Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genetic Diversity of CMV and ToMV Isolates in Tomato and Pepper Production Areas in Hakkari, Turkey

Nevin AKDURA (nevinakdura@hakkari.edu.tr)
Hakkari University: Hakkari Universitesi https://orcid.org/0000-0001-6162-0500
Handan ÇULAL KILIÇ
Isparta Uygulamali Bilimler Universitesi

Research Article

Keywords: CMV, ToMV, DAS-ELISA, phylogenetic analysis, Hakkari

Posted Date: March 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1451598/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Background Tomato (*Lycopersicum esculentum* Mill.) and pepper (*Capsicum anuum* L.) are the host species for the Cucumber mosaic virus (CMV) and Tomato mosaic virus (ToMV). This article aims to report the detection of serolocigally and molecularly and identification of genetic diversity these viruses in Hakkari province, Turkey.

Methods and results A total of 184 leaf samples were collected from tomato and pepper plants that showed virus-like symptoms. Sample collection was carried out in Hakkari province in October, 2020. CMV and ToMV factors were investigated by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). DAS-ELISA test showed that 106 (57.60%) of the samples were infected with one or more viruses. For phylogenetic analyses, 10 samples which were positive for CMV and ToMV were tested using Reverse Transcription Polymerase Chain Reaction (RT-PCR) method. Coat protein (CP) and replicase genes of Hakkari CMV and ToMV isolates were amplified by RT-PCR using specific primers. The resulting DNA sequences were deposited in NCBI GenBank (Accession Nos: OM286731, OM286732, OM286733, OM286734, OM418629). When the replicase and coat protein gene sequences of four ToMV isolates and one CMV isolate were compared with the isolates in GenBank, a nucleotide homology of 98.1-99.6% and 91.7-99.4% were found, respectively. Phylogenetic analysis revealed a strong similarity between Hakkari ToMV and CMV isolates and other Turkish isolates. Consistent with the molecular studies conducted in Turkey on CMV and ToMV, the isolates detected in this study also clustered in subgroup Ia.

Conclusions With this study, CMV and ToMV factors were serologically and molecularly detected for the first time in Hakkari and the first nation-wide report for ToMV replicase sequences in infected tomato and peppers were submitted.

Introduction

In tomato and pepper production areas, various fungal, bacterial and viral diseased lead to severe losses during the period between seedling and harvest. CMV and ToMV are the most important viruses encountered in tomato production areas [1, 2]. CMV, which was first reported in the United States of America in 1934 by Price, belongs to the *Cucumovirus* genus in the family *Bromoviridae*. It has an isometric structure and a diameter of 29 nm. It has a linear genome composed of single-stranded RNA [3, 4]. CMV can infect more than 1200 different plants belonging to 500 genera from 100 families and is non-persistently transmitted by more than 60 aphid species belonging to the family of *Aphididae* [3, 5, 6, 7]. It can also be transmitted through mechanical inoculation and in seeds. In infected plants, CMV leads to mosaics, leaf and fruit distortion, and even to plant death [8]. It is serologically categorized in subgroups I and II and in the phylogenetic analysis of the coat protein, it clusters in the subgroup Ia [9]. CMV subgroups Ia and II can be found worldwide [10].

Tobamovirus is the largest genus in the family *Virgaviridae* [11, 12]. Belonging to this genus, ToMV is a disease agent with a linear single-stranded RNA genome and has rod-shaped virions. It has a wide host range including members of the family *Solanaceae* such as tomato and pepper and it causes significant crop losses in tomato plants worldwide [2, 12, 13, 14, 15, 16]. It is the most persistent virus in terms of its ability to survive outside of plant cells or in dead tissues [17]. ToMV is known to decrease tomato production by up to 20% [1, 2]. ToMV causes mosaic, necrosis and senescence in pepper leaves, and light or dark green mosaic and distortion in tomato leaves. ToMV infection has also been detected in tomato and pepper seeds [14, 15]. There is a number of methods employed to manage tobamoviruses, including using resistant varieties, and resorting to biological or chemical control. It is difficult to control the spread of ToMV as it can be transmitted in seeds or through mechanical inoculation, contaminated tools or workers [2, 12, 13]. Within the three pathological groups of different ToMV populations, global ToMV isolates in Group I mostly infected solanaceous host species tested while the isolates in Group II did also infect other plants [18, 19].

Although CMV and ToMV are common in tomato and pepper production areas in our country, information about the diversity of these viruses is highly limited. The aim of this 2020 study is to demonstrate the genetic relationship and diversity of the coat protein (CP) and replicase genes of CMV and ToMV present in our country. To that end, viruses were detected by DAS-ELISA method and selected samples were used in molecular studies conducted by using RT-PCR test. In this study, researchers also tried to determine the phylogenetic diversity of CP and replicase genes of CMV and ToMV isolates from tomato and

pepper. This is, to our knowledge, the second phylogenetic analysis conducted in our country on CMV and ToMV isolates from tomato and pepper production areas.

Materials And Methods

Field surveys

During the field surveys carried out in 10 villages of Hakkari province (Durankaya, Geçitli, Kırıkdağ, Otluca, Üzümcü, and Çimenli villages in the city center of Hakkari; and Geçimli, Taşbaşı, Doğanlı, and Gelinli villages in Çukurca district) in October 2020, 184 leaf samples were collected from tomato (94 samples) and pepper (90 samples) plants that showed symptoms like mosaic, deformation, warty formations, local necrotic lesions, growth reduction, vein banding, wilting, chlorosis and fruit distortion. The areas to be surveyed were selected according to the intensity of vegetable production. Leaf samples were taken from plants showing common symptoms caused by plant viruses, placed in sterile polyethylene bags together with the labels containing information on the land area, brought to the laboratory and stored at -20 ^{o}C for testing.

DAS-ELISA test

184 leaf samples were subjected to DAS-ELISA test. For serological tests, ELISA kits (Bioreba, Switzerland) that are specific to CMV and ToMV were used. The results were evaluated at 405 nm on the Versamax ELISA reader by using positive and negative controls. Samples with an absorbance value of at least twice the mean absorbance value of the negative control were considered positive.

Preparation of primers and total nucleic acid extraction

The primer pairs were synthesized to amplify a 678 bp fragment of CP and a 318 bp fragment of the replicase gene and these were used during PCR. Primers used in the study are shown in Table 1. RNA extraction from leaf samples of tomato and pepper plants were conducted by using the EURX GeneMATRIX Universal RNA Purification Kit (Poland) as specified in the manufacturer's protocol.

RT-PCR test

5 samples were taken from each group of CMV and ToMV positive samples, and CMV CP and ToMV replicase gene were amplified using RT-PCR method. For RT reaction, a mixture of 20 µl was prepared. The mixture consisted of 6 µl of RNA (10 ng-5 µg), 1 µl of primers, 1 µl of dNTP (10 mM), 4 µl of n5 x cDNA Buffer, 2 µl of DTT (0.1 M), 0.5 µl of RNase Inhibitor (50 U/µ), 1 µl of smART (200 U/µl), and PCR-grade water. It was incubated at 50 °C for 45 minutes and at 85°C for 5 minutes in the PCR device. 25 µl PCR reaction consisted of 3,54 µl of PCR buffer, 3,54 µl of MgCl2 (1,5 mM), 0,35 µl dNTP (0,2 mM), 1.77 µl of each primer (0,3 µM), 0,40 µl of Taq DNA Polymerase (2U), 3 µl of DNA template and 20,96 µl of PCR-grade water. PCR was conducted for 45 seconds at 94°C, for 45 seconds at 57°C (annealing) and for 1 minute at 72°C (elongation). 40 cycles were run followed by final extension for 5 minutes at 72°C and the temperature was decreased to 4°C. The resulting PCR products with 100 bp DNA markers were electrophoresed by 1.5% agarose gel and visualized under ultraviolet light using a Transilluminator (White/2UV) (UVP, USA).

Purification of PCR products

RT-PCR amplified DNA was purified with the MAGBIO "HighPrep™ PCR Clean-up System" (AC-60005) in accordance with the manufacturer's instructions.

Sequencing and sequence comparison

The nucleotide sequences of CMV CP and ToMV replicase gene were determined by using ABI 3730XL Sanger sequencer (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA). The obtained DNA sequences were transferred to the BioEdit program and aligned [22]. Hakkari CMV and ToMV isolates were

compared with sequences in the NCBI GenBank database. The similarities of the nucleotide sequences in the replicase genes of Hakkari ToMV isolates were determined by making multiple sequence comparisons in the Clustal W program [23].

Phylogenetic analysis

Phylogenetic analysis was performed to determine the relationship between Hakkari CMV and ToMV isolates and related isolates in the NCBI GenBank database. The partial sequence of the CP and replicase genes of CMV and ToMV isolates was compared using Mega (version X) program [24]. With data obtained from the phylogenetic analysis, a phylogenetic tree was constructed using the neighbor-joining method which is applied to Kimura two-parameter algorithm. 1000 repetitive bootstrap analyses were performed in order to statistically determine the accuracy of the generated parentage [25].

Results

Field observation

Field surveys were conducted in Çukurca district and city center of Hakkari province during the 2020 growing season. Suspicious plants thought to be contaminated with CMV and ToMV were photographed in the field (Fig. 1). Samples were collected in accordance with the common symptoms that are known to be caused by these viral agents on tomato and pepper plants.

DAS-ELISA test

DAS-ELISA test showed that out of 94 tomato samples, 34.04% was infected with CMV, 23.40% was infected with ToMV, and 5.31% had mixed infection of ToMV+CMV. In general, it was found that 62.77% of tomato samples were infected with one or more viruses. The rates of exposure to viral infections in tomato production areas were as follows: 100% in Doğanlı, 85.71% in Çimenli, 83.33% in Geçimli, 76.92% in Kırıkdağ, 50% in Üzümcü, 44% in Otluca and 25% in Gelinli. No infection was found in Durankaya. CMV+ToMV mixed infection was detected in 66.66% of the samples from Doğanlı and 4.76% of the samples from Çimenli.

DAS-ELISA test showed that out of 90 pepper samples, 11.11% was infected with CMV, 18.88% was infected with ToMV, and 1.11% had mixed infection of ToMV+CMV. In general, it was found that 31.11% of pepper samples were infected with one or more viruses. The rates of exposure to viral infections in pepper production areas were as follows: 66.67% in Doğanlı, 41.18% in Çimenli, 33.33% in Gelinli, 28.57% in Üzümcü, and 8.33% in Kırıkdağ. No infection was found in the pepper samples form Durankaya and Geçimli. In Otluca, single infection of CMV and ToMV was observed only and the infection rate was found as 45.83%. The rate of CMV infection was highest in Doğanlı and Gelinli with 33.33%, followed by Üzümcü with 21.42%, Çimenli with 11.76%, Kırıkdağ with 8.33%, and Otluca with 4.16%. No CMV infection was found in Durankaya and Geçimli. The rate of ToMV infection was highest in Otluca with 41.66% and Çimenli with 29.41%. No ToMV infection was found in Gelinli, Kırıkdağ, Durankaya and Geçimli. CMV+ToMV mixed infection was observed only in Doğanlı with a rate of 16.66% (Table 2).

Molecular detection

Total nucleic acids were isolated from 10 samples which were found positive for CMV and ToMV. Then these samples were tested for the presence of viral agents along with negative controls using RT-PCR. 678 bp and 318 bp fragments of CMV and ToMV CP and replicase genes were amplified (Fig. 2a, b). Results of these tests have definitely proven the existence of these agents in Hakkari.

Sequencing and phylogeny

DNA sequences of one tomato isolate for CMV and one pepper and three tomato isolates for ToMV were deposited in NCBI GenBank with accession numbers OM418629, OM286731, OM286732, OM286733 and OM286734. Hakkari CMV and ToMV isolates were named as NHP1, NHT1, NHT2, NHT3 and NHD1.

282 bp and 264 bp regions of the CP and replicase genes of Hakkari CMV and ToMV isolates were compared with the appropriate regions of the other CMV and ToMV isolates available in the NCBI GenBank database in order to determine the genetic diversity of Hakkari CMV and ToMV isolates. Partial replicase gene sequences of four Hakkari ToMV isolates were highly conserved with sequence identity of 99.6%-100% at the nucleotide level, respectively. A low variation (up to 0.4%) was found among the four Turkish isolates.

In order to determine the similarity in DNA sequence information, 21 CMV isolates deposited in NCBI GenBank from different countries including 2 whole genome, 5 partial genome and 14 coat protein gene sequences [China (Accession Nos: AJ575589, AJ575589), USA (Accession Nos: AJ271416, U20219), Hungary (Accession Nos: AJ511990, AJ517802), China (Accession No: AJ239098), Australia (Accession No: AJ585518), Germany (Accession Nos: AJ810262, AJ810263), Spain (Accession No: AJ829776), Vietnam (Accession No: AM048831), India (Accession No: DQ285569), Japan (Accession Nos: LC066467, LC066473), Korea (Accession Nos: MG251397, MG251398), Turkey (Accession Nos: MH426932, MZ711455, MZ711456, MZ711457), Italy (Accession No: Y10886)], were compared with Hakkari CMV (Accession No: OM418629) isolate (Table 3).

The sequences of the Hakkari CMV isolate and 21 international isolates were 91.7% to 99.4% identical in the nucleotide sequence of the partial coat protein gene. Thus, it was determined that the partial CP gene of the CMV isolate had a maximum sequence difference of 8.3%.

When the nucleotide sequence similarities were examined, it was seen that the maximum nucleotide sequence identities of Hakkari CMV strain was with isolates from Turkey (Accession Nos: MZ711455, MZ711456, MZ711456) with a rate of 99.2%-99.4%. The minimum nucleotide sequence similarity, on the other hand, was with isolates from Germany (Accession Nos: AJ810262, AJ810263) with a similarity rate of 91.7%-91.9%.

Phylogenetic analyses showed that CMV isolates are categorized in two major groups (I and II) according to their coat protein genes. These two groups are also categorized within themselves (Ia, Ib, IIa, IIb). When we examined the first major group according to the origins of the isolates, we saw that the Turkey strain in this study (Accession No: OM418629) clustered in the first group together with Korea (Accession Nos: MG251397, MG251398), Vietnam (Accession No: AM048831) and USA (Accession No: AJ271416) strains and in the subgroup Ia together with Turkey (Accession Nos: MZ711455, MZ711456, MZ711457), Iran (Accession Nos: LC066467, LC066473), India (Accession No: DQ285569) and USA (Accession No: U20219) strains. In the second major group, another strain from our country, Turkey (MH426932), was in the same subgroup (subgroup IIb) together with Spain (Accession No: AJ829776), Australia (Accession Nos: AJ517802, AJ585518), and Hungary (Accession No: AJ517802) strains.

The branches of the phylogenetic trees created were supported with bootstrap values ranging from 20-100, showing that the evaluations were not random and that most of the branches were statistically supported. The phylogenetic tree of Hakkari NHD1 isolate, which was constructed in accordance with the nucleic acid sequence of the coat protein genes of CMV isolates around the world, is shown in Fig. 3.

In NCBI GenBank, 29 different ToMV isolates were present including 21 whole genome, 3 partial genome and 5 replicase gene sequences. In order to determine the similarity in DNA sequence information, these 29 ToMV isolates from different countries [Japan (Accession Nos: AB083196, AB355139), Australia (Accession No: AF332868), Kazakhstan (Accession No: AJ243571), China (Accession No: FN985165, KY967219, KY967222, KY967225, KY967228, MF002479, MF002480, MF002490), USA (KR537870), Egypt (Accession No: KU321698), South Africa (Accession No: KX711903), Slovakia (Accession No: KY912162), Uganda (Accession No: MG456601), India (Accession No: MH038047), Korea (Accession Nos: MH063883, MH063884, MH063885, MH393621, MH393622, MH393623), South Korea (Accession Nos: MH507165, MH507166), Turkey (Accession No: MK652756), Slovenia (Accession No: MN267904), Russia (Accession No: Z92909)] were compared with the Hakkari ToMV isolates (Table 4).

Hakkari ToMV isolates and 29 international ToMV isolates were found to be 98.1% to 99.6% identical in the nucleotide sequence of the partial replicase gene. Thus, it was determined that the partial replicase gene of the Turkish CMV isolates had

a sequence difference of 1.9%.

When the nucleotide sequence similarities were examined, it was seen that the maximum nucleotide sequence identities of Hakkari ToMV strains was with isolates from USA (Accession No: KR537870), Australia (Accession No: AF332868), South Africa (Accession No: KX711903), Kazakhstan (Accession No: AJ243571), Russia (Accession No: Z92909), Slovakia (Accession No: KY912162), Turkey (Accession No: MK652756) and Korea (Accession No: MH393621) with a rate of 99.2%-99.6%. The minimum nucleotide sequence similarity, on the other hand, was with isolates from Korea (Accession No: MH063884), China (Accession No: KY967225) and Japan (Accession No: AB083196) with a similarity rate of 98.1%-98.4%.

Phylogenetic analyses are highly effective in determining genetic relationships between different isolates. Using more and diverse isolates increases the reliability of phylogenetic trees constructed for identification and classification of isolates. Using maximum likelihood method, phylogenetic analyse showed that there are two major phylogenetic clusters (I and II) of the ToMV isolates (Fig. 4). Among these groups, group I is divided into subgroups Ia and Ib. Of these, Group I includes the majority of isolates. ToMV isolates from Turkey are in Group I. A phylogenetic tree based on partial replicase gene sequences revealed that the ToMV isolates used in this study belonged to Group Ia by clustering with USA (Accession No: KR537870) isolate. The second group, on the other hand, included strains from Vietnam (Accession Nos: MH063883, MH063884, MH063885, MH393622, MH393623). The branches of the phylogenetic trees created were supported with bootstrap values ranging from 20-86, showing that the evaluations were not random and that most of the branches were statistically supported.

Discussion

CMV and ToMV are plant viruses that cause diseases in tomato and pepper plants all around the world, leading to economic losses. The presence of CMV and ToMV viral agents in these plants in our country has previously been detected. However, there is no study in the literature on the existence, prevalence and economic impacts of these agents in Hakkari province. In this study, we tried to fill this gap in the literature by conducting a survey study in different regions of Hakkari province and managed to detect for the first time the CMV and ToMV agents in the samples collected from these areas by DAS-ELISA method. We conducted PCR tests and phylogenetic analyses on the samples that were serologically found to be positive for these viruses and found which groups these samples belong to.

However, the number of molecular studies and phylogenetic analyses on CMV is limited. In a study conducted in Çanakkale province, 6 of 22 plants collected from cowpea and bean cultivation areas and subjected to DAS-ELISA test were found to be infected with CMV. CP gene of the CMV isolates was amplified, the RT-PCR products of the selected CMV isolates were purified and partial base sequencing was performed. Phylogenetic analyses determined that CWP17 isolate was in subgroup Ib. Similarly, a close phylogenetic relationship was found between CMV isolates from melon plants in our country and other isolates in subgroup Ib that were obtained from various plant sources in Turkey, Thailand, India and China [38, 39]. In another study, 66 spinach samples were collected from the spinach production areas in Çanakkale province, tested by DAS-ELISA method and CMV was detected in 4 of the samples. One of the infected isolates was randomly selected for molecular characterization and the phylogenetic analysis conducted determined that this isolate was in subgroup Ia [40]. Karanfil [31] conducted survey studies in tomato production areas in Marmara region and applied DAS-ELISA test on 113 tomato plants that showed virus and virus-like symptoms. Of 34 plants that were found to have CMV infection, 10 were selected for amplification of CP and movement protein (MP) genes by RT-PCR. Phylogenetic analyses showed that tomato CMV isolates were closely related for both gene regions. CMV isolates in Marmara region were found to be in subgroup Ia. Molecular studies of CMV showed that isolates in subgroup Ia are more common.

Borah et al. [41] detected the presence of CMV in *Capsicum chinense* Jacq. in India by RT-PCR and prepared the first report on molecular characterization of the virus. Phylogenetic analysis of the 593 bp coat protein gene of CMV demonstrated that the isolate studied was closely related to the African isolates in subgroup Ib and other global isolates. Mishchenko et al. [42] deposited the 443 bp CP gene sequence of the CMV isolate P-EP-Ukr-19, which infects *Echinacea purpurea* (coneflower) plants in Ukraine, in the NCBI GenBank with the accession no. MT978189. Phylogenetic analysis revealed that P-EP-Ukr-19 clustered in subgroup IB with a nucliotide sequence similarity of 93.7–99.7%. In a study conducted in Colombia, leaves of three chili

pepper varieties (tabasco, cayenne and habanero) with viral symptoms were sampled, total RNA was purified and a fragment of CP of CMV was amplified by RT-PCR. It was then sequenced and analysed bioinformatically. 37 of 71 chili pepper samples (52.1%) were found positive for CMV. CMV sequence analysis showed the maximum identity (98.5%) of the isolates in the study was with a CMV isolated from bananas from Ecuador. CP analysis of CMV also demonstrated that it can be transmitted by the species *Aphis gossypii* [43]. CMV is one of the most harmful viruses causing disease outbreaks in agricultural products all around the world [21]. Studies to be conducted in other countries will be helpful in understanding the epidemiology of CMV and in designing an appropriate management program [41].

A study conducted in the Mediterranean region of Turkey showed that tomato CMV isolate clustered in subgroup Ia [44]. Hakkari CMV isolates in the present study also clustered in subgroup Ia. It is suspicious that the CMV isolates from our country cluster in the same group with the isolates from Iran in the phylogenetic classification. However, they also show similarity with the USA isolate, suggesting that geographical origins may not be very important in this case.

Studies conducted in our country on the genetic diversity of ToMV are limited. Yılmaz and Sipahioğlu [45] carried out a large-scale survey study to determine the presence and prevalence of ToMV, TYLCV, TSWV, ToRSV and PVY in tomato production areas in Diyarbakır province. In multiplex RT-PCR tests carried out to detect the presence of ToMV and PVY, DNA bands of 621 bp and 480 bp were obtained from infected plant samples, respectively. The nucleotide sequences of four isolates were deposited in the NCBI GenBank with the accession numbers MK992250, MK992251, MK992252 and MK992253. The ToMV isolates in the study were 99.2–99.8% identical with other ToMV isolates in GenBank. In the phylogenetic classification, DT1 and DT2 isolates clustered in the same group with the USA (KR537870) isolate. The fact that Hakkari ToMV isolates were also in the same group with the USA (KR537870) isolate suggests that there is not much difference according to geographical origin and hosts, as in CMV.

Duarte et al. [46] isolated ToMV from *Hemerocallis sp.* (tobamo-H) and *Impatiens hawkeri* (tobamo-I). They performed RNA extraction, RT-PCR amplification, CP gene sequencing and phylogenetic to compare and characterize homologous sequences. The isolates obtained were deposited in NCBI GenBank with accession numbers DQ230836 and AY063743 and they showed 98,5% nucleotide sequence identity with ToMV. Regarding Tobamo-H, 100% identity was found with ToMV from Australian and Peruvian tomatoes. It was also observed that variences in hosts did not lead to a difference in similarity rates. Fillmer et al. [35] deposited the first whole genome sequence of a ToMV isolate from jasmine in the NCBI GenBank with the accession number KR537870. In phylogenetic analysis, they found 99% identity with the reference ToMV sequence. Arinaitwe et al. [37] performed the first study on whole genome molecular characterization of ToMV isolates from Uganda, as well as from the East and Middle East. They showed that the isolate from Africa was 98–100% identical to the ToMV strains especially from China, Japan and Germany, and that this strain was a strain of the same virus species seen in Asia and Europe. Bae et al. [47] amplified the ToMV-specific RdRp region from tomato and pepper and deposited the ToMV strains isolated from tomato (Accession No: MH393621), pepper leaves (Accession No: MH393623), and chili seeds (Accession No: MH393622) in NCBI Genbank. They found that these isolates had 38–43 nucleotide differences and 99% similarity to the Queensland ToMV (Accession No: AF332868) isolate. Four Hakkari ToMV isolates in the present study were also found to be identical with Queensland ToMV isolats at rates ranging from 99.2–99.6%. In order to investigate the host range and genetic variability of ToMV in Iran, Aghamohammadi et al. [18] collected 849 symptomatic samples from different vegetables and weeds and they found ToMV infection in 45 of the samples. They also performed molecular characterization of the ToMV CP gene. An analysis of the biological characteristics of the selected ToMV isolates revealed three pathological groups, indicating the presence of different ToMV populations in Iran. It was found that Iranian isolates from group II (NL93 and NO1) did not infect solanecous host plants, while four Iranian ToMV isolates in group I, together with other global isolates, infected solanecous host species.

Hakkari ToMV strains from our country clustered in the same group with strains from other countries, except for those from Vietnam. This demonstrated that Hakkari ToMV isolates were independent of geographical origin, suggesting that strains from other countries were introduced into our country. This may have happened due to the exchange of plant material between countries. It has been suggested that plant-virus interaction may play an important role in the genetic differentiation of tobamoviruses [48]. The variation potential of ToMV may lead to outbreaks of virulent and resistance-breaking strains.

Knowledge of genetic diversity and population structure is a prerequisite for the successful development of disease resistance against viruses [49]. The development of successful techniques to control ToMV infection is urgently needed for the protection of agriculture and food production [2, 12, 13].

Conclusions

The present study was carried out to detect CMV and ToMV in tomato and pepper production areas in Hakkari and to compare the CMV and ToMV isolates in Turkey with other isolates from different parts of the world by using CP and replicase genes of the viruses. The fact that, in the surveyed areas, the use of pesticides is not common and that local seeds are used was noted. Since these viral agents can be carried mechanically or by graft, vector, seed and pollen, it would be appropriate to comply with the internal quarantine rules. The number of isolates used in phylogenetic analyses in this study is sufficient. The fact that the compared isolates were from different geographical regions of the world increased the diversity of CMV and ToMV isolates. Whole or partial genome sequences are generally used to group isolates of a particular virus. The availability of whole genome sequences of CMV and ToMV isolates in NCBI GenBank allowed us to use the CP and replicase gene alone. The data presented here, along with other available sequences, provides useful information on CMV and ToMV isolates in Turkey. These findings are particularly relevant to the recent reports on the presence of CMV and ToMV in vegetables in Turkey. CMV CP gene and ToMV replicase gene are highly conserved in different hosts. For a more efficient grouping of CMV and ToMV isolates, studies on various isolates combined with their biological characterization and further analyses with different genomic regions or whole genome sequences of these isolates are needed.

Declarations

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nevin AKDURA and Handan ÇULAL KILIÇ. All authors read and approved the final manuscript.

Funding This work was supported by Scientific Research Projects Unit of Hakkari University, Project number: EF2021BAP1.

Data availability All data will be available on request.

Conflict of interest Author Nevin AKDURA and Handan ÇULAL KILIÇ have no relevant financial or non-financial interests to disclose. The authors declare that they have no conflict of interests.

Consent to participate All authors read and approved the manuscript.

Consent to publish All authors read and approved the manuscript.

References

1. Valizadeh J, Jafar M (2011) Identification, distribution and incidence of important tomato and cucur-bits viruses in Southeast of Iran. Am J Plant Physiol 6(5):242–251
2. Broadbent L (1965) The epidemiology of tomato mosaic. Ann Appl Biol 56:177–205
3. Francki RIB, Mossop DW, Hatta T (1979) Cucumber mosaic virus. CMI/AAB Description Viruses No 213:6
4. Habili N, Francki RIB (1974) Comparative studies on tomato aspermy and cucumber mosaic viruses. I. Physical and chemical properties. Virology 57:392–401
5. Jacquemond M (2012) Cucumber mosaic virus. Adv Virus Res 84:439–504
6. Zitter TA, Murphy JF (2009) Cucumber mosaic. Plant Health Instructor, DOI: 10.1094. PHI-I-2009-0518-01
7. Raccah B, Galon A, Eastop VF (1985) The role of Flying Aphid Vectors in the Transmission of Cucumber mosaic virus and Potato virus Y to Peppers in Israel. Ann Appl Biol 106:451–460

8. Kosaka Y, Fukunishi T (1997) Multiple İnoculation with Three Attenuated Viruses for the Control of Cucumber Virus Disease. Plant Dis 81:733–738
9. Roossinck MJ, Zhang L, Hellward K (1999) Rearrangements in the 5'non translated region and phylogenetic analyses of Cucu.mber mosaic virus RNA3 indicate radial evolution of three subgroups. J Virol 73:6752–6758
10. Roossinck MJ (2002) Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. J Virol 76:3382–3387
11. Megahed AA, El-Dougdoug KA, Othman BA, Lashin SM, Ibrahim MA, Sofy AR (2013) Induction of resistance in tomato plants against Tomato mosaic tobamovirus using beneficial microbial isolates. Pak J Biol Sci 16:385–390
12. Sofy AR, Sofy MR, Hmed AA, El-Dougdoug NK (2019) Potential Effect of Plant Growth-Promoting Rhizobacteria (PGPR) on Enhancing Protection against Viral Diseases. In: Maheshwari DK, Dheeman S (eds) Field Crops: Sustainable Management by PGPR. Sustainable Development and Bi-Odiversity, vol 23. Springer, Cham, Switzerland, pp 411–445
13. Adams MJ, Heinze C, Jackson AO, Kreuze JF, Macfarlane SA, Torrance L (2012) Family virgaviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus Taxonomy – Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, Netherlands, pp 1139–1162
14. Chitra T, Prakash H, Albrechtsen S, Shetty HS, Mathur S (1999) Infection of tomato and bell pepper by ToMVand TMV at different growth stages and establishment of virus in seeds. J Plant Pathol 81:123–126
15. Jones JB, Zitter TA, Momol TM, Miller SA, Hansen AJ (2014) 1990. Report on consultancy on fruit trees and vegetables virology UGA/87/003, FAO / UNDP. 43pp
16. Hansen AJ (1990) Report on consultancy on fruit trees and vegetables virology. UGA/87/003, FAO / UNDP. 43pp
17. Lanter JM, McGuire JM, Goode MJ (1982) Persistence of tomato mosaic virus in tomato debris and soil under field conditions. Plant Dis 66:552–555
18. Aghamohammadi V, Rakhshandehroo F, Shams-bakhsh M, Palukaitis P (2013) Distribution and Genetic Diversity of Tomato Mosaic Virus in Iran. J Plant Pathol 95(2):339–347
19. Karavina C, Ibaba JD, Gubba A (2018) Genome sequence of a tomato-infecting tomato mosaic virus isolate from Zimbabwe. Genome Announc 6:e01457–e01417. https://doi.org/10.1128/genomeA.01457-17
20. Han-Xin L, Rubi L, Smythe AB, Bryce WF (2004) Molecular population genetics of Cucumber Mosaic Virus in California: Evidence for founder effects and reassortment. J Virol 78(12):6666–6675
21. Kumar MS, Udaya Shankar AC, Nayaka SC, Lund OS, Prakash HS (2011) Detection of Tobacco mosaic virus and Tomato mosaic virus in pepper and tomato by multiplex RT–PCR. Lett. Appl Microbiol 53:359–363
22. Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98
23. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673–4680
24. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549
25. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Mol Evol 16(2):111–120
26. Li F, XuePing Z, HaiRu C, DeBao L (2000) cDNA cloning and sequence analysis of coat protein genes of ToMV and CMV isolated from tobacco in Yunnan province. J Zhejiang Univ (Agriculture Life Sciences) 26(3):261–265
27. Divéki Z, Salánki K, Balázs E (2004) The Necrotic Pathotype of the Cucumber mosaic virus (CMV) Ns Strain Is Solely Determined by Amino Acid 461 of the 1a Protein. Mol Plant Microbe Interact 17(8):837–845
28. Deyong Z, Willingmann P, Heinze C, Adam G, Pfunder M, Frey B, Frey JE (2005) Differentiation of Cucumber mosaic virus isolates by hybridization to oligonucleotides in a microarray format. J Virol Methods 123(1):101–108

29. Bonnet J, Fraile A, Sacrist´an S, Malpica JM, Garc´ıa-Arenal F (2005) Role of recombination in theevolution of natural populations of Cucumber mosaic virus, a tripartite RNA plant virus. Virology 332:359–368
30. Ohshima K, Matsumoto K, Yasaka R, Nishiyama M, Soejima K, Korkmaz S, Ho SYW, Gibbs AJ, Takeshita M (2016) Temporal analysis of reassortment and molecular evolution of cucumber mosaic virus: Extra clues from its segmented genome. Virology 487:188–197
31. Karanfil A (2021) Prevalence and molecular characterization of Cucumber mosaic virus isolates infecting tomato plants in Marmara region of Turkey. Plant Prot Bull 61(4):19–25
32. McGarvey P, Tousignant M, Geletka L, Cellini F, Kaper JM (1995) The complete sequence of a cucumber mosaic virus from Ixora that is deficient in the replication of satellite RNAs. J Gen Virol 76 (9):2257-70 doi: 10.1099/0022-1317-76-9-2257. PMID: 7561763
33. Yamamoto H, Abe T, Ueda K, Inoue M, Matsumoto T (2002) A Missense Mutation in Tomato mosaic virus L11A-Fukushima Genome Determines Its Symptomless Systemic Infection of Tomato. J Gen Plant Pathology: JGPP; Tokyo 68(4):385–389
34. Ohnishi J, Hirai K, Kanda A, Usugi T, Meshi T, Tsuda S (2009) The coat protein of Tomato mosaic virus L11Y is associated with virus-induced chlorosis on infected tobacco plants. J Gen Plant Pathol 75:297–306. DOI 10.1007/s10327-009-0177-x
35. Fillmer K, Adkins S, Pongam P, D'Elia T (2015) Complete Genome Sequence of Tomato Mosaic Virus Isolated from Jasmine in the United States. Genome Announc 9(4):e00706–e00715. doi: 10.1128/genomeA.00706-15
36. Sihelská N, Vozárová Z, Predajňa L, Šoltys K, Hudcovicová M, Mihálik D, Kraic J, Mrkvová M, Kúdela O, Glasa M (2017) Experimental Infection of Different Tomato Genotypes with Tomato mosaic virus Led to a Low Viral Population Heterogeneity in the Capsid Protein Encoding Region. Plant Pathol J 33(5):508–513. DOI: https://doi.org/10.5423/PPJ.NT.04.2017.0082
37. Arinaitwe W, Ochwo-Ssemakula M, Mbewe WK, Sseruwagi P, Kyamanywa S (2018) Molecular characteristics of Tomato mosaic virus infecting tomato in Uganda. Afr Crop Sci J 26(3):433–445
38. Güller A, Usta M (2020) Occurrence of Cucumber mosaic cucumovirus and Watermelon mosaic potyvirus on Melon exhibiting viral symptoms in Bingöl province of Turkey and Their Phylogenetic Affinities. Turkish J Agric Nat Sci 7(4):948–958
39. Karanfil A, Korkmaz S (2017) Detection and molecular characterization based on coat protein gene of Cucumber mosaic virus (CMV) from cowpea production fields of Çanakkale province in Turkey. Plant Prot Bull 57(3):293–304
40. Kurtoğlu A, Korkmaz S (2018) Determination and Molecular Characterization of Cucumber Mosaic Virus (CMV) Infection on Spinach Production Fields of Çanakkale Province, Turkey. J Turk Phytopath 47(2):43–51
41. Borah M, Kumar G, Siddappa S (2019) Molecular Detection and Phylogenetic Analysis of Cucumber Mosaic Virus Infecting Bhut Jolokia (Capsicum chinense Jacq.) of Assam. Int J Economic Plants 6(3):126–129
42. Mishchenko LT, Dunich AA, Dashchenko AV, Bondus RO (2021) Molecular characterization of cucumber mosaic virus infecting Echinacea purpurea in Ukraine. Archives of Phytopathology and Plant Protection 54:15–16. DOI:10.1080/03235408.2021.1899378
43. Rivera-Toro DM, Vaca-Vaca JC, López-López K (2020) Detection and molecular characterization of the cucumber mosaic virus in chili pepper (Capsicum spp. L.) crops. Agronomía Colombiana 38(2):218–225
44. Çağlar BK (2006) Characterization of melon (CMV-K), tomato (CMV-D), pepper (CMV-B) isolates of cucumber mosaic virus (CMV) by biological, serological, molecular methods and effect of satellite-RNAs on virus. Çukurova University, PhD Thesis, 89 p Adana
45. Yılmaz F, Sipahioğlu M (2020) Detection of some important viruses in tomato production areas of Diyarbakır province by molecular methods and their molecular characterization. Plant Prot Bull 60 – 4. DOI: 10.16955/bitkorb.682860
46. Duarte LML, Alexandre MAV, Rivas EB, Cattai MB, Soares RM, Harakava R, Fernandes FMC (2007) Phylogenetic analysis of *Tomato mosaic virus* from *Hemerocallis* sp. and *Impatiens hawkeri*. *Summa Phytopathol*, *33*:409–413 https://doi.org/10.1590/S0100-54052007000400016

47. Bae M, Jo Y, Choi H, Tran PT, Kim KH (2018) First report of tomato mosaic virus isolated from tomato and pepper in Vietnam. J Plant Pathol 101:181
48. Aldaoud R, Dawson WO, Jones GE (1989) Rapid, random evolution of the genetic structure of replicating Tobacco mosaic virus populations. Intervirology 30:227–233
49. García-Arenal F, Escriu F, Aranda MA, Alonso-Prados JL, Malpica JM, Fraile A (2000) Molecular epidemiology of Cucumber mosaicvirus and its satellite RNA. Virus Res 71:1–8

Tables

Table 1
Primer pairs used in the molecular characterization of CMV and ToMV isolates

Primer	Sequence	Specific Gene	Reference
CMV_Forward	5´-TTGAGTCGAGTCATGGACAAATC-3´	Coat protein gene	[20]
CMV_Reverse	5´-AACACGGAATCAGACTGGGAG – 3´	Coat protein gene	
ToMV_Forward	5´-CGAGAGGGGCAACAAACAT – 3´	Replicase gene	[21]
ToMV_Reverse	5´-ACCTGTCTCCATCTCTTTGG – 3´	Replicase gene	

Table 2
Places where the tomato and pepper samples were collected, number of samples taken and presence of the viruses

District	Collected Samples Tomato	Infected Samples			Infection rate (%)	Collected Samples Pepper	Infected Samples			Infection rate (%)
		CMV	ToMV	CMV + ToMV			CMV	ToMV	CMV + ToMV	
Çimenli	21	8	9	1	85.71	17	2	5	0	41.18
Otluca	25	0	11	0	44.00	24	1	10	0	45.83
Üzümcü	16	7	1	0	50.00	14	3	1	0	28.57
Doğanlı	6	2	0	4	100.00	6	2	1	1	66.67
Gelinli	4	0	1	0	25.00	3	1	0	0	33.33
Kırıkdağ	13	10	0	0	76.92	12	1	0	0	8.33
Durankaya	3	0	0	0	0.00	4	0	0	0	0.00
Geçimli	6	5	0	0	83.33	10	0	0	0	0.00
Total	**94**	**32**	**22**	**5**	**62.77**	**90**	**10**	**17**	**1**	**31.11**

Table 3
List of the CMV isolates used in this study

Accession Number	Country	CMV Gene Region	Host	Isolate	Origin	Reference
AJ239098	China	coat protein	tobacco	YN	China	[26]
AJ271416	USA	coat protein	-	-	USA	unpublished
AJ511990	Hungary	partial genome	*Nicotiana glutinosa*	Ns	Hungary	[27]
AJ517802	Hungary	partial genome	*Raphanus sativus*	Rs	Hungary	[27]
AJ575589	China	coat protein	*Brassica juncea*	ZJZC-CMV	China	unpublished
AJ585518	Australia	partial genome	rockmelon	237	Australia	unpublished
AJ810262	Germany	coat protein	bean	RT144	Germany	[28]
AJ810263	Germany	coat protein	cornflower	RT54	Germany	[28]
AJ829776	Spain	partial genome	melon	MAD99/1	Spain	[29]
AM048831	Vietnam	coat protein	*Nicotiana tabaccum*	VN-Bavi	Vietnam	unpublished
DQ285569	India	coat protein	Piper longum	-	India	unpublished
LC066467	Japan	complete sequence	*Raphanus sativus*	IRN-REY4	Iran	[30]
LC066473	Japan	complete sequence	*Raphanus sativus*	IRN-REY10	Iran	[30]
MG251397	Korea	coat protein	*Gentiana scabra*	CMV-YD2	South Korea	unpublished
MG251398	Korea	coat protein	*Gentiana scabra*	CMV-YD3	South Korea	unpublished
MH426932	Turkey	coat protein	canola	KNLc32	Turkey	Unpublished
MZ711455	Turkey	coat protein	tomato	TKR-81	Turkey	[31]
MZ711456	Turkey	coat protein	tomato	EDR-91	Turkey	[31]
MZ711457	Turkey	coat protein	tomato	EDR-97	Turkey	[31]
OM418629	Turkey	coat protein	tomato	NHD1	Turkey	this study
Y10886	Italy	coat protein	tomato	Tfn	Italy	unpublished
U20219	USA	3a protein and coat protein	tomato	Ixora	USA	[32]

Table 4. List of the ToMV isolates used in this study

Accession Number	Country	ToMV Gene Region	Host	Isolate	Origin	Reference
AB083196	Japan	complete genome	-	Japan	L11A-Fukushima	[33]
AB355139	Japan	complete genome	tobacco	Japan	L11Y	[34]
AF332868	Australia	complete genome	tomato	Australia	Queensland	unpublished
AJ243571	Kazakhstan	complete genome	-	Kazakhstan	K1	unpublished
FN985165	China	complete genome	-	China	XJT-1	unpublished
KR537870	USA	complete genome	jasmine	USA	99-1	[35]
KU321698	Egypt	complete genome	tomato	Egypt	AH4	unpublished
KX711903	South Africa	complete genome	tomato	South Africa	mutoko	[19]
KY912162	Slovakia	complete genome	tomato	Slovakia	SL-1	[36]
KY967219	China	complete genome	tomato	China	SX	direct submission
KY967222	China	complete genome	tomato	China	Tai'an	direct submission
KY967225	China	complete genome	tomato	China	LY	direct submission
KY967228	China	complete genome	tomato	China	HHHT	direct submission
MF002479	China	complete genome	tomato	China	HHHT4	direct submission
MF002480	China	complete genome	tomato	China	HHHT3	direct submission
MF002490	China	complete genome	tomato	China	JX	direct submission
MG456601	Uganda	complete genome	Solanum	Uganda	ToMV-Ug	[37]
MH038047	India	partial replicase gene	tomato	India	B10 Jodhe	direct submission
MH063883	Korea	partial replicase gene	tomato	Vietnam	BaoLoc	unpublished
MH063884	Korea	partial replicase gene	chili pepper	Vietnam	CPS	unpublished
MH063885	Korea	partial replicase gene	bell pepper	Vietnam	DonDuong	unpublished

MH393621	Korea	partial genome	tomato	Vietnam	ToMV_BaoLoc	unpublished
MH393622	Korea	partial genome	chili pepper seed	Vietnam	ToMV_chili	unpublished
MH393623	Korea	partial genome	bell pepper	Vietnam	ToMV_DonDoung	unpublished
MH507165	South Korea	complete genome	tomato	South Korea	GW1	unpublished
MH507166	South Korea	complete genome	tomato	South Korea	GW2	unpublished
MK652756	Turkey	partial replicase gene	eggplant	Turkey	TRAntToMVEgp	Unpublished
MN267904	Slovenia	complete genome	tobacco	Slovenia	ToMV_WW17-I-f16-18-Nocc-pos2	direct submission
OM286731	Turkey	partial replicase gene	pepper	Turkey	NHP1	this study
OM286732	Turkey	partial replicase gene	tomato	Turkey	NHT1	this study
OM286733	Turkey	partial replicase gene	tomato	Turkey	NHT2	this study
OM286734	Turkey	partial replicase gene	tomato	Turkey	NHT3	this study
Z92909	Russia	complete genome		Russia	K2 (Kazakhstan strain)	unpublished

Figures

Figure 1

Examples of pepper and tomato leaves showing common virus symptoms

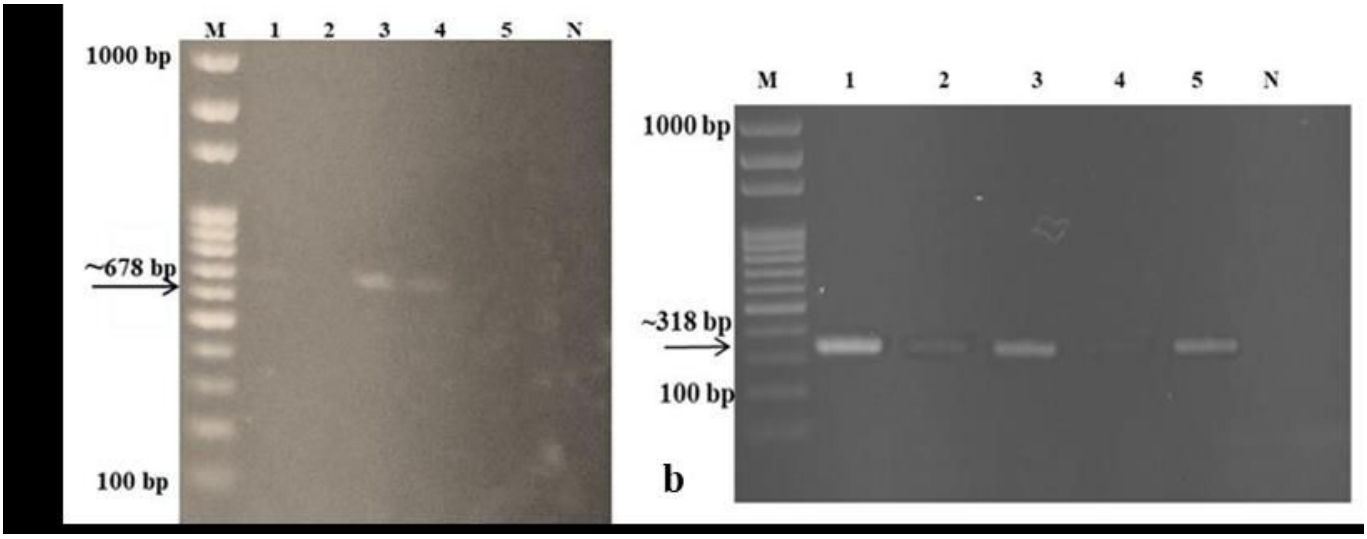

Figure 2

a. Amplification of the partial CP gene of CMV from tomato and pepper samples using RT-PCR. M-100 bp DNA marker, 1 (tomato/Çimenli), 2 (tomato/Çimenli), 3 (tomato/Çimenli), 4 (pepper/Çimenli), 5 (tomato/Geçimli) **b.** Amplification of the partial replicase gene of ToMV from tomato and pepper samples using RT-PCR. 1 (tomato/Çimenli), 2 (pepper/Çimenli), 3 (pepper/Otluca), 4 (tomato/Çimenli), 5 (tomato/Otluca)

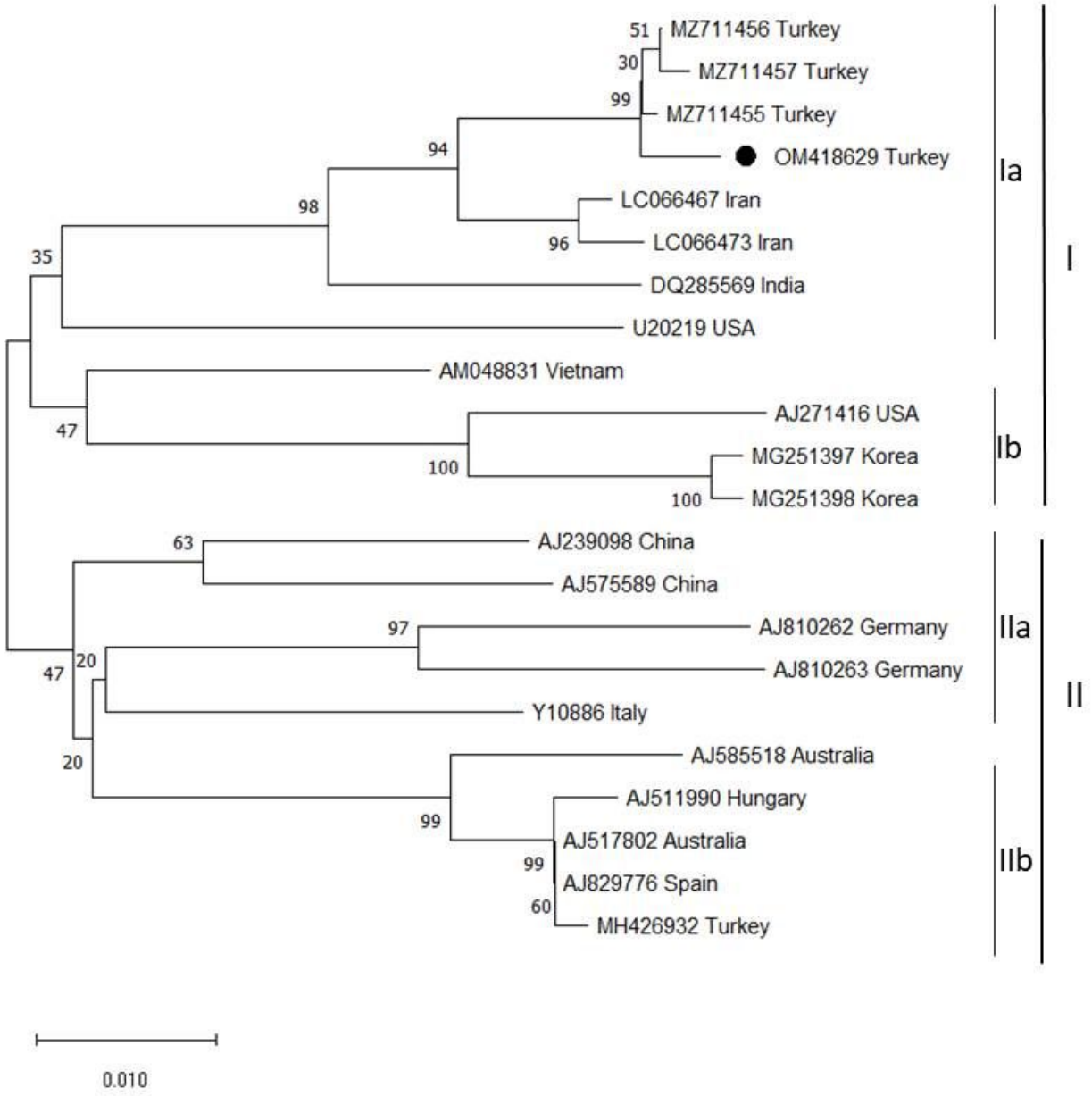

Figure 3

Phylogenetic tree based on the nucleotide sequence of the CMV CP gene. All data from GenBank are indicated by nucleotide database accession numbers. Data analysis and tree construction were carried out using the MEGA (Version X) program.

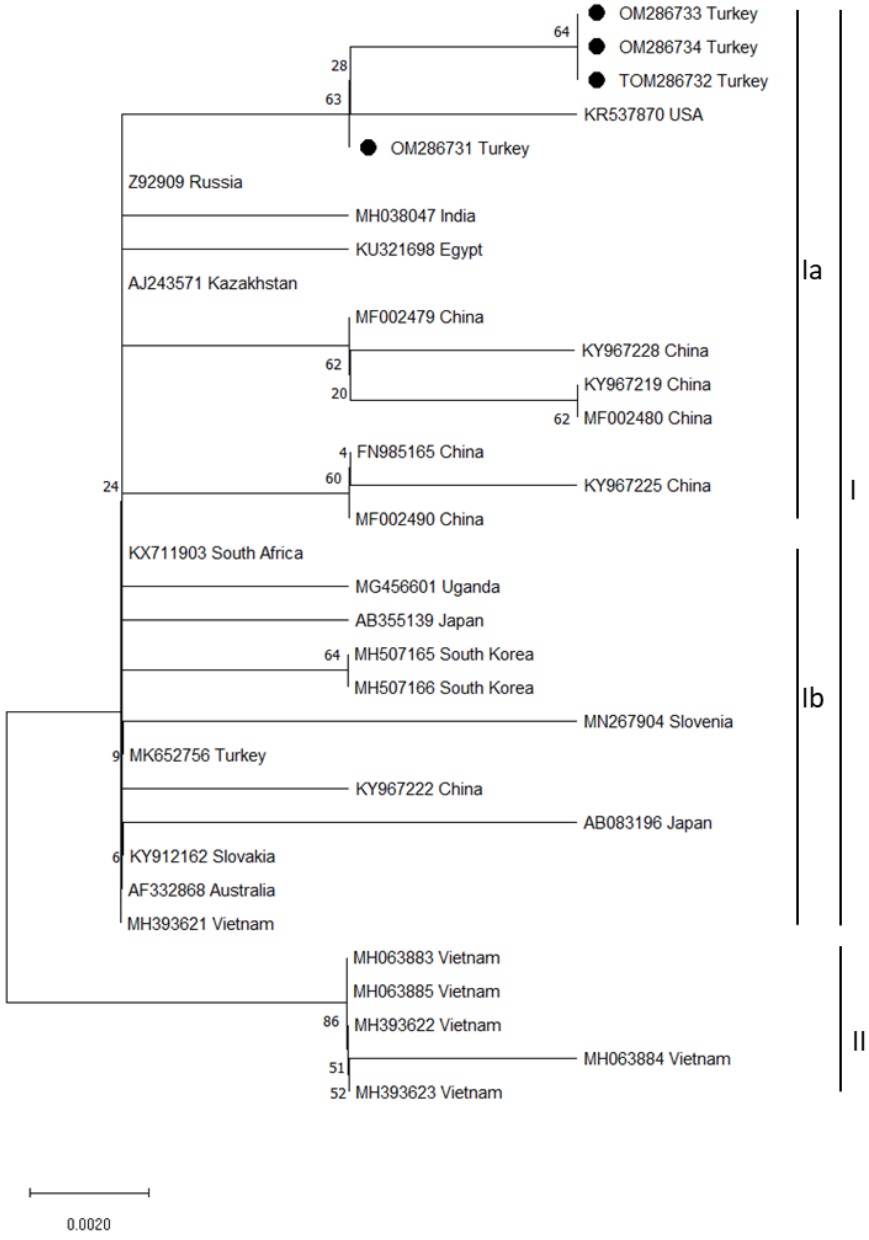

Figure 4

Phylogenetic tree based on the nucleotide sequence of the ToMV replicase gene. All data from GenBank are indicated by nucleotide database accession numbers. Data analysis and tree construction were carried out using the MEGA (Version X) program.