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Araştırma Makalesi

Detection of Cucumber Mosaic Virus (CMV) in Cucurbits by TaqMan Real-Time RT-PCR in Hakkari Province, Türkiye

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ABSTRACT

Cucumber mosaic virus (CMV) is among the important viruses that can infect many crops. In 2022, this study was conducted in the Kırıkdağ, Otluca, and Merzan areas of the Hakkari province of Türkiye to investigate the presence of CMV in cucurbits exhibiting viral disease symptoms (yellowing, necrosis, mosaic, and deformity). A total of 50 samples; 7 watermelons (*Citrullus lanatus*), 3 melons (*Cucumis melo* L.), 17 cucumbers (*Cucumis sativus* L.), 8 zucchinis (*Cucurbita pepo* L.), 9 pumpkins (*Cucurbita moschata*), 2 gourds (*Lagenaria siceraria*) and 4 gherkins (*Cucumis melo* var. *flexuous*) were collected. The presence of CMV in the samples was investigated by TaqMan real-time RT-PCR using probes and primers specific to the 105 bp region of partial the coat protein gene of the virus. CMV-specific viral RNA was detected in 6 (12%) of the 50 samples analyzed. CMV was detected most commonly in the Otluca area and melon (33.3%) among cucurbit varieties. CMV agent was not detected in the samples collected from the Merzan area. The TaqMan real-time RT-PCR assay used in this study demonstrated that the method can be widely used for the general detection of CMV in a wide range of hosts. This is the first report on the detection of CMV in cucurbits in the Hakkari province of Türkiye.

Key words: Cucumber mosaic virus; cucurbits; TaqMan real-time RT-PCR; Hakkari

Hakkari İlinde Kabakgillerde TaqMan Real-Time RT-PCR ile Hıyar Mozaik Virüsü (CMV)'nün Tespiti

ÖZ

Hıyar mozaik virüsü (Cucumber mosaic virus; CMV) birçok ürünü enfekte edebilen önemli virüsler arasındadır. Bu çalışma, 2022 yılında Hakkari ili Kırıkdağ, Otluca ve Merzan alanlarında viral hastalık belirtileri (sararma, nekroz, mozaik ve şekil bozukluğu) gösteren kabakgillerde CMV varlığının araştırılması amacı ile yapılmıştır. Toplam 50 örnek; 7 karpuz (*Citrullus lanatus*), 3 kavun (*Cucumis melo* L.), 17 hıyar (*Cucumis sativus* L.), 8 kabak (*Cucurbita pepo*), 9 kabak (*Cucurbita moschata*), 2 su kabağı (*Lagenaria siceraria*) ve 4 acur (*Cucumis melo* var. *flexuous*) toplanmıştır. Örneklerde CMV varlığı, virüsün kısmi kılıf protein geninin 105 bç bölgesine spesifik problar ve primerler kullanılarak TaqMan real-time RT-PCR ile araştırılmıştır. Analizi yapılan 50 örneğin 6'sında (%12) CMV spesifik viral RNA tespit edilmiştir. CMV en sık Otluca alanında ve kabakgil çeşitleri arasında da kavunda (%33.3) tespit edilmiştir. Merzan'dan toplanan örneklerde CMV etmenine rastlanmamıştır. Bu çalışmada kullanılan TaqMan real-time RT-PCR testi, CMV'nin genel tespiti için bu yöntemin çok çeşitli konukçularda yaygın olarak kullanılabileceğini göstermiştir. Bu çalışma Türkiye'nin Hakkari ilinde kabakgillerde CMV'nin tespitine ilişkin ilk rapordur.

Anahtar kelimeler: Hıyar mozaik virüsü; kabakgiller; TaqMan real-time RT-PCR; Hakkari

INTRODUCTION

The Cucurbitaceae family includes most of the plant species used as human food (Lira Saade and Montes Hernández, 1994). Among the cucurbits in this family, watermelon (*Citrullus lanatus* L.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), and squash (*Cucurbita moschata* Duch., *Cucurbita pepo* L., and *Cucurbita maxima* Duch. ex Lam) are the most widely known crops worldwide (Andres, 2004; Simson, 2006). Cucurbits originated in the New World and Asia about 11.000 years ago and have recently been cultivated in Africa (Chomicki et al., 2020). There are about 125 genera and 960 species belonging to this family in tropical and temperate climatic regions of the world (Jeffrey, 2005). Various diseases that limit the production of cucurbit crops are caused by bacteria, fungi, viruses, and virus-like organisms. Approximately 60 viruses and virus-like disease agents have been identified. These include cucumber mosaic virus (CMV), squash mosaic virus (SqMV), zucchini yellow mosaic (ZYMV), watermelon mosaic 1 (WMV-1), watermelon mosaic 2 (WMV-2), cucurbit aphid-borne yellows (CABYV), tobacco mosaic virus (TMV) and papaya ringspot virus (PRSV) are among the major viruses damaging cucurbits (Kaya and Erkan, 2011; Lecoq and Desbiez, 2012; Karanfil, 2022).

CMV is a spherical virus belonging to the genus Cucumovirus in the family *Bromoviridae* and is a positive sense single-strand RNA (ssRNA) virus. The viral genome consists of three segments. The RNA1, RNA2, and RNA3 segments of the viral genome consist of approximately 3350, 3050, and 2200 nucleotides, respectively. RNA3 can also contain a fourth RNA (RNA4; ~1030 nucleotides). Another RNA structure related to CMV is RNA5, which is called satellite RNA (Kaper and Waterworth, 1981; Martelli and Quacquarelli, 1988). CMV isolates are divided into two subgroups (I and II) according to the genetic structure and nucleotide sequence similarity of their RNAs (Gallitelli, 2000; Hsu et al., 2000). According to the classification made in recent years, CMV isolates are classified as IA and IB in subgroup I (Roossinck, 2001). CMV is a virus with a wide host range that can infect 1200 plant species in more than 100 families (Zitter and Murphy, 2009; Jacquemond, 2012). Among its natural hosts, cucurbits, legumes (Fabaceae), and eggplants (Solanaceae) have been reported (Brunt et al., 1996). CMV has the largest host range in the world after tobacco mosaic virus (TMV) (Zitter and Murphy, 2009). CMV is non-persistently transmitted by more than 80 aphid species (Insecta: Hemiptera: *Aphidoidae*) (Francki et al., 1979; Raccah et al., 1985; Kaper and Waterworth, 1981; Palukaitis et al., 1992). *Aphis gossypii, Myzus persicae,* and *Acyrthosiphon pisum* are the most important aphids (Palukaitis et al., 1992; Grube et al., 2000). In addition to aphid transmission, CMV can also be transmitted from plant to plant by mechanical inoculation, grafting, and seeds of different plant species (Tomlinson and Carter, 1970; Neergaard, 1977; Zitter and Murphy, 2009). In cucurbits, CMV is one of the most important viral agents causing severe mosaic and deformities and causing economic losses (Harth et al., 2017). In plants infected with CMV, plant mortality can occur as a result of symptoms such as mosaic, fruit, and leaf deformities.

In this study, molecular detection of CMV agent in Hakkari province, where cucurbit crops are cultivated, was carried out by TaqMan real-time RT-PCR test. The prevalence and disease rate of CMV were determined in Kırıkdağ, Merzan, and Otluca areas where the samples were collected.

MATERIAL and METHODS

Field studies and sample collection

In 2022, in August-September, a survey was conducted in the Kırıkdağ, Otluca, and Merzan areas of Hakkari where cucurbit crops are cultivated and leaf samples were collected from cucumber, zucchini, watermelon, melon, pumpkin, gourd, and gherkin exhibiting symptoms such as yellowing, mosaic, deformity, and stunting. The samples were labelled with field information in polyethylene bags and transported to the laboratory in cool conditions and stored at 4°C until tested.

Total nucleic acid extraction

Total nucleic acid extraction was performed from leaf samples collected during the field study using a High Pure Viral Nucleic Acid Kit as specified in the manufacturer's (Roche, Germany) protocol.

Detection of CMV by TaqMan real-time RT-PCR

Probe and primers targeting the partial coat protein of the virus were used for CMV detection (Xu et al., 2022). Primer and probe sequences are given in Table 1. The reaction mixture for real-time RT-PCR analysis was performed using AgPath-ID™ One-Step RT-PCR kit (Thermo, USA). In the study, a 20 µl reaction mixture was prepared for each sample for real-time RT-PCR analysis. 2X RT-PCR buffer 10 µl, forward primer: 5'-GACAGTCCGTAAAGTTCC-3' (20 µM) 0.4 µl, reverse primer: 5'-GATGCAGCGTACTGATAA-3' (20 µM) 0.4 µl, TaqMan probe: Texas Red-5'-TATCCGTTGCCGCCATCTCT-3'-BHQ2 (20 µM) 0.4 µl, 25X RT-PCR enzyme mix 0.8 µl, RNA 3 µl and nuclease-free water 5 µl were used. Real-time RT-PCR analysis was performed using BIORAD CFX96

Real-Time System C1000 Touch. Amplification conditions were reverse transcription at 45°C for 10 minutes, and initial denaturation at 95°C for 10 minutes. The amplification step consisted of 40 cycles in total; 95°C for 15 seconds and 58.4°C for 45 seconds. In PCR optimization studies, CMV isolates previously found to have high absorption values in serological tests were used as positive control, and sterile nuclease-free water was used as negative control.

RESULTS and DISCUSSION

Field observation

General disease symptoms such as yellowing, mosaic, deformity, curling, and shrinking of leaves and necrotic spots were observed during surveys conducted in cucurbit growing areas in Merzan, Otluca, and Kırıkdağ of Hakkari province (Fig. 1).

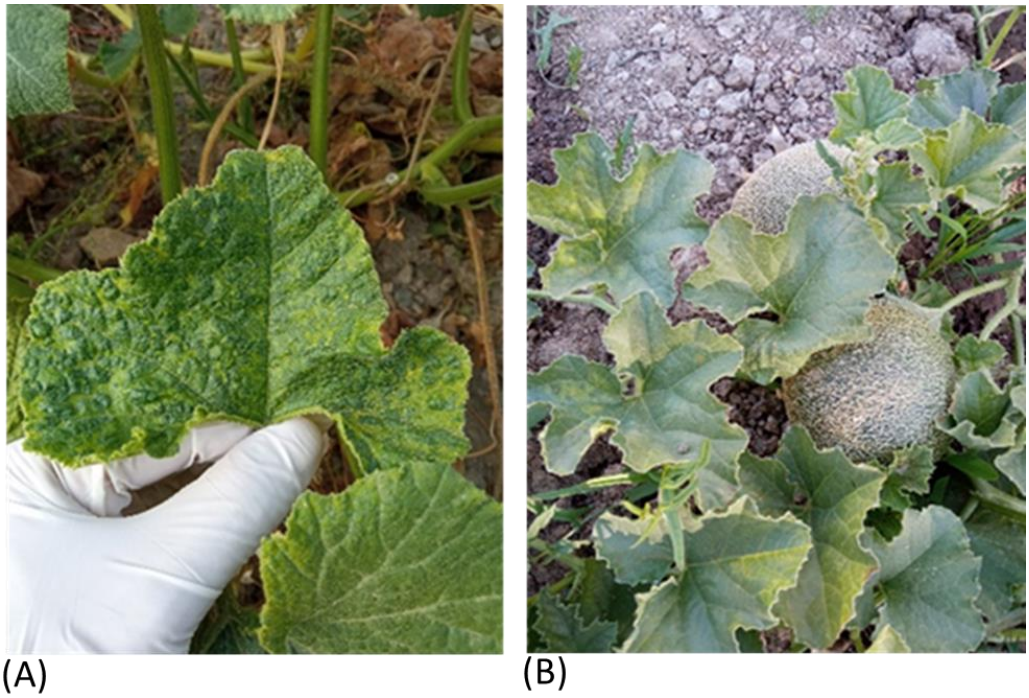

Figure 1. Symptomatic plants collected in the field survey from Hakkari province; (A) Cucumber, (B) Melon

In the survey study, 50 samples were collected from 15 fields shown in Table 1 from the cucurbit production areas of Hakkari Province. The distribution of cucurbits according to areas and varieties is given in Table 2.

Table 1. List of cucurbit samples collected from Hakkari province for TaqMan real-time RT-PCR

Number	Host	Location
1	Pumpkin	Kırıkdağ
2	Melon	Kırıkdağ
3	Gherkin	Kırıkdağ
4	Gourd	Kırıkdağ
5	Zucchini	Kırıkdağ
6	Cucumber	Kırıkdağ
7	Zucchini	Kırıkdağ
8	Cucumber	Kırıkdağ
9	Gourd	Kırıkdağ
10	Watermelon	Kırıkdağ
11	Watermelon	Kırıkdağ
12	Watermelon	Kırıkdağ
13	Pumpkin	Kırıkdağ
14	Watermelon	Kırıkdağ

15	Cucumber	Kırıkdağ
16	Watermelon	Kırıkdağ
17	Pumpkin	Kırıkdağ
18	Cucumber	Kırıkdağ
19	Pumpkin	Kırıkdağ
20	Cucumber	Kırıkdağ
21	Zucchini	Kırıkdağ
22	Zucchini	Kırıkdağ
23	Pumpkin	Kırıkdağ
24	Cucumber	Kırıkdağ
25	Gherkin	Kırıkdağ
26	Watermelon	Kırıkdağ
27	Cucumber	Kırıkdağ
28	Gherkin	Kırıkdağ
29	Watermelon	Kırıkdağ
30	Cucumber	Kırıkdağ
31	Cucumber	Kırıkdağ
32	Cucumber	Kırıkdağ
33	Pumpkin	Merzan
34	Melon	Merzan
35	Zucchini	Merzan
36	Zucchini	Merzan
37	Cucumber	Merzan
38	Cucumber	Merzan
39	Cucumber	Merzan
40	Pumpkin	Merzan
41	Zucchini	Merzan
42	Cucumber	Otluca
43	Cucumber	Otluca
44	Pumpkin	Otluca
45	Pumpkin	Otluca
46	Cucumber	Otluca
47	Gherkin	Otluca
48	Melon	Otluca
49	Cucumber	Otluca
50	Gherkin	Otluca

Table 2. Distribution of cucurbits collected from Hakkari province according to areas and hosts

Area	Watermelon	Melon	Cucumber	Zucchini	Pumpkin	Gherkin	Gourd	Total
Kırıkdağ	7	1	10	5	5	2	2	**32**
Merzan	-	1	3	3	2	-	-	**9**
Otluca	-	1	4	-	2	2	-	**9**
Total	**7**	**3**	**17**	**8**	**9**	**4**	**2**	**50**

Molecular detection

The presence of CMV-specific RNA was detected in 6 (12%) of the 50 cucurbit samples (samples numbered 15, 18, 20, 47, 48, 49) (Fig. 2). When the infected sample types were examined, it was determined that samples 15, 18, and 20 were cucumber varieties and collected from the Kırıkdağ, and samples 47, 48, and 49 were collected from the Otluca and were determined to be gherkin, melon and cucumber varieties, respectively. CMV was not detected in the samples collected from the Merzan area. The samples in which CMV was detected and Cq (Cycle quantification) values are given in Table 3.

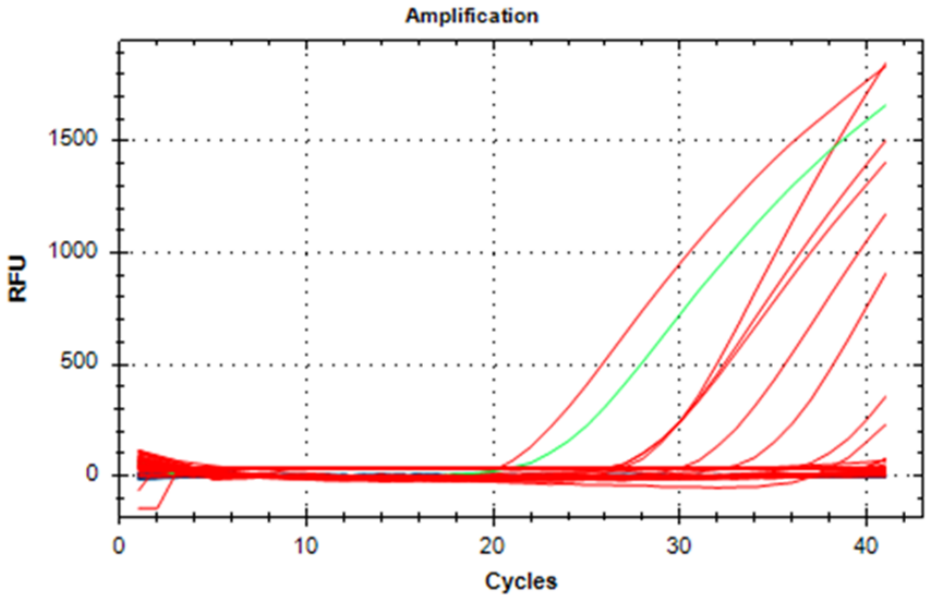

Figure 2. Result of TaqMan real-time RT-PCR analysis. Red: Amplification curves of samples 15, 18, 20, 47, 48, 49; Green: Positive control; Black: Negative control

Table 3. Cq values obtained from TaqMan real-time RT-PCR analyses of different plant samples collected from Hakkari province

No	Host	The collected field	Cq Value
15	Cucumber	Kırıkdağ	29.66
18	Cucumber	Kırıkdağ	26.62
20	Cucumber	Kırıkdağ	32.06
47	Gherkin	Otluca	25.63
48	Melon	Otluca	20.11
49	Cucumber	Otluca	25.88

In this study, the presence of CMV was detected in different hosts. Samples with a Cq value ≤32.06 were considered to be infected with CMV. The Cq value of the positive control was 17.53, but the Cq values of other samples considered positive could be higher. Three of the 32 samples tested from the Kırıkdağ and 3 of the 6 samples tested from the Otluca area were found to be infected with CMV. CMV infection was not detected in the Merzan (Table 4). According to the cucurbit plant varieties tested, CMV prevalence was found to be 25% in 4 gherkin plants, 21.4% in 14 cucumber plants, and 33.3% in 3 melon plants. It is worth noting that CMV infected regions are rare. CMV infected cucurbits are generally concentrated in Otluca. The CMV infection rate was generally low in cucurbit samples collected during this survey in Hakkari province.

Table 4. CMV infection rate in cucurbit samples collected from Hakkari province

Region	Samples tested	CMV infected samples	Infection rate (%)
Kırıkdağ	32	3	9,3
Merzan	12	-	-
Otluca	6	3	50
TOTAL	**50**	**6**	**12**

The CMV is one of the important plant viruses that can infect different host varieties. After being reported by Doolittle on cucumber plant in the USA in 1916, it has been reported in China, Iran, Tunisia, Brazil, Serbia, Syria, India, Nigeria, and Egypt (Francki et al., 1979; Brunt et al., 1996; Li et al., 2004; Bashir et al., 2006; Mnari-Hattab et al., 2008; Silveira et al., 2009; Vučurović et al., 2012; Mando et al., 2011; Jacquemond, 2012; Suresh et al., 2013; Ayo-John et al., 2014; Shehata et al., 2023).

Plant viruses have long been diagnosed by serological methods. The enzyme-linked immunosorbent assay (ELISA) method is preferred as a very useful method when the number of samples is large (Abdalla et al., 1991), but the ELISA method is not sensitive enough and it is necessary to produce antiserum for viruses in this test. In recent years, molecular techniques have been more widely used in the diagnosis of plant virus diseases. Using molecular methods, it is possible to detect viral DNA and RNA present directly in samples (Clementi et al., 1993). Since the majority of plant viruses have a single-stranded RNA genome, the reverse transcription-polymerase chain reaction (RT-PCR) method has been used to identify a large number of viruses (Henson and French, 1993). However, real-time RT-PCR has the advantage over conventional PCR in that it is faster, more sensitive, and minimizes the risk of contamination in its application (Mackay, 2004).

CMV has been detected in various regions of Türkiye by serological and molecular detection methods. In many studies conducted in Türkiye, the presence of CMV has been detected in different hosts other than bulbous ornamental plants, greenhouse peppers, tobacco, spinach, tomatoes, and different cucurbits (Soylu, 2014; Can, 2019; Kurtoğlu and Korkmaz, 2018; Daş, 2020; Günay and Usta, 2020; Papan, 2022; Sharma, 2023). The methods used in these studies are generally DAS-ELISA and RT-PCR methods. Among the studies conducted in cucurbits; cucurbit plants were collected from the Marmara region and tested serologically. CMV was detected at a rate of 52% in cucumber. It has been reported that CMV causes local and systemic infections in cucumber, squash, melon, and watermelon (Nogay and Yorgancı, 1984; 1985). It is seen that geographical location may affect the results in the investigation of CMV prevalence in our country. The CMV detection rate is quite high at 52%. Arlı-Sökmen and Şevik (2004) detected the region containing the coat protein gene of the CMV agent isolated from samples collected from cucumber production areas in Samsun province using the RT-PCR method. Yeşil (2013) collected 652 cucurbits, 92 seed, and 85 weed samples from cucurbit production areas of Konya, Karaman, and Aksaray provinces in 2009 and 2010. Using DAS-ELISA and RT PCR tests, viral infection was detected in 83.4% of the plant samples, 50.6% of the weed samples, and 8.7% of the seed samples. CMV was detected in 21.3% of the cucurbit plant samples and 1.1% of the seed samples tested. In this study, CMV was detected serologically and molecularly and CMV was detected at a higher rate than in our study. It is thought that this situation may be affected by geographical location. Hakkari province is isolated from nearby provinces due to its geographical location. CMV was detected in the cucumber plant in Diyarbakır province, which is geographically close to Hakkari province (Güller et al., 2024). In Antalya province and its districts, the presence of CMV was detected by serological and biological methods in 455 samples collected from greenhouse cucumber and zucchini production areas between 2014 and 2015. CMV was detected at 3.9% in cucumber samples and 6.35% in zucchini samples (Çat et al., 2016). In our study, CMV was detected at a higher rate of 21.4% in cucumber samples collected, while CMV was not detected in zucchini samples. Cucumber cultivation is more common in Hakkari province and the necessary measures to increase production are insufficient. Due to this situation, CMV rates have shown variability. Güller and Usta (2020) took samples from melon plants showing deformation, mosaic pattern, and vein banding on leaves in field studies conducted in Bingöl province in 2019 and tested by RT-PCR method and CMV was detected. Bingöl CMV isolates were grouped with CMV-IB subgroup isolates. Karanfil and Korkmaz (2021) collected 72 plant samples from cucurbit production areas for the detection of CMV infection in Çanakkale, Balıkesir, and Bursa provinces of the South Marmara Region and determined CMV infection in 10 samples by DAS-ELISA method. To perform molecular characterization of the CMV isolates obtained, 3 of the samples were selected according to their geographical origin, and all coat protein genes were amplified, cloned, and sequenced by RT-PCR. As a result of the phylogenetic analysis, it was determined that two of the CMV isolates were in subgroup IA and one was in subgroup IB. In this study, the molecular detection rate of CMV was 1.4%. The low rate of samples collected from different provinces suggests that there is crop production based on integrated pest management principles. In our country, studies on the detection of CMV by real-time RT-PCR are also very limited. Uzunoğulları and Gümüş (2015) collected a total of 235 tomato, pepper, cucumber, zucchini and gladiolus leaf samples from Çanakkale, Bursa, Yalova, İstanbul, Bilecik and Sakarya in June and July 2013. The samples were first analyzed by the DAS-ELISA method. 69% of the pepper samples, 87% of the tomato samples, 75% of the squash samples, 58% of the cucumber samples, and 69% of the gladiolus samples were found to be infected with CMV. They then compared the results of real-time RT-PCR, conventional RT-PCR analysis, and DAS-ELISA. In real-time RT-PCR analysis, they reported that CMV-positive gladiolus, pepper, tomato, zucchini, and cucumber samples gave a melting peak between 84.0-88.7ºC while the negative control gave a melting peak at 76.0ºC. In their study, real-time RT-PCR analysis was performed with the dye called Syber Green. In our study, the TaqMan probe was used.

Apart from our country, the CMV agent has also been detected in other countries, and precautions are being taken. Nagendran et al. (2017) conducted surveys in the southern Indian state of Tamil Nadu between 2012 and 2014 to determine the virus diseases infecting cucurbits. The collected plant samples were tested by classical PCR, RT-PCR, and DIBA (Dot Immuno Binding Assay) methods to detect CMV, PRSV, ZYMV, CGMMV, and

begomoviruses. CMV was detected at a rate of 5%. This rate is low compared to the rate of 12% in our study. Omar and Fadel (2022) found that the most common symptoms in naturally infected plants in Libya were severe mosaic, and mild mosaic in cucumbers, peppers, tomatoes, and eggplants, and tested samples with RT-PCR, suspecting that CMV caused these symptoms. One tomato and one cucumber sample were detected as CMV positive. As a result of phylogenetic analysis, a comparison of the CP sequences of two Libyan isolates obtained from tomato and cucumber plants with other isolates reported in the gene bank revealed that these isolates were clustered together in the same branch, but there was a higher genetic difference between the two Libyan isolates. Xu et al. (2022) developed a fast, effective, and sensitive detection method for the simultaneous detection and specific quantification of these viruses to prevent the emergence and spread of 5 viruses, including CMV, which are of worldwide importance in lilies (*Lilium* spp.). For the simultaneous detection of these viruses in Chinese lily samples, primers and probes for multiplex TaqMan real-time RT-PCR analyses designed from conserved regions of the coat protein sequence of each virus were used. In our study, Cq values increased as virus density decreased in cucurbits. This may be due to the evaluation of different hosts. Borah et al. (2023), squash plants showing symptoms such as chlorotic spots, mosaic, mottling, and leaf deformation were collected and analyzed for the presence of viruses using RT-PCR. Sequence similarity analysis and phylogenetic studies revealed that the isolated virus belonged to subgroup IB of CMV. Ali et al. (2023) in their study in Iraq, detected CMV isolates in cucumber and cowpea plants exhibiting mosaic, mottling, and leaf disorders in local fields in Baghdad province. CMV was characterized by RT-PCR. Phylogenetic analysis based on the CMV CP gene sequence clustered the Iraqi CMV isolate with members of subgroup IB. Nowadays, PCR is one of the most reliable and accurate methods for the diagnosis of plant virus diseases. Although the ELISA method is still used in the diagnosis of diseases, negative results in the ELISA test can turn into positive results with PCR. The studies are based on molecular detection of CMV and phylogenetic analysis of selected isolates.

CONCLUSION

CMV infection rate was generally low in cucurbit samples collected during this survey in Hakkari province. It is noteworthy that local seed use was common in the observed areas. Although there have been several attempts to detect the presence of CMV in Türkiye, this is the first report on the molecular detection of CMV in different hosts by TaqMan real-time RT-PCR in Hakkari. In conclusion, the TaqMan real-time RT-PCR assay demonstrated that the method can be widely used for the general detection of CMV in a wide range of hosts.

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REFERENCES

Abdalla, O. A., Desjardins, P. R., Dodds, J. A. 1991. Identification, disease incidence and distribution of viruses infecting peppers in California. *Plant Disease,* 75: 1019-1023.

Ali, A. K., Ahmed, I. A., Al-Kuwaiti, N. A. 2023. Molecular of Cucumber Mosaic Virus Subgroup IB in Iraq. *Plant Protection*, 7(2), 289-294.

Andres, T.C. 2004. The Cucurbit Network. http://www.cucurbit.org. http://www.cucurbit.org/family.html.

Arlı-Sökmen, M., Şevik, M. A. 2004. Reverse transkriptaz-polimeraz zincir reaksiyon (RT-PCR) yöntemi kullanılarak hıyar mozayik virüsü enfeksiyonunun belirlenmesi. *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi*, 19(3): 14-18. https://doi/full/10.5555/20043205625

Ayo-John, E.I., Olorunmaiye, P. M., Odedara, O.O., Dada, O. B., Abiola, K. O., Oladokun, J. O. 2014. Assessment of field-grown cucurbit crops and weeds within farms in South West Nigeria for viral diseases. Academic

Pres, *Cluj-Napoca, Romania, Notulae Scientia Biologicae*, 6(3): 321-325. https://doi.org/10.15835/nsb639315

Bashir, N. S., Kalhor, M. R., Zarghani, S. N. 2006. Detection, differentiation and phylogenetic analysis of cucumber mosaic virus isolates from cucurbits in the northwest region of Iran. *Virus Genes*, 32(3): 277-288. https://doi.org/10.1007/s11262-005-6912-2

Borah, M., Gowtham, K. R., Rupsanatan, M., Palash, D. N. 2023. Molecular characterization and Efficacy Study of Different Treatments Against Cucumber mosaic virus in Pumpkin (Cucurbita moschata). *Biological Forum-An International Journal*, 15(2): 1053-1063.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., Zurcher, E. 1996. Plant Virus Online. Descriptions and Lists from the Vide Database. http://biology.anu.edu.au/Groups/MES/vide/.

Can, S. 2019. Akdeniz bölgesinde örtü altı biber üretim alanlarında hıyar mozaik virüsü (Cucumber Mosaic Virus, CMV)'nün saptanması ve karakterizasyonu, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Adana.

Chomicki, G., Kiers, E. T., Renner, S. S. 2020. The evolution of mutualistic dependence. *Annual Review of Ecology, Evolution, and Systematics,* 51, 409-432. https://doi.org/10.1146/annurev-ecolsys-110218-024629

Clementi, M., Menzo, S., Bagnarelli, P., Manzin, A., Valenza, A., Varaldo, P.E. 1993. Quantitative PCR and RT-PCR in virology. *PCR methods and applications*, 2, 191-191.

Çat, A., Yardımcı, N., Çulal Kılıç, H. 2016. Antalya İli ve İlçelerindeki Örtüaltı Hıyar (Cucumis sativus L.) ve Kabak (Cucurbita pepo L.) Üretim Alanlarında Viral Etmenlerin Saptanması. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi,* 20(1): 129-132. DOI: 10.19113/sdufbed.53635

Daş, F. 2020. Adana ve Mersin illeri domates alanlarında hıyar mozaik virüsü (Cucumber mosaic virus, CMV) nün karakterizasyonu. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Adana.

Doolittle, S. P. 1916. A new infectious mosaic disease of cucumber. *Phytopathology,* 6(2), 145-147.

Francki, R. I. B., Mossop D. W., Hatta, T. 1979. Cucumber mosaic virus. CMI/AAB Description Viruses No. 213: 6p.

Gallitelli, D. 2000. The ecology of Cucumber mosaic virus and sustainable agriculture. *Virus Research,* 71: 9-21. https://doi.org/10.1016/S0168-1702(00)00184-2

Grube, R. C., Zhang, Y., Murphy, J. F., Loaıza-Fıgueara, F., Lackney, V. K., Provvidenti, R., Jahn, M. K. 2000. New Source of Resistance to Cucumber mosaic virus in Capsicum Frutescens. *Plant Disease,* 84: 885-891. https://doi.org/10.1094/PDIS.2000.84.8.885

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. *Türk Tarım ve Doğa Bilimleri Dergisi*, 7(4): 948-958. https://doi.org/10.30910/turkjans.740195

Güller, A., Demirel, S., Usta, M., Korkmaz, G. 2024. Genetic Diversity of Cucumber mosaic virus in Cucumber Plants Grown in Diyarbakır. *Turkish Journal of Nature and Science*, 13(1):1–6.

Günay, A., Usta, M. 2020. First Investigation of Five Tobacco Viruses using Pcr Based Methods In Tobacco Plants Grown In Adıyaman Turkey. *Fresenius Environmental Bulletin*, 29, 11624–11632.

Henson, J. M., French, R. 1993. The polymerase chain reaction and plant disease diagnosis. *Annual Rev. Phytopathology*, 31: 81-109.

Harth, J. E., Simmons, H. E., Stephenson, A. G. 2017. Vertical infection of Zucchini yellow mosaic virus via pollen transmission occurs at a lower frequency than ovule transmission. *European Journal of Plant Pathology,* 147(3): 717-720. https://doi.org/10.1007/s10658-016-1024-5

Hsu, H. T., Barzuna, L., Hsu, Y. H., Bliss, W., Perry, K. L. 2000. Identification and Subgrouping of Cucumber Mosaic Virus with Mouse Monoclonal Antibodies. *Phytopathology,* 90, 615- 620. https://doi.org/10.1094/PHYTO.2000.90.6.615

Jacquemond, M. 2012. Cucumber Mosaic Virus, *Advances in Virus Research*, 84, 439–504. https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Jeffrey, C. 2005. A new system of Cucurbitaceae. Botanicheskii Zhurnal, 90: 332-335.

Kaper J. M., Waterworth H. M. 1981. Cucumoviruses. Hand Book of Plant Virus Infections, 257-263.

Karanfil, A., Korkmaz, S. 2021. Güney Marmara Bölgesi kabakgil üretim alanlarında cucumber virus enfeksiyonunun tespiti ve kılıf protein gen diziliminin filogenetik analizi. *Ege Üniversitesi Ziraat Fakültesi Dergisi,* 58(2), 239-246. https://doi.org/10.20289/zfdergi.682293

Karanfil, A. 2022. Marmara Bölgesi Kabak Üretim Alanlarında Tobacco Mosaic Virus İzolatlarının Yaygınlığı ve Moleküler Karakterizasyonu. *Journal of Advanced Research in Natural and Applied Sciences,* 8(2), 163-170. https://doi.org/10.28979/jarnas.981619

Kaya, A., Erkan, S. 2011. İzmir, Aydın, Manisa ve Balıkesir İllerinde üretilen kabakgillerdeki viral etmenlerin tanılanması ve yaygınlıklarının belirlenmesi. *Bitki Koruma Bülteni,* 51(4): 387-405.

Kurtoğlu, A., Korkmaz, S. 2018. Çanakkale İli Ispanak Üretim Alanlarındaki Hıyar Mozaik Virüsü (Cucumber Mosaic Virus; CMV) Enfeksiyonunun Belirlenmesi ve Moleküler Karakterizasyonu. *J. Turk. Phytopath.,* 47(2): 43-51.

Lecoq, H., Desbiez, C. 2012. Virus of cucurbit crops in the Mediterranean Region: an ever-changing picture. In: Loebenstein, G., Lecoq, H. (Eds.), Viruses and Virus Diseases of Vegetables in the Mediterranean Basin. *Adv. Virus Res.,* 84. Elsevier, USA, 67-126. https://doi.org/10.1016/B978-0-12-394314-9.00003-8

Li, S., Wang, H. Huo, Z. Pang, J. 2004. Detection of main casual virus in cucumber by RT-PCR. *Acta Agriculturae Boreali-Sinica,* 19(3): 100-102.

Lira-Saade, R., Montes-Hernandez, M. 1994. Cucurbits (Cucurbita spp.). In J. Hernando Bermejo, J. Leon (Eds.), Neglected crops: 1492 from a different perspective, plant production and protection 26 (pp. 63–77). Rome, Italy: FAO.

Mackay, I. M. 2004. Real-time PCR in the microbiology laboratory. *Clinical microbiology and infection*, 10(3): 190-212.

Mando, M. J., Kasem, A. A. H., Al-Chaabi, S., Kumari, S. G., Turina, M. 2011. Survey of some mosaic viruses on cucurbits in Syria and molecular detection of Zucchini yellow mosaic virus. *Arab Journal of Plant Protection,* 29(1): 14-20.

Martelli, G. P., Quacquarelli, A. 1988. The Present Status of Tomato and Pepper Viruses. *ACTA Horticulture,* 127: 39-64. DOI: 10.17660/ActaHortic.1983.127.3

Mnari-Hattab, M., Jebari, H., Zouba, A. 2008. Identification and distribution of viruses responsible for mosaic diseases affecting cucurbits in Tunisia. *Bulletin OEPP/EPPO,* 38(3): 497-506. DOI: 10.1111/j.1365-2338.2008.01269.x

Nagendran, K., Mohankumar, S., Aravintharaj, R., Balaji, C. G., Manorenjitham, S. K., Singh, A. K., Rai, A. B., Singh, B., Karthikeyan, G. 2017. The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu State, India. *Crop Protection,* 99, 10-16.

Neergaard, P. 1977. Seed-borne viruses. Chapter 3 in "seed Pathology, vol. I." Macmillan Press, London, and Madras, 839 p. https://doi.org/10.1016/j.cropro.2017.05.006

Nogay, A., Yorgancı, Ü. 1984. Investigations on the identification, seed transmission and host range of viruses infecting the culture plants in the Cucurbitaceae in Marmara region. 1. The identification of viruses infecting cucurbits in Marmara region. *Journal of Turkish Phytopathology,* 13(1): 9-27.

Nogay, A., Yorgancı, Ü. 1985. Investigations on the identification, seed transmission, and host range of viruses infecting the culture plants in the Cucurbitaceae in Marmara region. 2- The seed transmissibilities and cucurbit hosts of CMV and WMV-2 isolated from the culture plants in the Cucurbitaceae. *Journal of Turkish Phytopathology*, 14(1): 9-16.

Papan, A. N. 2022. Bandırma yöresinde salçalık domates yetiştirilen alanlarda Hıyar mozaik virüsü (Cucumber mosaic virus, CMV)' nün saptanması ve karakterizasyonu. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 96s, Adana.

Omar, S. M., Fadel, S. F. 2022. Molecular Characterization of Cucumber mosaic virus (CMV) local isolates in Libya. *Libyan Journal of Basic Sciences (LJBS),* 18: 93-105.

Palukaitis, P., Rossinck, M. J., Dietzgen, R. G., Francki, R. I. B. 1992. Cucumber mosaic virus. *Advances in Virus Research,* 41: 281-348. DOI: 10.1016/s0065-3527(08)60039-1

Raccah, B., Galon, A., Eastop, V. F. 1985. The role of flying aphid vectors in the transmission of cucumber mosaic virus and potato virus to peppers in Israel. *Ann. Appl. Biol.,* 106: 451-460. https://doi.org/10.1111/j.1744-7348.1985.tb03135.x

Roossinck, M. J. 2001. Cucumber mosaic virus, a model for RNA virus evolution. *Molecular Plant Pathology,* 22: 59-63. https://doi.org/10.1046/j.1364-3703.2001.00058.x

Sharma, S. 2023. Domateste Cucumber mosaic virus (CMV)'un Biyolojik ve Moleküler Karakterizasyonu. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İzmir.

Shehata, W. F., Iqbal, Z., Abdelbaset, T. E., Saker, K. I., El Shorbagy, A. E., Soliman, A. M., Sattar, M. N., El-Ganainy, S. M. 2023. Identification of a Cucumber Mosaic Virus from Cucurbita pepo on New Reclamation Land in Egypt and the Changes Induced in Pumpkin Plants. *Sustainability,* 15(12): 9751. https://doi.org/10.3390/su15129751

Silveira, L. M., Queiroz, M. A., Lima, J. A. A., Nascimento, A. K. Q., Lima Neto, I. S. 2009. Serological survey of virus in cucurbit species in the Lower Middle São Francisco River Basin, Brazil. *Tropical Plant Pathology,* 34(2): 123-126. https://doi.org/10.1590/S1982-56762009000200008

Simson, M. G. 2006. Plant Systematics. Elsevier Academic Pres., ISBN: 978-0-12-644460-9, 599. California, USA.

Soylu, B. 2014. Çanakkale ili Ev Bahçelerinde Yetiştirilen Soğanlı Süs Bitkilerinde Hıyar Mozaik Virüsü'nün Serolojik ve Moleküler teşhisi. Çannakkel Onsekiz Mart Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Çanakkale.

Suresh, L. M., Malathi, V. G., Shivanna, M. B. 2013. Serological diagnosis and host range studies of important viral diseases of a few cucurbitaceous crops in Maharashtra, India. *Archives of Phytopathology and Plant Protection,* 46(16): 1919-1930. https://doi.org/10.1080/03235408.2013.780841

Tomlinson, J. A., Carter, A. L. 1970. Studies on the seed transmission of cucumber mosaic virus in chickweed (Steleria media) in relation to the ecology of the virus. *Annals of Applied Biology,* 73: 293-298. https://doi.org/10.1111/j.1744-7348.1970.tb04617.x

Uzunoğulları, N., Gümüş, M. 2015. Marmara Bölgesi'nde bazı kültür bitkilerinde doğal enfeksiyona neden olan Hıyar mozaik virüsü (Cucumber mosaic virus, CMV)'nün tespiti. *Trakya University Journal of Natural Sciences,* 16(1): 9-15.

Vučurović, A., Bulajic,´A., Stankovic´, I., Ristic´, D., Berenji, J., Jovic´, J., Krstic´, B. 2012. Nonpersistently aphid-borne viruses infecting pumpkin and squash in Serbia and partial characterization of Zucchini yellow mosaic virus isolates. *European Journal of Plant Pathology,* 133(4): 935-947. https://doi.org/10.1007/s10658-012-9964-x

Yeşil, S. 2013. Konya, Karaman ve Aksaray illeri kabakgil ekim alanlarında görülen virüs hastalıklarının serolojik ve moleküler yöntemlerle tespiti ve enfeksiyon kaynaklarının belirlenmesi. Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, Konya.

Xu, L., Song, M., Ming, J. 2022. Application of Multiplex TaqMan Real-Time PCR Assay in Survey of Five Lily Viruses Infecting Lilium spp. *Agronomy,* 12, 47. https://doi.org/10.3390/agronomy12010047

Zitter, T. A., Murphy, J. F. 2009. Cucumber mosaic. The Plant Health Instructor. DOI: 10.1094/PHI-I-2009-0518-01.