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Investigation of activity of *Tobamovirus* in pepper plants containing *L4* resistance gene

Hakan FIDAN, Ailar GONBADI, Pelin SARIKAYA, Ozer CALIS

Department of Plant Protection, Faculty of Agriculture, Akdeniz University, 07059, The Campus, Konyaalti, Antalya, Türkiye

Corresponding author: A. Gonbadi, e-mail: a.gonbadi@yahoo.com
Author(s) e-mail: hakanfidan@akdeniz.edu.tr, pelinsarikaya75@gmail.com, ozercalis@akdeniz.edu.tr

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ABSTRACT

Pepper mild mottle virus (PMMoV) is a plant virus belonging to the *Virgaviridae* family; it significantly reduces pepper yield production worldwide. The PMMoV is spread by contaminated seeds and there is no chemical treatment available. Therefore, resistant pepper varieties containing the *L4* gene are recommended for the management of PMMoV. A considerable amount of evidence suggests that the *L4* gene confers resistance to PMMoV in pepper. The aim of the project is to confirm the status of the *L4* gene for resistance to PMMoV in pepper varieties, several inoculations were performed on pepper plants containing *L3*, *L4* resistant genes and susceptible pepper plants without the resistance genes. The *L4* resistant plants produced mottling, mosaic, leaf curl, stem necrosis symptoms in the tested pepper plants but there was no amplicon observed with specific primers of PMMoV in RT-PCR analyses. To determine if the *L3* and *L4* genes are controlling resistance to PMMoV, RT-PCR analyzes were conducted using PMMoV and *Tomato brown rugose fruit virus* (ToBRFV) where both viruses belong to the same family. The molecular studies revealed that the *L4* gene controls resistance mechanisms to PMMoV but it is not able to govern *Tobamovirus*, ToBRFV. We showed that pepper plants harboring the *L3* and *L4* gene have the ability to precisely control the mechanism of resistance to PMMoV compared to pepper plants carrying only the *L3* gene. A complete genome sequence of PMMoV was obtained and submitted to Genbank with MW523006 accession number in the NCBI system.

1. Introduction

The *Solanaceae* is a unique family within agronomically important members who are infected with the same or very closely related plant pathogens. Pepper (*Capsicum annuum* L.) is one of the most diverse vegetables in this family (Tsuda et al. 2007). The capsicum plants are roughly infected with 68 viruses belonging to *Potyvirus*, *Carlavirus*, *Potexvirus*, *Tobamovirus*, *Tobravirus*, *Luteovirus*, *Tospovirus*, and *Cucumovirus* genera. Among them, about 20 viruses are reported to cause extensive damage to this valuable vegetable (Moury et al. 2012). One of these viruses, which has been reported from different countries around the world in the last 40 years (Genda et al. 2007; Antignus et al. 2008) and restricts pepper production, is *Pepper mild mottle virus* (PMMoV), which belongs to the *Tobamovirus* genus of the *Virgaviridae* family (Secrist et al. 2018). This virus was first detected in commercial pepper varieties grown in field conditions in Turkey in 1994 (Guldur et al. 1994). The PMMoV is characterized with a typical rod-shaped particle morphology spanning 6357 bp single-stranded RNA (+ssRNA) genome which is encoding four open reading frames (ORFs). The ORF1 and ORF2 are separated by a stop codon and encode non-structural proteins that constructed a replicase complex. The ORF3 is on a large subgenomic RNA producing a non-structural movement protein (MP). The last ORF4 is on the small subgenomic RNA, encodes 17 to 18 kDa coat protein (Tsuda et al. 2007; Rialch et al. 2015). The genus *Tobamovirus* also contains ToBRFV which is another important pathogen causing serious diseases on pepper plants. The ToBRFV transmission is mainly mechanical but it can also be transmitted via contaminated seeds or fruits over long distances likely common to other *Tobamoviruses* (King et al. 2011). The virus is capable of being in direct contact with diseased plants, or infected sap from various surfaces such as harvesting, clothing, pots, packaging which can result in the mechanical transmission of the novel virus within crops (Oladokun et al. 2019). Therefore, in order to control the disease pathogen in pepper production areas, suitable cultural precautions and resistant varieties have to be used (Petrovic et al. 2010). Nowadays, pepper resistance to the viral pathogens is broken except the *L4* resistance gene which still mediates resistance to the viruses in dynamic mechanisms. On the other hand, *Tobamovirus*-tolerant varieties are available in pepper plantations, *L3* resistance breaking isolate and new *Tobamoviruses* like ToBRFV are creating potential problems in the agricultural sector. This study aims to understand the genome organization of *Tobamoviruses* and to determine whether the *L4* gene mediates resistance mechanisms in pepper plants. Therefore, since ToBRFV had not yet been reported in Turkey at the beginning of our study (2018), the route of our research shifted to the activity of the *L4* resistance gene in existing resistant pepper lines (Fidan et al. 2021).

2. Material and Methods

2.1. Preparation of infected PMMoV plants and symptomatological studies

PMMoV isolate was obtained from greenhouses where pepper is grown intensively in the Antalya province and its districts. Intense complaints, especially from the Kumluca region, determined the direction of the study. In the study, it was requested to determine whether the *L4* gene works efficiently or not. With this aim, pepper varieties used as plant material had *L3* and *L4* resistance genes used in pathogenicity tests and the results were observed in greenhouses. *L4* resistance gene source *Capsicum chacoense* pepper genotype and *L4* resistance gene, Koray F1, Mustang, Doğanay, Ozan, Vergase pepper varieties and non-resistant Caltı standard varieties were used. In the experiment established in the greenhouse, the number of plants used per cultivar was ten.

At the beginning of molecular studies, first of all, the resistance status of the cultivars declared *L4* resistance by the companies was determined using the *L4* Locus primers developed by Kim et al. (2008). *Capsicum chacoense*, which is the source of resistance, was obtained from the Alata Horticultural Research Institute (ALATA). Before starting the mechanical inoculation procedures, molecular studies were carried out using 15 different virus-specific primer pairs identified in Table 1 to determine that the source of the inoculum was only infected with PMMoV and free from other viruses. After making sure that our source of inoculum was only infected with PMMoV, mechanical inoculation processes were carried out at regular intervals both on plant materials carrying *L3* and *L4* resistance genes and on sensitive plants lacking these genes. Also, control plants were included in the experiment.

Inoculated plants were kept at 23±3°C for 16 hours during the day and 8 hours at night with appropriate culture management such as irrigation, fertilization, and pest control at 7-day intervals throughout the trial period. The entire experiment was set up in a greenhouse with no artificial lighting or heating used during the studies in 3 replications. While the plant materials were in the true second leaf stage, they were inoculated with PMMoV isolate obtained from the Akdeniz University Virology Laboratory, while in the control plants, distilled sterile water was preferred for inoculation, and finally the complementary Koch's postulates were executed.

Table 1. The 15 viruses were tested for understanding which virus causes disease on pepper plants in RT-PCR analyzes

Virus Name	Primer Name	Primer Sequences (5'→3')	Product Length (Bp)	Reference
AMV	AMV (F)	GTGGTTGGAAAGCTGGTAAA	700	(Buzkan and Yuzer 2009)
	AMV (R)	CCCCCAGTGGAGGTCAGCATT		
ChiVMV	D (F)	GGAAAGGCGATCCCGATCTACTAT	788	
	E (R)	CGCGCTAATGACATATCGGT		
CMV	CMV (F)	TAACCTCCCAGTTCTCACCGT	513	
	CMV (R)	CCATCACCTTAGCTTCCATGT		
PMMoV	P12/3 (F)	ACAGCGTTTGGATCTTAGTAT	836	
	P12/3A (R)	GTGCGGTCTTAATAACCTCA		
PepMoV	P3 (F)	AATGCAAAGCCAACATTC	345	
	M4 (R)	CTAATACGAACACCAAGCAT		
PVMV	D (F)	GGAAAGGCGATCCCGATCTACTAT	737	
	E (R)	CGCGCTAATGACATATCGGT		
PVX	PVX (F)	TAGCACAACACAGGCCACAG	562	
	PVX (R)	GGCAGCATTCATTTCAGCTTC		
PVY	PVY (F)	ACGTCCAAAATAGAGATGCC	480	
	PVY (R)	TGGTGTTCGTGATGTGACCT		
TEV	TEV-CP2-F	CTAAATGGATTTATGGTGGTGGTG	391	
	TEV-CP2-R	CAGTACCCACGTTGCCATCA		
TMV	TMV(F)	GCACATCAGCCGATGCAGC	880	
	TMV(R)	ACCGTTTTCGAACCGAGACT		
ToMV	ToMV(F)	CGAGAGGGGCAACAAACAT	318	
	ToMV(R)	ACCTGTCTCCATCTCTTTG		
TSWV	L1TSWVR	AATTGCCTTGCAACCAATTC	276	
	L2TSWVF	ATCAGTCGAAATGGTCGGCA		
TYLCV	VP2715	ATACTTGGACACCTAATGGCTATTTGG	543	
ToBRFV	ToBRFV1F	CTTCCAAACGTGTACGCACC	475	(Fidan et al. 2021)
	ToBRFV1R	ATGCATCTTCCATTGCGCTG		
General Tobamo virus	R-4718	CAATCCTTGATGTGTTTAGCAC	1052	(Tsuda et al. 2007)
	F-3666	ATGGTACGAACGGCGGCAG		

The development of symptoms was monitored from the initiation of the first symptoms to full appearance, and the inoculated plants were photographed at all stages in a greenhouse located at Akdeniz University (Figure 1). The inoculated samples were collected from fresh leaves and fruits showing typical symptoms such as chlorosis, mild mosaic on the leaf, reduced fruit size, mottling, brown necrotic spots, and streaks on fruit. The collected leaf samples were crushed in an extraction buffer in a mortar, and their total nucleic acids were isolated. In the total nucleic acids extracted, DNA and RNA ratios were measured in a one microliter (1 µl) solution and then their concentrations were optimized for further molecular studies. The mechanical inoculation procedure was repeated 3 times in 2 week intervals, the inoculated leaves were analyzed to confirm PMMoV inoculation with RT-PCR tests. Subsequently, all inoculations described above were carried out with an isolate obtained from plants showing ToBRFV symptom and collected from pepper growing areas. The ToBRFV isolate was identified and used in our trials with precautions to avoid contamination during inoculations as previously described (Davino et al. 2020). Similar mechanical inoculations were repeated using inocula from PMMoV negative, but ToBFRV positive, plant samples in RT-PCR analyses in 2019.

2.2. Verification of the L4 gene and determination of PMMoV infection by RT-PCR

Pepper plants with the *L4* gene were tested with PCR amplification using *L4* gene-specific primers to confirm the presence of the *L4* gene (Kim et al. 2008). After mechanical inoculation, total nucleic acid isolation was performed from plants with typical virus symptoms. The nucleic acid extractions from PMMoV and ToBRFV inoculated pepper plants were conducted using GeneJET Plant RNA Purification Kit (Thermo Fisher Scientific, USA). For *L3* and *L4* gene analyzes, GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) was used according to the manufacturer's instructions. All inoculated plant materials were tested for PMMoV infection with PMMoV-specific primers using Verso One-step RT-PCR ReddyMix kit (Thermo Fisher Scientific, USA) in RT-PCR analyzes.

2.3. Screening of plants containing L4 gene in terms of Tobamoviruses

We observed disease symptoms on pepper plants containing the *L4* gene; they were showing typical leaf and fruit symptoms inoculated with the *Tobamovirus* genus. ToBRFV which belongs to the *Tobamoviruses* was first reported on tomato plants (Caglar et al. 2013). The virus is known to infect the *Solanaceae* family's plants, with this information a separate trial was immediately conducted with ToBRFV using all these plants. Resistant plants with both the *L3* and *L4* genes and susceptible pepper plants without any of these genes were mechanically inoculated with the ToBRFV isolate, followed by inoculated plants transferred in a growth chamber. After symptoms developed on the inoculated pepper plants, RT-PCR analyzes were carried out using specific primers to ToBRF (Fidan et al. 2021).

2.4. Designing of PMMoV specific primers

For PMMoV, a complete genome was constructed with specific primers using the Primer-BLAST program from the National Center for Biotechnology Information (NCBI) system. Specific primer pairs (Table 2) were designated and synthesized in a commercial company (Nanogen Medical, Turkey). After minor errors were corrected using the MEGA 7.0 (Stecher et al. 2020) and Chromas (version 2.6) programs, the whole genome of PMMoV was aligned with these specific primer combinations (Table 2). Additionally, to obtain sequences from the 5'-ends, the FirstChoice® RLM-RACE Kit (Thermo Fisher Scientific, USA) was used according to the manufacturer's instructions. This generated a single sequence overlapping and bidirectional, with the forward and complementary sequences provided 6357 bp length complete genome of PMMoV. The 6313 bp complete genome of the ToBRFV isolate in pepper was obtained using specific primers as previously described (Fidan et al. 2021).

Figure 1. Symptoms of mottling, chlorosis, and curl signs are observed in leaves with mechanical inoculation with *Pepper mild mottle virus* (1, 2, 3, A). A hypersensitive response (HR) is appeared on leaf harboring *L4* resistance gene (B). The pepper plants have typical trunk necrosis on stems (C).

Table 2. Primer pairs are designed within the primer BLAST program at National Center for Biotechnology Information (NCBI) system

Primer pairs	Sequence (5'->3')		Starting point	Ending point	TM	Product length (bp)
Primer 1	Forward	GGGAATAACCCCTTGGTGAA	121	140	57.09	153
	Reverse	CTCAGGGTAGGCCTTAGTTG	273	254	57.01	
Primer 2	Forward	GGGAATAACCCCTTGGTGAA	121	140	57.09	1194
	Reverse	TAAGCGCTTTCGACTGGTAT	1315	1296	57.05	
Primer 3	Forward	CTGTCGCTTTGCACAGTTTA	662	681	56.96	654
	Reverse	TAAGCGCTTTCGACTGGTAT	1315	1296	57.05	
Primer 4	Forward	ACATAGGCGCCTTCTTCTCG	803	822	59.90	1047
	Reverse	TTGCTGCCACCAATGGATCT	1849	1830	59.96	
Primer 5	Forward	TGGGATGAGATTACAGCCGC	1525	1544	59.89	751
	Reverse	TCGCAGCTGTGTCCTTGATT	2275	2256	59.96	
Primer 6	Forward	ATTTAGACAGCCTGGTAGCC	2201	2220	56.99	743
	Reverse	GACCTCGAGTTGACTCACAT	2943	2924	56.98	
Primer 7	Forward	ATGTTACACCCTGGTTGTGT	2800	2819	56.96	729
	Reverse	CGGCAAACACTTGTCGTAAT	3528	3509	57.04	
Primer 8	Forward	GTGTTAACCTTTTCGTCGCA	3452	3471	56.98	622
	Reverse	AGCGCATTGATTTTCTTGCT	4073	4054	56.98	
Primer 9	Forward	CCGTTGATCAATACAGGCAC	3953	3972	56.89	607
	Reverse	CCCTGTTGAATATCGGGGAA	4559	4540	56.98	
Primer 10	Forward	GGTGCGAACCTTCTCTGGAA	4558	4577	59.97	1098
	Reverse	CGACTCCGAGTTCAACCCAA	5655	5636	59.97	
Primer 11	Forward	ATCAGTTCCAATGGCTGACA	5505	5524	57.11	799
	Reverse	CGTTCGCTAATACACGTCAC	6303	6284	57.05	

2.5. RT-PCR amplification, sequencing and phylogenetic studies

RT-PCR amplification was carried out in a total volume of 15 µL containing: 1 µL template RNA, 200 nmol of each primer, 0.25 µL Verso enzyme mix, 0.75 µL RT-Enhancer, 7.5 µL One-Step RT-PCR ReddyMix (Thermo Fisher Scientific, USA), and 3.5 µL nuclease-free water. The amplified products were run on 1.5% agarose gel then amplified fragments were cut from the gel and purified using the GeneJet Gel Extraction Kit (Thermo Fisher Scientific, USA). The sequences of the amplified and gel-purified PCR products were obtained from Medsantek Company (Istanbul, Turkey).

The RT-PCR program executed the reverse transcription of RNA at 50 °C for 30 min, and performed PCR step at 95°C for 2 min followed by 35 cycles at 95 °C for 30 s, 52 °C for PMMoV and 59 °C for ToBRFV for 30 s, and 72 °C for 1 min, followed by a final 72 °C extension step for 5 min. The entire PMMoV sequences were deposited on pepper (Ailar3, MW523006) in the GeneBank Database at NCBI. Furthermore, the whole PMMoV sequence was compared with 10 available sequences from different countries in the world in the NCBI database. A phylogenetic tree was constructed to understand the relationships of PMMoV to other PMMoV isolates (Table 3).

3. Results and Discussion

Pepper plants containing the *L4* gene were resistant to PMMoV without any leaf symptoms but the capsicum plants showed brown streaks in fruits during warm periods in the Mediterranean region, Turkey. The fruit symptoms seem strange; they are likely produced by PMMoV creating misconceived situations. Further symptomotological observations revealed that there were no virus symptoms developed until fruiting on which hypersensitive reactions (HR) were observed on the plants harboring the *L4* gene (Figure 1). Two weeks after mechanical inoculations, typical virus disease symptoms such as dwarfing on young plants, puckering, and yellow mottling on leaves appeared (Figure 2).

When inoculated pepper plants reached the fruiting period, their fruits were deformed and their size slightly reduced than older fruits which exhibited brown streaks with undesirable colors. The detection of ToBRFV by RT-PCR confirmed the presence of ToBRFV in tested pepper plants displaying similar symptoms with PMMoV inoculated resistant plants (Figure 3). The experiment was started in spring 2018 under controlled conditions and continued until the first days of summer. With the warming of the weather, the symptoms seen in the material plants began to appear more intensely. This situation was attributed to the fact that both the viruses had enough time to multiply in the plant and that the resistance might have been broken as a result of the increase in temperature.

Although, the inoculated resistant pepper plants were free from PMMoV infection, indicating that the *L4* gene is still conferring resistance to PMMoV, the *L4* gene-mediated resistance was no longer controlling resistance to the ToBRFV isolates either above 32 °C temperatures or repetitive inoculations (Figure 3). In mixed infections, it was very difficult to discriminate the PMMoV symptoms from *Tomato spotted wilt virus* (TSWV) symptoms (Fidan and Sarı 2019) which has been causing epidemics on-field and greenhouse grown pepper plants. Although, pepper seeds in fruits did not darken withTSWV infection, pepper seeds darkened from light to bold brown color as observed in PMMoV infections (Figure 4).

Visually, this is one of the best ways to distinguish the two viral diseases symptomatologically. We ensured that the *L4* gene still mediates resistance to PMMoV but it is not responsible to control resistance to ToBRFV. Producers have problems in mixed infections with TSWV and ToBRFV causes epidemics in all pepper-growing areas in the world. In the study, the *L4* gene

Table 3. Complete genome sequences of *Pepper mild mottle virus* isolates used in phylogenetic analyzis

Isolate Name	Origin	Source	GenBank Accession Number	Identity%
BL14	U.S.A	Pepper	MH063882	94.31
Chaff RNA	Korea	*Achyranthes aspera*	LC538100	94.49
ZJ2	China	Pepper	MN616927	94.65
BR-DF01	Brazil	Pepper	AB550911	94.31
PMMoV-16.9	India	Pepper	MN496154	94.60
VE	Venezuela	Pepper	KU312319	94.34
PMMoV-WW17	Slovenia	Tobacco	MN267900	94.37
IW	Japan	Pepper	AB254821	94.70
Spainish isolate	Spain	Pepper	AJ308228	100
Ailar3	Turkey	Pepper	MW523006	100
TBRFV-Ant-Pep	Turkey	Pepper	MT118666	93.33

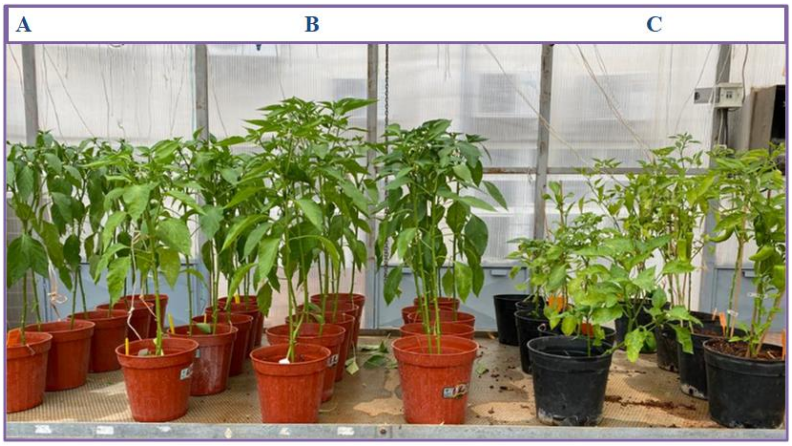

Figure 2. Plants carrying the *L3*, *L4* resistance gene and susceptible pepper plants lacking any of these genes, thet are mechanically inoculated with PMMoV. A) *L3* gene mediated resistant pepper plants, B) *L4* gene mediated resistant capsicums and C) Susceptible pepper plants containing any resistance gene.

Figure 3. Plants carrying the *L3*, *L4* resistance gene and susceptible pepper plants lacking any of these genes. are mechanically inoculated with ToBRFV. Their phenotypic reactions are observed at 30 days post inoculation. a) *L3* gene containing pepper fruits are infected with ToBRFV with typical Kebab appearance; b) *L3* resistance gene containing pepper plants infected with ToBRFV show mosaic symptoms on leaves; c) Healthy control pepper plants are inoculated with distilled water without any symptoms; d) Mottling symptoms in pepper plants infected with ToBRFV carrying the *L3* resistance gene. e) L3 and L4 gene containing pepper plants' stems are showing trunk necrosis, and f) L4 resistance gene containing capsicums are exhibiting HR after ToBRFV inoculations. The inoculated plants are kept below 32 °C in the growth chamber.

still provided resistance to PMMoV but further molecular analyzes revealed that ToBRFV was not able to control the *L4* resistance gene. Molecular studies with RT-PCR-based amplification using PMMoV specific primers showed that PMMoV infection is not detected in *L4* resistant plants, whereas viral infection is confirmed in no gene containing and *L3* gene containing pepper plants with amplifying 836 bp fragment to PMMoV (Figure 4).

Therefore, these results indicate that the *L4* gene mediates resistance against PMMoV infection and the *L4* resistance gene will be able to be used to control PMMoV infection for breeding studies in Turkey. Sequence data analysis revealed that there is no mutation in the genome of the PMMoV isolate (Ailar3) When comparing open reading frame (ORF) regions; no mutation was found in the (Ailar3). There is no mutation seen and the *L4* gene effectively mediates resistance against PMMoV in pepper plants. The sequence of the PMMoV was submitted to the NCBI GenBank with an MW523006 accession number.

A phylogenetic tree was constructed using our PMMoV sequence and other available sequences on the NCBI database, the constructed phylogenetic tree was divided into two main groups as Group 1 and Group 2 (Figure 5). Group 1 is further subdivided into subgroups 1a and 1b, respectively. The PMMoV isolate used in our study was in the same group (Group 2) as the Spanish and Korean isolates. These results indicate that there are close relationships among Turkish, Spanish, and Korean PMMoV isolates.

The results also suggest a divergent group of PMMoV isolates which share specific clustering motifs. When all the obtained ToBRFV (MT118666) and PMMoV (MW523006) genomes were compared, it was determined that they were typical *Tobamovirus* members with 4 ORFs as the genome structure, but when their ORF structures were analyzed on the basis of nucleotides, they were found to be separate viruses. These results also ensured that the two viruses were included in two separate branches in the phylogenetic tree (Figure 5).

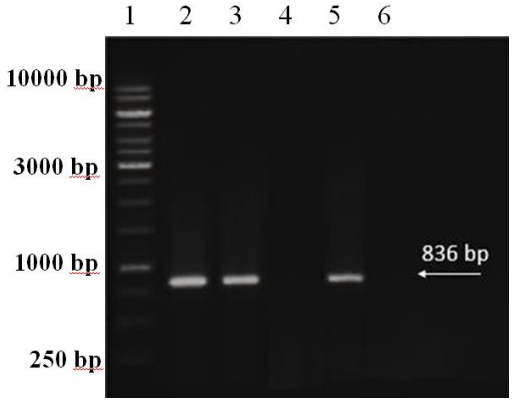

Figure 4. The *L3*, *L4* resistance gene and no gene containing susceptible pepper plants are inoculated with PMMoV and their total nucleic acids are studies in RT-PCR analyzes. 1) 1kb DNA ladder; 2) *L3* gene containing pepper plant; 3) *L4* gene containing pepper plant, 4) None of a gene containing susceptible pepper plant; 5) The PMMoV positive control pepper plant, 6) The PMMoV negative control pepper plant.

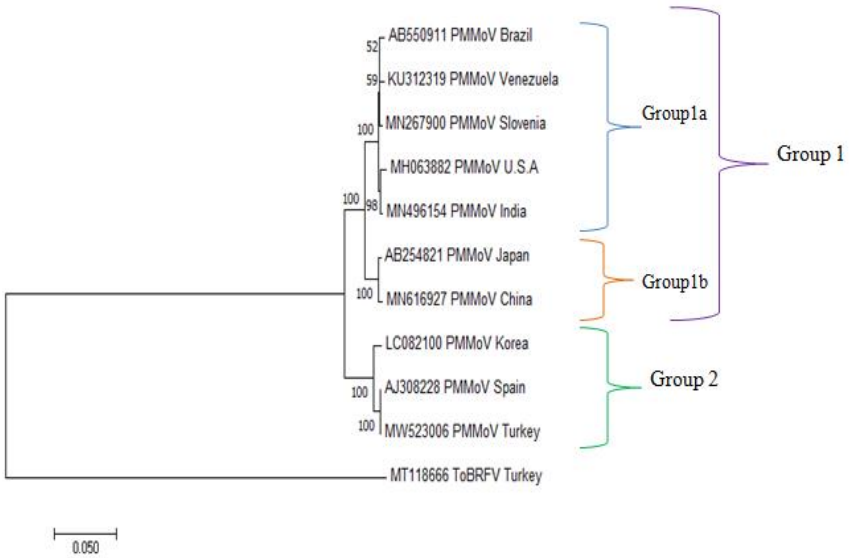

Figure 5. A phylogenetic tree was constructed with known PMMoV and ToBRFV Turkey sequences. It is clear that PMMoV is different from ToBRFV. All sequences were analyzed using MEGA 7.0 software according to the neighbor-joining method.

Although PMMoV and ToBRFV are two separate types of viruses in the *Tobamovirus* genus, it is difficult to distinguish their symptoms on pepper plants. Both viruses can be transmitted viainfected seeds, mechanical inoculations, Bombus bees, and irrigation. These viruses cause morphological changes in host cells resulting in dwarfism (Afaf et al. 2017), chlorosis, mottling, deformations, bleaching. It is known that all viruses are insensitive to certain chemicals; therefore, resistant pepper varieties are the only effective method for viral disease management. For the production of resistant pepper varieties, reliable sources of resistance are needed in the breeding studies of pepper seeds.

4. Conclusion

We observed the presence of PMMoV in the tested pepper plants under different temperature conditions during four seasons. As a result of the typical symptoms similar to the *Tobamovirus* group in peppers in 2018, it was thought that the *L4* gene-mediated resistance was broken in these plants. however, with the report of another virus belonging to the same family (ToBRFV) that caused similardisease symptoms in Turkey in 2019 (Fidan et al. 2021), the course of the study was shifted to this new virus, which has caused epidemics in pepper growing areas around the world. The molecular analysis performed revealed that the *L4* gene is most likely to control resistance to PMMoV (Hamada et al. 2002), but the *L4* resistance gene is not responsible for controlling ToBRFV. In RT-PCR assays utilizing specific primers, ToBRFV was found in plants with *L4* resistance.

This result revealed that the ToBRFV overcame the *L4* mediated resistance and it is likely that the *L3* resistant pepper plants are very susceptible to both PMMoV and ToBRFV infections with severe symptoms. Molecular studies were carried out by giving priority to the *Tobamoviruses* in studies conducted to investigate the source of infection. Accordingly, if there is an *L4* resistance gene in the infected pepper plant and symptoms are seen, it can be said that the cause of this infection is ToBRFV. If the infected pepper plant has *L3* resistance and *L4* resistance, the cause of the infection may be PMMoV and ToBRFV, respectively. The source of infection can easily be detected by the RT-PCR method using PMMoV and ToBRFV specific primers (Fidan et al. 2021). In our study, we aimed to determine the susceptibility or resistance levels of pepper fields against PMMoV infections, scanned samples using PMMoV genome primers in molecular studies, and obtained the complete genome sequence from samples with positive results.

Additionally, it has been found that the infections which cause browning and necrosis around the seed in plants containing the *L4* gene that provides resistance to PMMoV were not caused by PMMoV but by another virus in the *Tobamovirus* group, namely ToBRFV. In Turkey, PMMoV has been identified on pepper several times since 2013 (Caglar et al. 2013). In a study conducted in Antalya, it was reported that genes that provide monogenic resistance to TSWV such as *Tsw* and *Sw-5* become inactive at high temperatures and the state of resistance disappears (Kabas et al. 2021).

As a result of this study, it was revealed that the *L4* gene was broken by the ToBRFV in infections above 32 °C and consecutive infections. In other words, while resistance to the *Tobamovirus* group is effective under 32 °C, it can break at high temperatures (Kabas et al. 2022). The similarity of these symptoms with PMMoV showed that the *L4* gene still retained its activity against PMMoV. In cases, where the temperature limit of 32°C is exceeded, it is of great importance to conduct resistance studies against ToBRFV disease, which causes severe symptoms, and to find a new source of resistance.

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