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Prevalence and molecular characterization of Cucumber mosaic virus isolates infecting tomato plants in Marmara region of Turkey

Marmara Bölgesi domates üretim alanlarında Cucumber mosaic virus izolatlarının yaygınlığı ve moleküler karakterizasyonu

Ali KARANFİL[a*]

[a]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, 17100 Canakkale, Turkeyy

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* Corresponding author: Ali KARANFİL
ali.karanfil@hotmail.com

ABSTRACT

There are many studies carried out on tomato viruses in Turkey. However, there is no study on the prevalence and genetic diversity of *Cucumber mosaic virus* (CMV), one of the most important viral diseases of tomatoes. In this context, surveys were conducted in the tomato production areas of the Marmara region, and samples were taken from 113 tomato plants showing virus and virus-like symptoms, and tests were carried out to determine CMV infection by DAS-ELISA. As a result of the tests, CMV infection was detected in 34 plants. And, 10 CMV isolates were selected for further studies. Coat protein (CP) and movement protein (MP) genes of selected isolates were amplified by RT-PCR and sequenced. As a result of multiple sequence analysis, CMV isolates from the Marmara region showed 97-100% similarity in nucleotide and amino acid levels within themselves, 77-100% in nucleotide level, and 79-100% in amino acid level in the world isolates according to the CP gene. According to the MP gene region, it was determined that the CMV isolates showed 97-100% and 96-100% similarities at the nucleotide and amino acid levels in each other, respectively. The similarity rates with world isolates were determined as 79-100% at the nucleotide level and 81-100% at the amino acid level. As a result of the phylogenetic analyses performed, tomato CMV isolates were closely related to each other according to both gene regions and were in the Ia subgroup.

INTRODUCTION

Cucumber mosaic virus (CMV) was first reported on the cucumber (*Cucumis sativus* L.) plant by Price in the USA in 1934. CMV is a viral agent belonging to the *cucumovirus* genus of the *Bromoviridae* family. The host range of the causal agent is probably the largest among plant viruses. CMV can infect more than 1200 plants belonging to 500 genera from 100 families (Jacquemond 2012, Zitter and Murphy 2009). The causal agent can be transmitted mechanically by plant sap, non-persistent by aphids, and by seeds (Palukaitis and Garcia-Arenal 2003, Palukaitis et al. 1992).

CMV has a positive susceptibility single-stranded and triple-segmented RNA genome. Of these RNA segments, RNA1 and RNA2 contain complex genes related to replicase

and movement, while RNA3 encodes movement (MP) and coat (CP) protein genes (Palukaitis and Garcia-Arenal 2003, Zitter and Murphy 2009). It has also been reported that the agent is transmitted by more than 75 aphid species (Palukaitis et al. 1992).

CMV, one of the most important viral diseases of tomatoes, has been reported to be divided into 2 groups based on their serological relationships and genetic diversity rates. In addition, these two groups are symbolized as I and II (Palukaitis et al. 1992). As a result of the studies carried out in the world in recent years, it was stated that subgroup I was divided into two as Ia and Ib, with the analysis of the CP gene and non-protein-coding regions at the 5' end of the agent (Roossinck et al. 1999).

It has been reported that CMV infections in different hosts have been detected previously in Turkey (Balsak et al. 2021, Güneş and Gümüş 2019, Kurtoğlu and Korkmaz 2018, Özdemir and Erilmez 2012, Usta et al. 2020). In a limited number of studies, it was stated that subgroup IA isolates are commonly seen as a result of molecular studies performed with CMV (Çaglar 2006, Ergün et al. 2013, Ohshima et al. 2016). In recent years, the presence of group II and subgroup IB has been reported in Turkey (Karanfil and Korkmaz 2017, Sarı 2015). However, although it has been reported that CMV infection is frequently detected in tomato production areas of our country, the genetic diversity of these isolates is not known.

In this context, no research has been conducted on the identification and genetic diversity of CMV in the tomato production areas of Çanakkale, Balıkesir, Bursa, Tekirdağ, and Edirne provinces in the Marmara region of Turkey. For this purpose, the presence and genetic diversity of CMV were investigated by taking samples from plants showing virus and virus-like symptoms from the specified areas.

MATERIALS AND METHODS

Field and virus diagnostic studies

Field studies were carried out in the tomato production areas of Çanakkale, Balıkesir, Bursa, Tekirdağ, and Edirne provinces and their districts in 2020-2021. During the production season, the plants were visually examined by making land exits to the tomato production areas, and samples were taken from the plants showing viral disease-like symptoms. The selection of production areas were made randomly. The collected samples were brought to the laboratory in the cold chain in silica gels and stored at 4°C for further analysis.

The presence of CMV in the collected samples was determined by DAS-ELISA test. For this purpose, tests were carried out in line with the recommendations of the company from which the ELISA kits were purchased, based on the method specified by Clark and Adams (1977) (Bioreba, Switzerland).

Molecular characterization studies

As a result of DAS-ELISA tests, a total of 10 isolates, two isolates for each province, were selected among the isolates infected with CMV, considering their geographical origins, and were used within the scope of molecular characterization studies. Molecular characterization studies were performed based on the coat protein (CP) and movement protein (MP) gene regions of the isolates.

Reverse transcriptase-polymerase chain reaction

Total RNA was first isolated from the selected samples, which were found to be infected with CMV, using the CTAB method (Li et al. 2008). Total RNAs obtained were first synthesized as complimentary DNA (cDNA) for PCR studies using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). Using the obtained cDNAs, the CP and MP genes of the CMV isolates were amplified in 2X Emerald Master Mix (Takara, Japan) and MJ Mini Thermal Cycler (Biorad, USA) using the primer pairs in Table 1.

Sequencing studies

RT-PCR products containing the amplified CP and MP genes were directly sequenced in a bidirectional manner by purchasing service from BM Labosis (Ankara, Turkey). The obtained raw sequence data were assembled in the CLC Main Workbench V.20 program and deposited into the GenBank (Table 2).

Table 1. Primer pairs used in molecular characterization of *Cucumber mosaic virus* isolates

Code	Primer Sequence	Sense	Target Gene	Amplicon Size	Reference
CMV_ MPF	ATGGCTTTCCAAGGTACCAG	Forward	Movement Protein	1165 bp	Karanfil and Korkmaz 2021
CMV_MPR	YAGCAYTGGGAGATYCAGA	Reverse			
CMV_CPF	ATGGACAAATCTGAATCAACC	Forward	Coat Protein	638 bp	Karanfil and Korkmaz 2017
CMV_CPR	GATGTGGGAATGCGTTGGTGC	Reverse			

Table 2. Information of Turkish tomato *Cucumber mosaic virus* isolates obtained in this study

Isolate Name	Origin	GenBank Accession Number	
		CP	MP
CNK-3	Çanakkale	MZ711448	MZ711458
CNK-13		MZ711449	MZ711459
BLK-27	Balıkesir	MZ711450	MZ711460
BLK-38		MZ711451	MZ711461
BRS-51	Bursa	MZ711452	MZ711462
BRS-63		MZ711453	MZ711463
TKR-72	Tekirdağ	MZ711454	MZ711464
TKR-81		MZ711455	MZ711465
EDR-91	Edirne	MZ711456	MZ711466
EDR-97		MZ711457	MZ711467

Based on the nucleotide (nt) and amino acid (aa) sequences contained in the CP and MP genes of the CMV isolates selected using the consensus sequences obtained, the similarity ratios of the isolates with the isolates obtained from different countries of the world, retrieved from the GenBank (Table 3), were determined in the Sequence Demarcation Tool V 1.2 program (Muhire et al. 2014). Phylogenetic relationships of the selected isolates were determined in CLC Main Workbench V. 20.

Table 3.

RESULTS AND DISCUSSION

As a result of the field studies, a total of 113 samples showing virus and virus-like symptoms were collected from tomato production areas in the Marmara region. The distribution of the collected samples according to the provinces was obtained by taking 22 samples from Çanakkale, 24 samples from Bursa, 23 samples from Balıkesir, 25 samples from Tekirdağ, and 19 samples from Edirne.

As a result of testing the collected 113 samples with the DAS-ELISA test, 34 samples were found to be infected with CMV. The distribution of infected sample numbers based on the provinces was determined as 8 for Çanakkale, 5 for Balıkesir, 7 for Bursa, 8 for Edirne, and 6 for Tekirdağ. With this result, the infection rate in the collected samples was determined as 30.08%. While the highest infection rate was found in the province of Edirne with 42.10%, the lowest infection rate was obtained from the province of Balıkesir with 21.73% (Table 4).

In a study conducted in Çanakkale, Bilecik, and Bursa provinces of the Marmara region in 2013, researchers determined that 67 of the 77 samples were infected with CMV and reported the infection rate as 87% (Uzunoğulları and Gümüş 2015). In a study conducted in the West-Mediterranean region, it was reported that 53 (38.40%) of the 138 tomato samples collected were infected with CMV (Yardimci and Eryigit 2006). It is thought that the different infection rates obtained between studies vary in parallel with the year of sampling, the availability of aphid vectors, and the sanitation measures applied.

Table 4. The numbers of virus infected and collected samples and infection rate of *Cucumber mosaic virus* in the collected tomato samples from Marmara region of Turkey

Province	Infected sample	Collected sample	Infection rate (%)
Çanakkale	8	22	36.36
Balıkesir	5	23	21.73
Bursa	7	24	29.16
Tekirdağ	8	19	42.10
Edirne	6	25	24.00
Total	34	113	30.08

Although all the samples collected within the scope of this study showed very typical virus and virus-like symptoms, the presence of CMV infection in 69.92% of the collected samples strengthens the possibility of at least one viral disease infection in these collected samples. As a matter of fact, the reports of infections of some begomovirus species (Sertkaya and Yılmaz 2017, Yilmaz and Sipahioglu 2020), *Tobacco brown rugose fruit virus* (Fidan et al. 2019), *Tomato*

Table 3. The world *Cucumber mosaic virus* isolates used for references in molecular characterization studies

Isolate Name	Origin	Phylogenetic Group	GenBank Accession Number
IRN-REY4	Iran	Ia	LC066467
IRN-REY10	Iran		LC066473
TUR-54	Turkey		LC066503
TUR-81	Turkey		LC066506
Ixora	USA	Ib	U20219
CTL	China		EF213025
Nt9	Taiwan		D28780
YN	China		EF216865
LBO	The Netherlands	II	AJ304397
Hnt	China		KC407999
Palampur	India		HE583224
MT	Japan		AB189917

chlorosis virus (Yeşilyurt and Çevik 2019), and *Tomato spotted wilt virus* (Çulal-Kılıc et al. 2017) as a result of studies carried out in the tomato production areas of our country in recent years also confirm this idea.

As a result of the molecular characterization studies, it was determined that CMV isolates from the Marmara region showed 97-100% similarity with each other at the level of nucleotides and amino acids according to the CP gene region. As a result of the comparisons made with world isolates according to this gene region, it was determined that the isolates showed 77-100% similarity with each other at the nucleotide level and 79-100% at the amino acid level (Figure 1).

The similarity analyses of CMV isolates from the Marmara region based on the MP gene region, it was determined that the isolates showed similarities between themselves at the rate of 97-100% at the nucleotide level and 96-100% at the amino acid level. The similarity rates of CMV isolates from the Marmara region with the world isolates according to the MP gene region were determined as 79-100% at the nucleotide level and 81-100% at the amino acid level (Figure 2).

As a result of the studies carried out previously on the molecular characterization of CMV isolates in our country, it was stated that the CMV isolates in our country were generally 80-100% similar to the world isolates (Güneş and Gümüş 2019, Karanfil

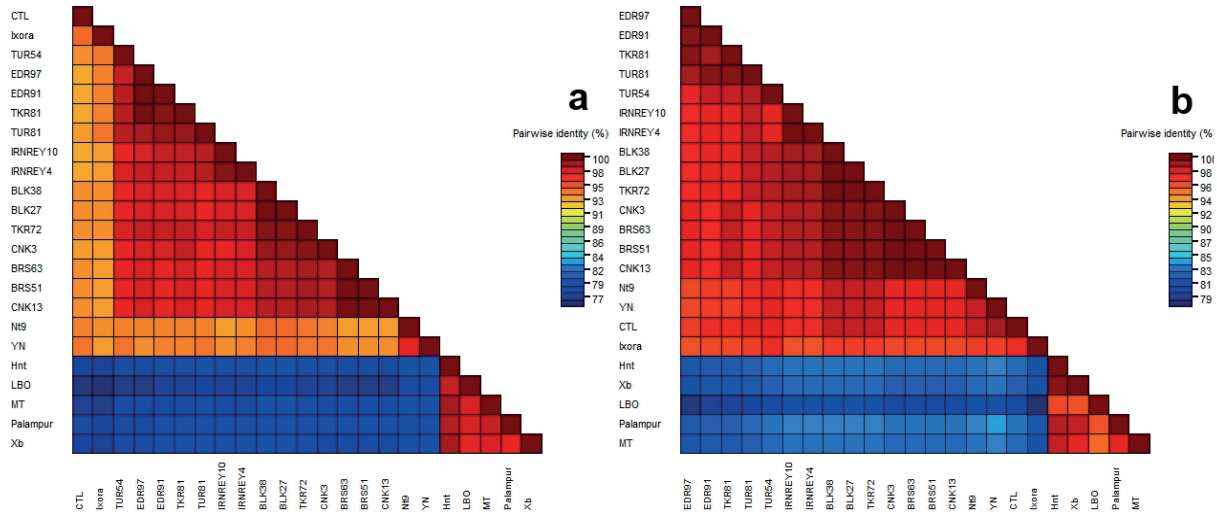

Figure 1. Similarity rates of *Cucumber mosaic virus* isolates infecting tomato plants from Marmara region of Turkey at the nucleotide (a) and amino acid (b) levels based on the coat protein gene region

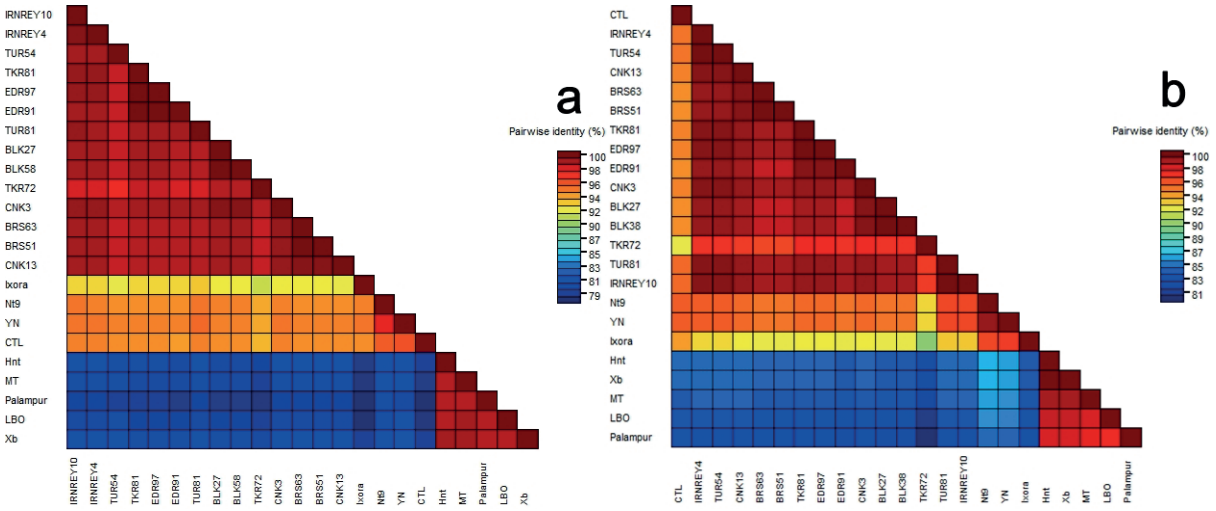

Figure 2. Similarity rates of *Cucumber mosaic virus* isolates infecting tomato plants from Marmara region of Turkey at the nucleotide (a) and amino acid (b) levels based on the movement protein gene region

and Korkmaz 2017). In this context, the results obtained within the scope of this study show parallelism with previous studies.

In addition, in general, it is seen that 3 colours are dominant in nt-based similarity matrices and 2 colours are dominant in aa-based similarity matrices. In the nucleotide-based similarity matrix, it is seen that the CMV subgroups IA, IB, and II are clearly separated, while in the aa-based similarity matrix, IA and IB converge to each other in terms of sequence similarity due to the increasing similarity rates, and the matrices are divided into I and II (Figure 1 and 2).

As a result of the phylogenetic analyses, it was determined that the selected tomato CMV isolates were in subgroup IA according to both CP and MP gene regions (Figure 3 and 4).

As a result of previous studies with CMV in different hosts in our country, it has been reported that subgroup IA isolates are common (Karanfil and Korkmaz 2021, Ohshima et al. 2016). In addition, the existence of subgroup IB and II isolates is also known in our country (Karanfil and Korkmaz 2017, Sarı 2015). However, the presence of tomato CMV isolate as subgroup IA within the scope of a study carried

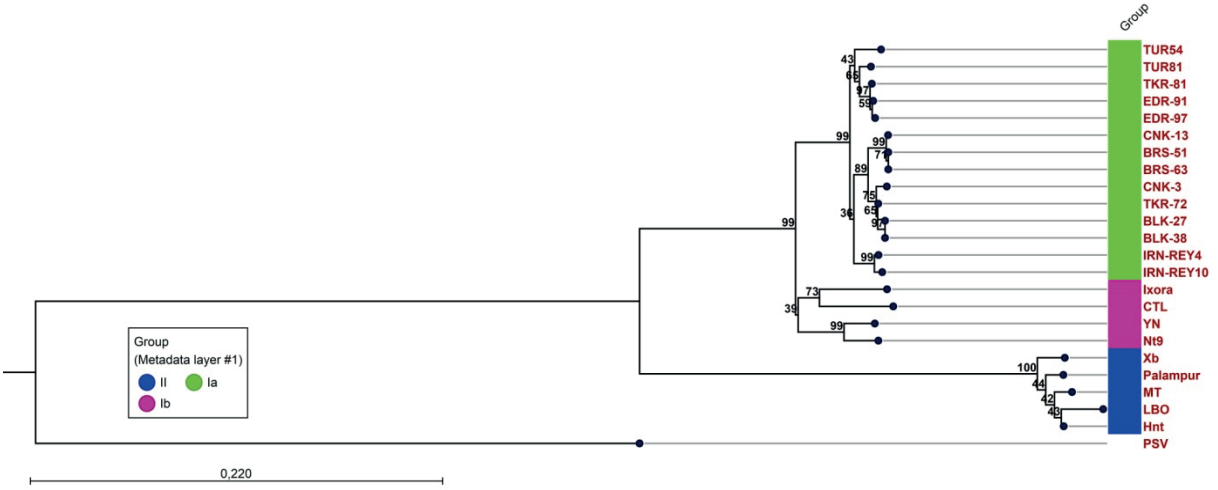

Figure 3. The phylogenetic tree of *Cucumber mosaic virus* isolates based on the coat protein gene region [The phylogenetic tree was constructed by the maximum likelihood method using HKY+G+T parameters and Peanut stunt virus (PSV) was used an out-group (Genbank accession no: JN135292)]

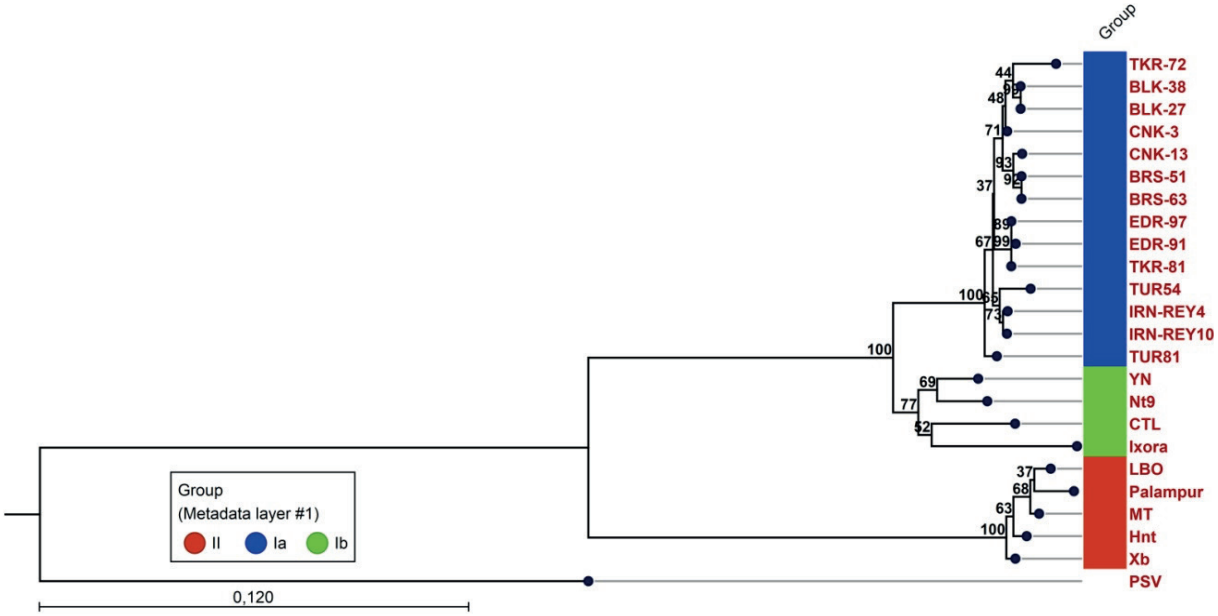

Figure 4. The phylogenetic tree of Cucumber mosaic virus isolates based on the movement protein gene region [The phylogenetic tree was constructed by the maximum likelihood method using HKY+G+T parameters and Peanut stunt virus (PSV) was used an out-group (Genbank accession no: JN135292)]

out in the Mediterranean region in our country (Caglar 2006), leads to the conclusion that CMV isolates that cause infection in tomato production areas of our country are predominantly Ia group. In addition, it was determined that CMV isolates in the Marmara region were identified as Ia group with this study, it is thought that geographical origins may affect the phylogenetic groups although it was reported that geographical origins are not very dominant in the phylogenetic classification of CMV isolates and that isolates from the same region may show the distribution in different phylogenetic groups (Ohshima et al. 2016). With this study, the molecular characterization of CMV isolates obtained from tomato production areas for the first time in Turkey was carried out according to two different gene regions. In addition, by depositing the sequences of the CP and MP genes of tomato CMV isolates to the GenBank, it was ensured that tomato CMV isolates originating in our country were included in the GenBank for the first time. Although the rate of CMV infection was found to be low in tomato production areas in the study area, it is thought that the genetic diversity of all segments of the isolates obtained by CMV surveys to be carried out at a national level should be determined. Thus, molecular population structures of CMV isolates in tomato production areas of Turkey will be able to be fully revealed.

ÖZET

Türkiye'de domates virüsleri ile ilgili olarak gerçekleştirilmiş çok sayıda çalışma vardır. Ancak domatesin en önemli virüs hastalıklarından bir tanesi olan Cucumber mosaic virus (CMV)'nin geniş alanlarda yaygınlığı ve genetik çeşitliliği üzerine gerçekleştirilmiş bir çalışma bulunmamaktadır. Bu bağlamda Marmara Bölgesi domates üretim alanlarında sürveyler düzenlenerek virüs ve virüs benzeri simptom gösteren 113 domates bitkisinden örnekler alınarak DAS-ELISA testi ile CMV enfeksiyonunun belirlenmesi amacı ile testlemeler gerçekleştirilmiştir. Gerçekleştirilen testlemeler sonucunda 34 bitkide CMV enfeksiyonu tespit edilmiştir. Enfekteli örnekler içerisinden elde edildikleri iller temel alınarak 10 CMV izolatı moleküler karakterizasyon çalışmaları için seçilmiştir. Seçilen bu izolatların kılıf protein (CP) ve hareket protein (MP) genleri RT-PCR ile amplifiye edilmiş ve sekanslanmıştır. Elde edilen sekanslar kullanılarak gerçekleştirilen çoklu dizi analiz çalışmaları sonucunda, Marmara Bölgesi CMV izolatlarının CP genine göre kendi içlerinde nükleotid ve amino asit düzeyinde %97-100, dünya izolatları ile nükleotid düzeyinde %77-100, amino asit düzeyinde ise %79-100 oranlarında benzerlikler gösterdiği tespit edilmiştir. MP gen bölgesine göre ise CMV izolatlarının kendi içlerinde nükleotid düzeyinde %97-100, amino asit düzeyinde ise %96-100 oranlarında benzerlikler gösterdiği belirlenmiştir, dünya izolatları ile gösterdikleri benzerlik oranları ise nükleotid düzeyinde %79-100, amino asit düzeyinde ise %81-100 olarak belirlenmiştir. Gerçekleştirilen filogenetik analizler sonucunda Marmara Bölgesi CMV izolatlarının hem CP hem de MP gen bölgesine göre birbirleri ile yakın ilişkili olduğu ve Ia altgrubunda olduğu belirlenmiştir.

Anahtar kelimeler: CMV, DAS-ELISA, RT-PCR, sekanslama

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