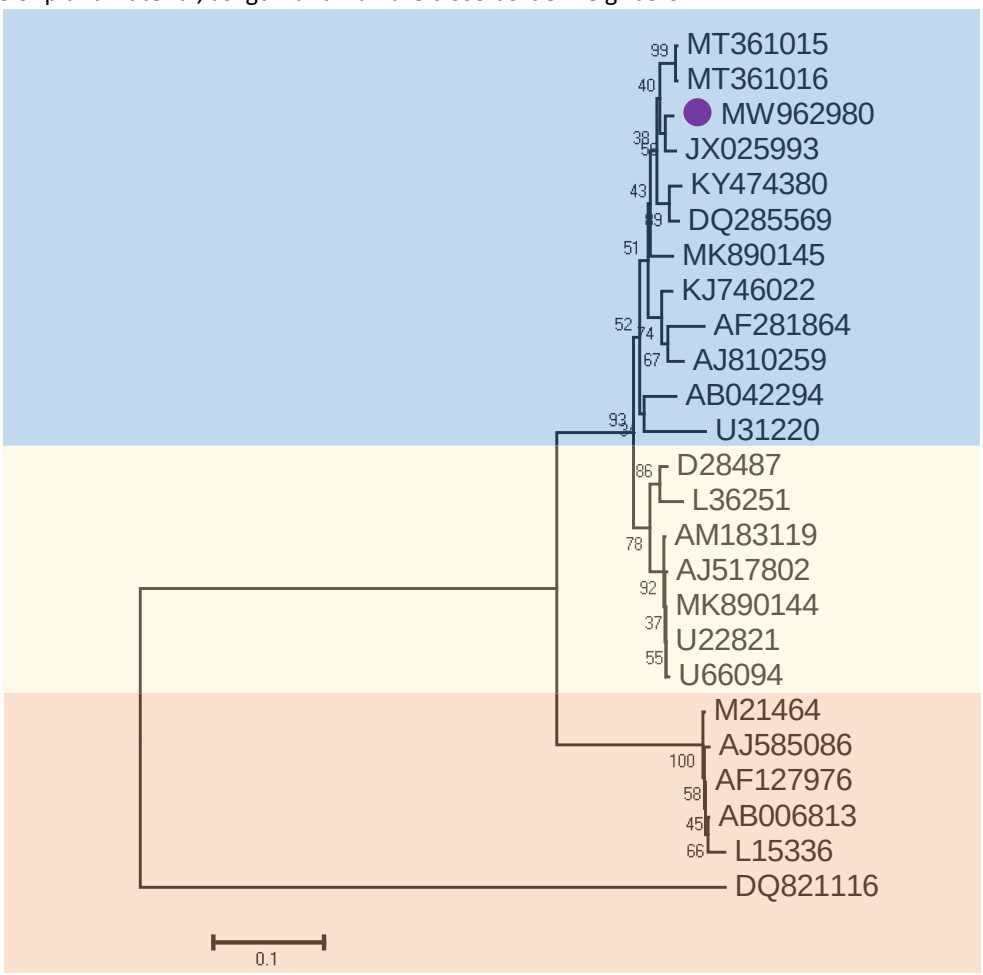

Figure 4. A phylogenetic dendrogram was created with CP gene region of the Iğdır-CMV isolate using the Neighbor-Joining model by MEGA 7 program. Boxes in blue, yellow, and orange indicate group IB, IA, and II isolates of CMV, respectively. Branches were supported by 1000 bootstrap analysis. MW962980 is the Iğdır 2-CMV isolate from melon marked with a claret red circle. A *Tobacco etch virus* (DQ821116) was used as the outgroup.

Phylogenetic tree and evolutionary inference of WMV isolates

Phylogenetic relationships of WMV-Iğdır isolates were determined using 28 WMV sequences from distinct agricultural ecosysytems, excluding those identified in this study and outgroup isolate (FJ376388) (Table 4).

Easy exchange of plant materials in the domestic and international market increases the probability of distribution of different WMV variants within the region. In addition, the cultivation of cucurbit crops in the Iğdır region, usually in private gardens or in small-scale fields where the virus spread cannot be controlled, may also support the genetic diversity of this virus. Accordingly, this may partly explain the diversity in the CP gene region of WMV isolated from melon and watermelon plants grown in the same region. However, based on Table 4, it was difficult to find a clear correlation between resulting molecular phylogeny and geographic origin and plant host. It should be noted that each group includes WMV members from Asia and Europe regions or different cucurbit hosts.

Table 4. Data on Turkish and other WMV isolates used in molecular phylogeny analysis

No	Accession no	Origin	Strain names	Host
1	EU660589	France	C05-337	-
2	EU660581	France	FMF00-LL1	-
3	AY437609	France	WMV-Fr	-
4	KJ461321	Ukraine	4K	*Cucumis sativus*
5	KF021300	Türkiye	W59	*Cucurbita pepo*
6	GQ421156	Iran	B-Torkaman 129	*Cucurbita pepo*
7	JN166706	Iran	Maz.Beh	Watermelon
8	JX262115	Serbia	256-09	*Cucurbita pepo* 'Tosca
9	EU660590	Italy	ITA00-G	-
10	EU660578	France	FMF00-LL2	-
11	AJ579497	Spain	VAL95.1	*Cucumis melo*
12	AB127934	Pakistan	Pak	Snake gourd
13	AB218280	Pakistan	WMV-Pk	*Cucumis melo* var *flexuosus*
14	MG952634	Türkiye	Alakoy 1	*Cucumis melo*
15	MG952635	Türkiye	Alakoy 2	*Cucumis melo*
16	D13913	USA	USA	Squash
17	JF273460	France	C05-465	Zucchini
18	JF273458	France	C05-463	Zucchini
19	KM527488	China	WTLF-3	Melon
20	FJ628395	Poland	WMV-GN	*Cucurbita pepo* L. *convar. giromantiina*
21	KT992086	South Korea	Yeongju6-1_2013	*Panax ginseng* C.A. Meyer
22	DQ399708	China	WMV-CHN	Watermelon
23	JF273467	France	Cg09-640	Zucchini
24	JF273461	France	C07-349	Melon
25	MT413451	Türkiye	Bingol W2	*Cucumis melo*
26	MT437295	Türkiye	Bingol W4	*Cucumis melo*
27	MT186267	Türkiye	Malatya/WMV1	Watermelon
28	KM527440	China	WCJ-3	Melon
29	FJ376388	South Korea	G5H	Soybean

The nucleotide sequence-based phylogenetic tree showed that WMV isolates were divided into two main phylogroups, with high confidence scores. Despite the fact that the plant materials were collected from the same location, Iğdır isolates were clustered with closely related isolates in different groups due to different nucleotide sequences, may be indicating the existence of two distinct evolutionary pathways of WMV isolates (Fig. 5). This is also consistent with pairwise nucleotide analysis, which showed that the two isolates had 94% nucleotide similarity (Fig. 3).

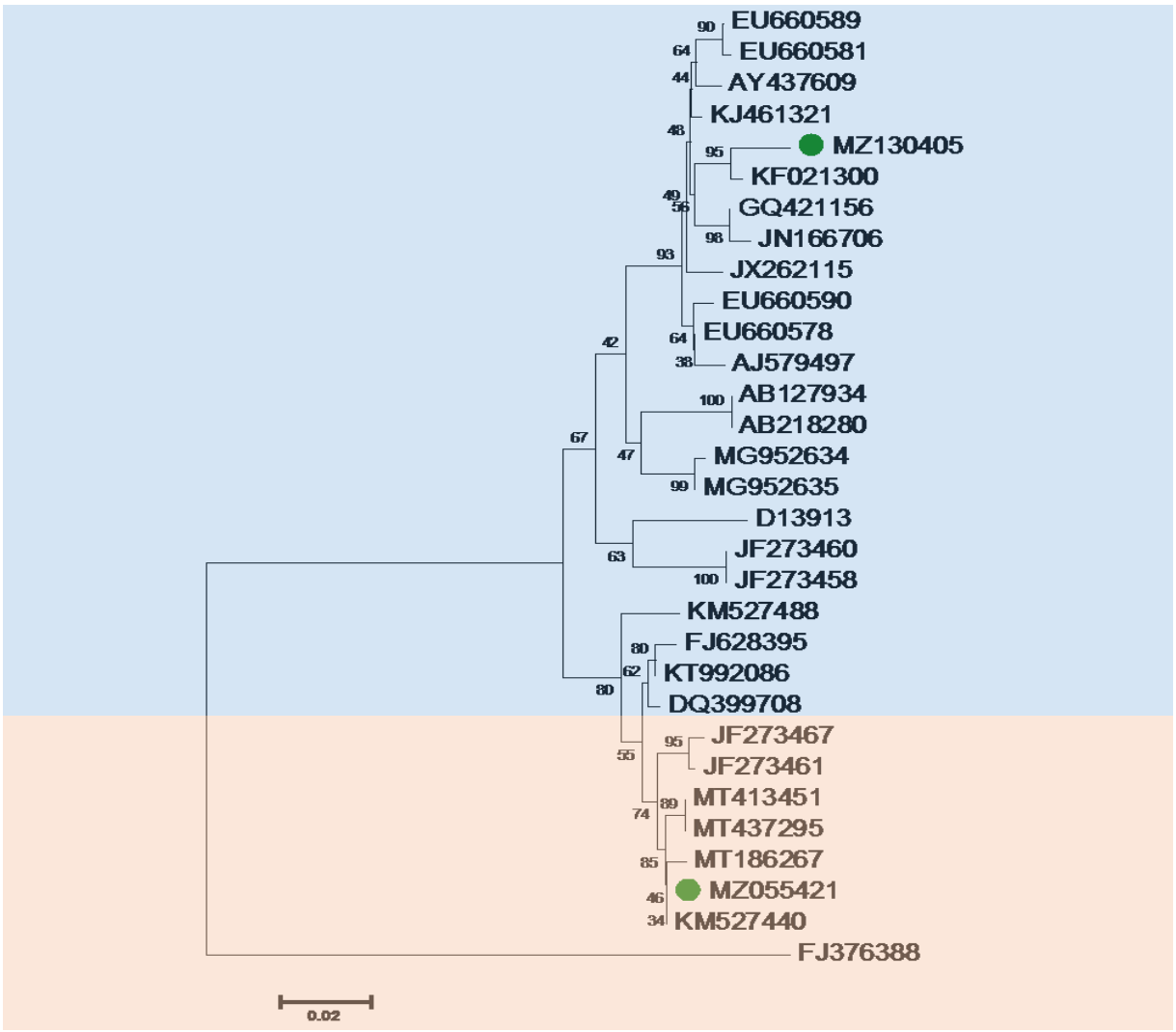

Figure 5. Phylogenetic dendrogram constructed with WMV-Iğdır 7-6 isolates and the other world isolates, retrieved from NCBI, formed by the neighbour-joining algorithm. Iğdır 7-6WMV isolates (MZ130405 and MZ055421) are indicated with a green circles. A Soybean mosaic virus (FJ376388) was used as an outgroup to root the tree. Bootstrap scores are indicated on each branch.

Differential clustering of two same-length WMV sequences may be due to recombination, which poses a new agricultural threat in natural populations of WMV (Desbiez et al., 2011). Recombination factor is one of the main evolutionary mechanism creating genetic variability in plant virus populations. In recent years, unpredictable recombination has the potential to introduce new viral strains that are particularly damaging to crops (Garcia-Andres et al, 2007). Recombination rates are significantly variable in plant RNA viruses. This may be the error-prone nature of the RNA polymerase, or the presence or absence of recombination-associated active sequences (Nagy, 2008). The increase in viral sequences in the GenBank and the advances in sequence analysis methods revealed that recombination events can be observed in many viral families in natural conditions, especially in the *Potyvirus* genus, of which WMV is a member (Tan et al., 2004; Chare and Holmes, 2006). Recombination events depending on the CP gene defined 3 WMV molecular groups: classic (CL or group 1), group 2 (G2), and emerging (EM) which includes 4 groups (EM1-EM4) (Desbiez and Lecoq, 2008). Indeed, some studies conducted in France, Argentina, Iran, and Spain have revealed the presence of recombination in some isolates of the WMV population (Moreno et al., 2004; Desbiez and Lecoq, 2008; Desbiez et al., 2007, 2011; Glasa et al., 2011; Pozzi et al., 2020). Further studies are needed to determine whether Iğdır-WMV isolates are recombinant isolates.

In Silico analyisis of Iğdır-WMV and CMV isolates

It was determined that other characteristics of coat protein of virus isolates (MZ130405 and MZ055421) were the same except molecular weight, Instability index (II), Aliphatic index Grand average of hydropathicity according to Protparam result. The pI above 7 demontrates that the protein will precipitate in basic buffers. Therefore, WMV coat protein precipitates in acidic buffer, while CMV coat protein precipitates in basic buffer. According to the instability index (II), WMV coat protein is stable, while CMV coat protein is not (Table 5). Grand average of hydropathicity (GRAVY) score of QWN55349.1, QWN55348.1 and QWN55360.1 have negative value, so this illustrated that coat proteins of WMV and CMV in the current study are hydrophilic and soluble in nature.

Table 5. Physiochemical properties of coat protein of virus isolates obtained from current study

Protein properties	**Accesion number (NCBI) of coat protein**		
	QWN55349.1 (WMV isolate Iğdır7)	**QWN55348.1 (WMV isolate Iğdır 6)**	**QWN55360.1 (CMV isolate Iğdır 2)**
Number of amino acids	281	281	218
PI	6.54	6.54	9.95
Total number of negativelycharged residues (Asp+Glu)	37	37	21
Total number of positively chargedresidues (Arg+Lys)	36	36	33
Molecular weight	31408.38	31387.45	24081.47
Instability index (II)	29.48	31.82	48.82
Aliphatic index	68.01	70.11	81.83
Extinction coefficient	29910	29910	17420
Estimated half-life	1.9 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli*, in vivo). (N-terminal ofthe sequence considered is M (Met))	1.9 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli*, in vivo). (The N-terminal of the sequence considered is S (Ser)).	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli*, in vivo). (N-terminal ofthe sequence considered is M (Met))
GRAVY	-0.720	-0.687	-0.381

3D structure of coat protein of virus isolates in this study was predicted by using homologymodeling (Fig. 6). 3D structure of proteins contributes valued comprehension into it's molecular function. The 3D structure information of the protein is critical in protein research such as understanding the function of an unknown protein, as well as enzyme kinetics, and ligand-protein interaction. According to the homology modeling analysis in Phyre server, the 3D structure of coat protein of WMV Iğdır 7 and WMV Iğdır 6 isolates showed 100% similarity with the c5odvB template and was created by aligning 207 residues.

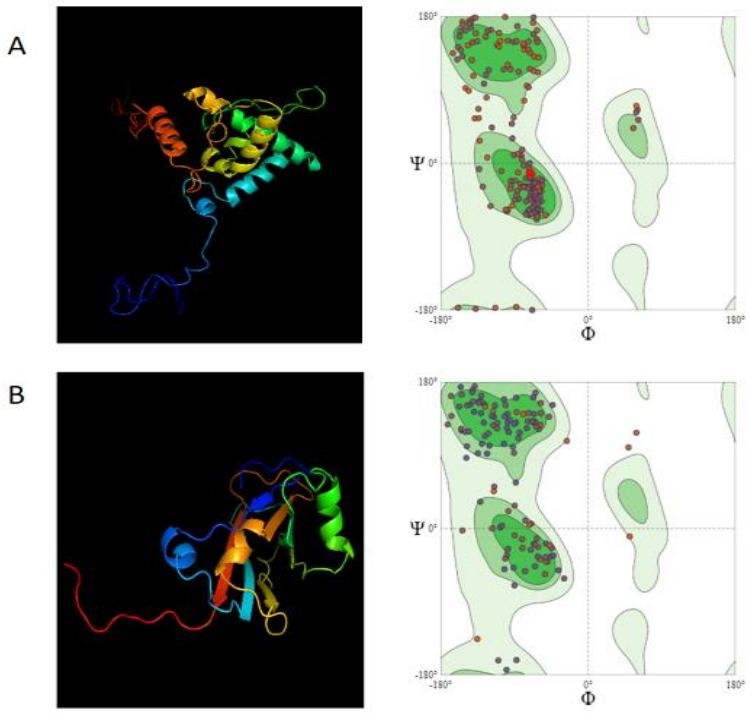

Figure 6. The 3D models existing as ribbon structure and Ramachandran Plots of coat protein of WMV and CMV virus isolates. A) 3D structure and Ramachandran Plot of coat protein of WMV Iğdır 7 and WMV Iğdır 6 isolates. B) 3D structure and Ramachandran Plot of coat protein of CMV Iğdır 2

The 3D structure of the coat protein of the CMV ığdır 2 isolate was also obtained in Phyre server with 100% similarity with the c1f15C template and 142 residue alignment. After obtaining pdb files of the proteins from the same server, the 3D structure was visualized using PyMOL. The illustrated model was confirmed with Ramachandran plot as shown in Figure 6. While the Ramachandran plot value of the coat protein of WMV iğdır 7 and WMV Iğdır 6 isolates calculated as 86.8%, the value of the CMV ığdır 2 isolate found as 80%. The Ramachandran plot analysis verified the good quality of model for coat proteins.

CONCLUSIONS and RECOMMENDATIONS

This study is the first report on CMV and WMV infection in the melon and watermelon province of Iğdır. Molecular characterization studies revealed that Iğdır-CMV melon isolate was in subgroup IB. Iğdır-WMV melon and watermelon isolates showed phylogenetic similarity with isolates from different countries in different hosts. However, there is a need for a more comprehensive survey study with plant materials obtained from larger areas to investigate other potential cucurbit viruses in this region.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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