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Research Article

Detection and Molecular Characterization of Viral Agents Infecting Cucurbits from South Marmara Region in Türkiye

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Abstract: Although cucurbit viruses have been widely investigated throughout different regions of Turkey, studies focusing on the molecular characterization of isolates from the South Marmara Region (SMR) are still scarce. To expand the current understanding of cucurbit viral diseases, a total of 76 leaf samples exhibiting virus-like symptoms were obtained from different cultivated cucurbit species in the provinces of Balıkesir, Bursa, and Çanakkale. These samples were screened for seven cucurbit-infecting viruses previously documented in Türkiye by employing reverse transcription polymerase chain reaction (RT-PCR). Among the tested samples, 52 were determined to be infected with at least one viral agent. The viruses identified included zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), and cucumber mosaic virus (CMV), while cucumber green mottle mosaic virus (CGMMV), cucumber vein yellowing virus (CVYV), cucurbit yellow stunting disorder virus (CYSDV), and cucurbit chlorotic yellows virus (CCYV) were not detected in any tested samples. Among the detected viruses, ZYMV was the most prevalent (34.21%), whereas CMV exhibited the lowest incidence (2.63%). The prevalence of WMV was found to be 14.47%. The most frequent symptom associated with infection was mosaic-type chlorotic mottling on the leaves. For further molecular characterization, nine representative isolates (three from each detected virus) were selected, and their coat protein gene sequences were determined. Comparative sequence analysis demonstrated that Turkish isolates shared 95–97% nucleotide identity with each other but differed by up to 10% when compared with reference isolates from other geographical regions. Phylogenetic reconstruction revealed that ZYMV isolates clustered closely within a single lineage, while WMV and CMV isolates were separated into multiple phylogenetic groups, suggesting distinct evolutionary histories. Overall, this research constitutes the first molecular characterization of ZYMV and WMV isolates from the South Marmara Region, thereby providing new insights into cucurbit virus diversity and epidemiology in Türkiye.

Keywords: Cucurbits, Virus, RT-PCR, Phylogenetics, Marmara Region.

1. Introduction

The Cucurbitaceae family represents an agriculturally significant group of plants cultivated across tropical, subtropical, and temperate regions of the world, holding great importance for global food security. Most species within this family play a fundamental role in human nutrition and are also utilized as animal feed, in oil production, and in the pharmaceutical industry. Cucurbits are rich in vitamin C, potassium, and magnesium, as well as dietary fiber, and can be easily cultivated under warm climatic conditions.

Yield and quality in cucurbit production systems are substantially constrained by the prevalence of fungal, bacterial, and viral pathogens. Due to their high economic value and widespread agricultural production, cucurbit species require protection against various biotic and abiotic stress factors. Among the biotic stresses, viral diseases are particularly significant, as they cause severe yield losses in these crops (Randa-Zelyüt and Karanfil, 2025). Plant viruses cause serious damage in cucurbits, leading to yield reduction, deterioration in fruit quality, and substantial economic losses (Keçe and Kamberoğlu, 2016). The most common viral diseases observed in cucurbit species include zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), cucumber mosaic virus (CMV), cucumber green mottle mosaic virus (CGMMV), cucumber vein yellowing virus (CVYV), cucurbit yellow stunting disorder virus (CYSDV), and cucurbit chlorotic yellows virus (CCYV) (Park, 2001; Ali et al., 2004; Desbiez et al., 2009; Güller and Usta, 2020; Örs et al., 2021).

Although numerous studies have been conducted in various regions of Türkiye regarding cucurbit viruses (Çat et al., 2016; Karanfil et al., 2023; Fidan et al., 2024), there are only a limited number of molecular characterization studies focusing on viral diseases affecting cucurbit production areas in the South Marmara Region (Karanfil and Korkmaz, 2020, 2021). Moreover, since there is no effective chemical control method for plant viral diseases, this study aims to provide a scientific basis for future resistance breeding studies by identifying isolates of ZYMV, WMV, CMV, CGMMV, CVYV, CYSDV, and CCYV. The study further seeks to determine the types and prevalence rates of virus-induced diseases in cucurbit production areas, and based on these data, to contribute to the development of preventive strategies essential for sustainable agricultural production systems in the region through molecular characterization studies.

2. Materials and Methods

2.1. Sampling

Field studies were conducted during the 2023 vegetation season in cucurbit growing areas located within the provinces and districts of Çanakkale, Balıkesir, and Bursa, which constitute the South Marmara Region. Throughout the cucurbit cultivation period, field inspections were carried out, and plants were visually examined for symptoms associated with virus and virus-like infections. Samples were collected from plants exhibiting such symptoms. The collected samples were transported to the laboratory under a cold chain and stored under appropriate conditions in a refrigerator until subsequent testing was performed.

2.2. Molecular detection of viruses

Infections caused by WMV, CMV, CGMMV, CVYV, CYSDV, and CCYV in the collected field samples were determined by RT-PCR assays. Accordingly, total RNA was first extracted by CTAB method (Li et al., 2008) from the collected samples, followed by RT-PCR analyses. In the RT-PCR tests, gene-specific primer pairs targeting the respective viruses were used (Table 1). The obtained amplification products were evaluated by agarose gel electrophoresis, and virus identification studies were finalized based on these results.

Table 1. Primer sets utilized for the detection of viral agents.

Virus Species	Primer Sequence	Direction	Referance
ZYMV	GAACAAGGAGACACTGTGAT	Forward	Khanal et al., 2021
	GCAGCGAAACAATAACCTAG	Reverse	
WMV	AACACACAACCAAGTGAATT	Forward	Ali et al., 2012
	TAACGACCCGAAATGCTAACT	Reverse	
CMV	ATGGACAAATCTGAATCAACC	Forward	Karanfil and Korkmaz, 2017
	GATGTGGGAATGCGTTGGTGC	Reverse	
CGMMV	GATTCCTTATCCGAGAAAGTT	Forward	Letschert et al., 2002

	ATTTAAGTGGASGGAAAAVCACT	Reverse	
CVYV	AGCTAGCGCGTATGGGGTGAC	Forward	Cuadrado et al., 2001
	GCGCCGCAAGTGCAAATAAAT	Reverse	
CYSDV	ATGGACATGCCTAACTGTTACTT	Forward	Choi et al., 2015
	ATAGCTGCTGCAGATGGTTC	Reverse	
CCYV	AGTGATCGCAATCAATAAG	Forward	Keshavarz et al., 2013
	GTGATCACTTGACCATCTC	Reverse	

2.3. Sequence analysis and phylogenetic evaluation

A total of nine isolates selected from the detected virus species were subjected to molecular characterization based on their target gene regions. The sequence similarity ratios and phylogenetic relationships of these isolates, both among themselves and with those from other parts of the world, were determined (Table 2). For this purpose, RT-PCR products containing the gene regions of interest for the target viruses were directly sequenced in both directions through a commercial sequencing service (BMLabosis, Ankara). The sequence data obtained in this study were analyzed for similarity and phylogenetic relatedness, both among the examined isolates and in comparison with isolates originating from various regions worldwide (their GenBank accession numbers are shown in the phylogenetic trees). These bioinformatic analyses were performed using programs such as MEGAX, CLC Main Workbench, and Sequence Demarcation Tool (SDT) (Muhire et al., 2014; Kumar et al., 2018). The additional isolates obtained from the GenBank database were incorporated into the analyses and visualized within the resulting phylogenetic trees.

Table 2. Information on isolates selected for molecular characterization studies.

Virus Species	Isolate Name	Host	Province
ZYMV	Çanakkale- ZYMV	Melon	Çanakkale
	Bursa-ZYMV	Zucchini	Bursa
	Balıkesir-ZYMV	Watermelon	Balıkesir
WMV	Çanakkale-WMV	Melon	Çanakkale
	Bursa-WMV	Melon	Bursa
	Balıkesir-WMV	Watermelon	Balıkesir
CMV	Çanakkale-CMV	Melon	Çanakkale
	Bursa-CMV	Melon	Bursa
	Balıkesir-CMV	Cucumber	Balıkesir

3. Results and Discussion

During the field surveys, 76 plant samples exhibiting virus and virus-like symptoms were obtained from cucurbit-growing areas across the South Marmara Region. Of these, 25 samples were collected from Balıkesir, another 25 from Bursa, and 26 from Çanakkale, encompassing various cultivated cucurbit species. Among the samples collected from Balıkesir, 8 were obtained from squash, 6 from melon, 8 from watermelon, and 3 from cucumber plants. Of the samples collected from Bursa, 6 were from squash, 14 from melon, 4 from watermelon, and 1 from cucumber. From Çanakkale province, 8 samples were collected from squash, 9 from melon, 7 from watermelon, and 2 from gherkin. During sample collection, the most commonly observed symptom type among cucurbit plants was mosaic patterns on the leaves. In addition, chlorosis, leaf blade reduction, and curling were also observed in some plants as other characteristic symptoms (Figure 1). The symptoms observed in cucurbits are similar to those documented in studies conducted across different regions and involving various viruses in our country (Karanfil, 2022; Sarı et al., 2024). In this context, there is no specific relationship between the symptom type and most virus species.

As a result of the virus detection studies conducted, the presence of three target viruses was identified in the collected samples. These were determined as CMV, ZYMV, and WMV. No infections of the other screened viruses were detected in the collected samples. Among the identified infections, ZYMV was found to be the most prevalent virus, while CMV was the least detected. CMV, which is among the viruses with the broadest host range, has been found at a low infection percentage in studies related to the detection of its infections in different hosts, particularly in the Marmara region. In this context, the obtained results are observed to be consistent with previous studies conducted in the region (Karanfil et al., 2016; Karanfil, 2021).The number and infection rates of these three viruses are presented in Tables 3. Based on the number of infected samples, the single infection rates of the identified virus species were calculated. The highest single infection rate was determined for ZYMV at 34.21%, while the lowest rate was found for CMV at 2.63%. The infection rate for WMV was calculated as 14.47%. When the infection rates were calculated based on mixed infections, the highest mixed infection rate was observed for the ZYMV + WMV combination at 6.57%, whereas the lowest mixed infection rate was recorded for the WMV + CMV combination at 3.94%. At the provincial level, the highest overall infection rate was recorded in Bursa province with 76.00%, while the lowest infection rate was observed in Çanakkale province with 53.84%. The infection rate in Balıkesir province was determined to be 56.00%. The overall infection rate across all samples was calculated as 61.84%. Based on these results, ZYMV was identified as the most prevalent virus in the collected samples, whereas CMV was the least detected. ZYMV infection was detected in 31 plants, WMV infection in 14 plants, and CMV infection in 5 plants. In contrast, no infections caused by CGMMV, CVYV, CYSDV, and CCYV were detected in any of the collected samples. Infection rates caused by different virus types can vary from region to region and from year to year. In this context, different results can be obtained from different studies (Şevik and Balkya, 2015; Aktaş and Akdura, 2023).

Figure 1. Some of the most frequently observed symptom types identified during the field studies (a: chlorotic discoloration on watermelon leaves; b–d: mosaic discolorations on melon leaves; c: mosaic discolorations on squash leaves).

Table 3. The numbers of single and mixed infections detected in the collected samples.

Province	ZYMV	WMV	CMV	ZYMV+WMV	WMV+CMV	Infection Rate (%)	Collected Samp
Balıkesir	7	2	-	2	3	56.00	25
Bursa	15	3	1	-	-	76.00	25
Çanakkale	4	6	1	3	-	53.84	26
Total	26	11	2	5	3	61.84	76

Bioinformatic analyses were conducted to determine the nucleotide and amino acid sequence similarities among WMV, ZYMV, and CMV isolates collected from the South Marmara Region of Turkey. The local WMV isolates exhibited a high degree of similarity among themselves, ranging from 91–93% at the nucleotide level and 98–100% at the amino acid level. ZYMV isolates demonstrated even higher homogeneity, with intra-regional similarities of 99–100% at the nucleotide level and 100% at the amino acid level. CMV isolates showed moderate to high intra-regional similarity, measuring 92–99% at both the nucleotide and amino acid levels. Comparative analyses with global isolates revealed that the South Marmara WMV isolates shared 91–95% nucleotide and 82–98% amino acid sequence similarity with international counterparts. The closest sequence affinity at the nucleotide level was observed with the Chinese isolate MW509731, while the closest amino acid similarity was found with the Chinese isolate OK050524. The ZYMV isolates demonstrated a sequence similarity of 83–100% (nucleotide) and 88–99% (amino acid) with world isolates, with the Hungarian/Chinese isolate ON604841 showing the highest overall sequence identity. For CMV, the regional isolates showed 90–92% nucleotide and 90–98% amino acid similarity globally. The closest nucleotide match was with the Iranian isolate KC122261, and the closest amino acid match was with the Polish isolate OR750501. Considering all isolates utilized in the bioinformatic study, the overall sequence similarity was determined to be 82–100% for WMV, 83–100% for ZYMV, and 90–100% for CMV. These findings indicate that the ZYMV population in the South Marmara Region is highly conserved locally, whereas WMV and CMV isolates exhibit greater genetic variability when compared to the broader global isolate pool. The analysis successfully identified specific closely and distantly related international isolates for each virus, providing valuable insights into their molecular epidemiology and phylogenetic relationships. In Türkiye, different studies conducted on WMV have also reported varying results regarding the genetic diversity of isolates. For instance, sequence similarity ratios obtained based on the P1 gene region were found to be consistent with the results of the present study (Aydın et al., 2025), whereas those derived from the CP gene region showed different patterns (Morca et al., 2025). This discrepancy is thought to be mainly related to differences in the selection of isolates retrieved from the GenBank database. In the case of ZYMV and CMV, the sequence similarity ratios obtained in this study were observed to be consistent with those reported in previous studies (Kamberoğlu et al., 2016; Karanfil and Korkmaz, 2021).

In the bioinformatic analyses conducted to determine the phylogenetic relationships of the WMV, ZYMV, and CMV isolates obtained from the South Marmara Region, distinct evolutionary relationships among the isolates were revealed. In case of WMV, the phylogenetic tree constructed to assess the relationships among isolates showed that they clustered into three main groups (Group 1, 2, and 3). It was observed that the South Marmara isolates, specifically those from Balıkesir and Bursa, belonged to Group 1, while one isolate was placed in Group 3. Although the Balıkesir and Bursa WMV isolates were located within the same major group, they were assigned to different subgroups. The distribution of Turkish isolates across different groups indicated no correlation with their geographic origin (Figure 2a). In this context, it was observed that the Turkish isolates were closely related to isolates obtained from various countries around the world (Sarı et al., 2024). For ZYMV, the phylogenetic tree constructed to determine evolutionary relationships revealed that the isolates clustered into two major groups (Group 1 and Group 2). All South Marmara isolates were found to belong to Group 2. The isolates most closely related to the Turkish isolates were those from Hungary, Brazil, and Egypt (Figure 2b). Phylogenetically, the isolate most closely related to the Turkish isolates was identified as the Hungarian isolate with accession number ON604841. In a different study conducted in our country, ZYMV isolates were found to be closely related to each other (Kamberoglu et al., 2016). For the CMV isolates, the phylogenetic analysis revealed that they were separated into three principal clusters, designated as Groups 1, 2, and 3. The South Marmara isolates from Çanakkale and Balıkesir were grouped within Group 1, while the Bursa isolate was placed in Group 3. Similar to the WMV results, the distribution of Turkish isolates across different groups suggested that there was no relationship with their geographic origin (Figure 2c). The isolate most closely related to the Çanakkale and Balıkesir isolates was identified as AB448696, while the isolate most closely related to the Bursa isolate was the Iranian isolate with accession number KC122261. It is known from different studies on CMV conducted in our country that the phylogeny of CMV isolates varies regardless of the host (Ohshima et al., 2016; Karanfil, 2021).

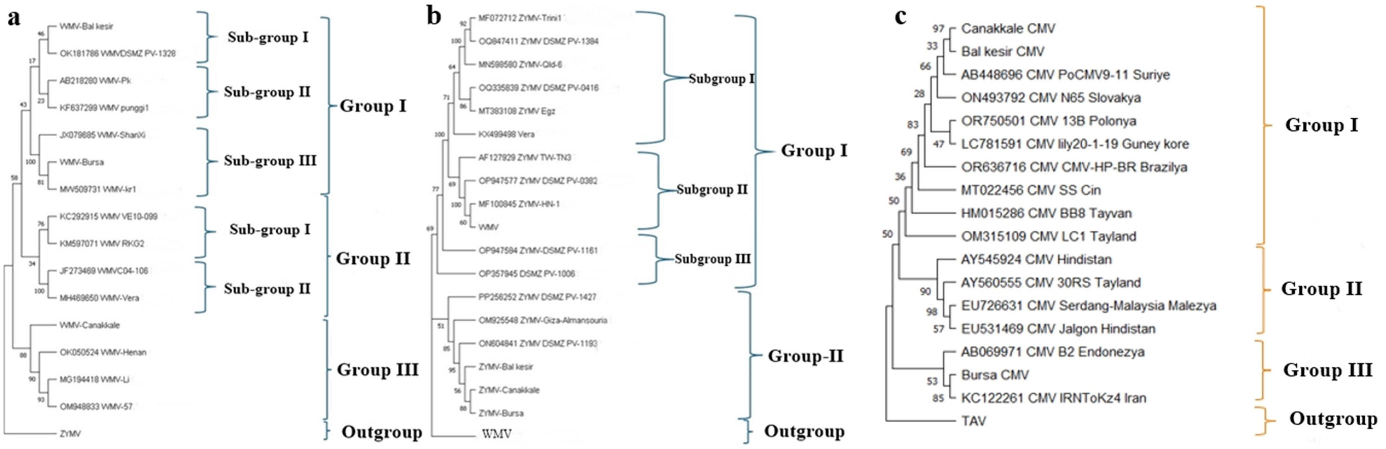

Figure 2. Phylogenetics relationships of watermelon mosaic virus (a), zucchini yellow mosaic virus (b) and cucumber mosaic virus (c).

4. Conclusions

In the South Marmara Region, viral infections—particularly those caused byZYMV were found to be widespread. Therefore, the establishment of a sustainable regional monitoring and early detection system is essential. The routine use of sensitive molecular diagnostic techniques, such as RT-PCR, is recommended for surveillance purposes. The high infection rates of ZYMV highlight the necessity of developing resistant cucurbit cultivars. In this context, breeding programs should be supported through the molecular screening of local genotypes and the selection of resistant individuals.

Integrated pest management strategies should be implemented against aphid species such as *Aphis gossypii* and *Myzus persicae*, which are the primary vectors of these viruses. Biological control, cultural practices, and crop rotation should be promoted as part of these management approaches. Training programs and seminars should be organized for farmers and agricultural workers to enhance their ability to recognize disease symptoms, understand transmission routes, and apply effective preventive measures. Such educational efforts are expected to improve early intervention capacity. Furthermore, it is known that WMV has an increasing host range in our country (Randa-Zelyut et al., 2022). Therefore, it is hypothesized that identifying and eradicating the alternative hosts of the detected viruses will enable the reduction of inoculum sources. Furthermore, advanced biotechnological studies focusing on complete genome sequencing, recombination analysis, and virus–plant interactions of cucurbit viruses should be encouraged. Research should also be conducted on the interactions between these viruses and environmental factors.

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Conflicts of Interests

Authors declare that there is no conflict of interests

Financial Disclosure

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Statement contribution of the authors

M.Ş. and S.K. collected samples, perfomed the laboratuary analyses. Both authors wrote the draft and approved the final version.

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