Journal of Environmental Sciences Studies
Journal home page: www.jess.ir

Infection cucurbit fields to most important plant viruses and phylogenetic analysis

Milad Yousefi[a], Davoud Koolivand[a*], Mohammad Hajizadeh[b]

[a]Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran
[*]d.koolivand@gmail.com
Received: (2022-09-29)
Accepted: (2023-10-28)

Abstract

Viral diseases are one of the limiting factors for cucurbit production around the world that some of these viruses can cause a severe reduction in crop yield. In the present study, 92 leaf and fruit samples of various species of cucurbits were collected from different farms in Zanjan and Hamedan provinces, based on descripted symptoms such as yellowing, mosaic, deformation, leaf asymmetry, and wrinkling of leaf veins, to detect and investigate the incidence of some important viruses in cucurbit fields. After total RNA extraction, RT-PCR were conducted using specific primers for *Cucumber mosaic virus* (CMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Cucumber green mottle mosaic virus* (CGMMV), *Squash mosaic virus* (SqMV), *Ourmia melon virus* (OuMV), *Zucchini yellow mosaic virus* (ZYMV), and general primers of the *Orthotospovirus* genus to amplify a part of the genome corresponded with each of these viruses. The results showed evidence of the presence of CMV, CGMMV, ZYMV, and CABYV, in the tested samples, while OuMV, SqMV and Orthotospoviruses were not detected in any of the tested samples. Among the detected viruses, CABYV was detected as the most common virus. Phylogenetic analysis of the new isolates and other isolates from different countries showed that CMV strains were placed in both subgroups I and II. The newly detected ZYMV strains from Zanjan were clustered with the Vietnamese strain, and CGMMV strains were grouped with strains from India, Pakistan, Uzbekistan, the Netherlands, and Spain in the cluster II. CABYV strains were grouped in the Mediterranean, Asian, and a new independent group, along with the Taiwanese and Indonesian strains. In addition, mixed infections of those virus infections were detected commonly in squash and cucumber. Especially, CGMMV+CABYV, CMV+CABYV and the infection of ZYMV+CABYV+CMV, ZYMV+CGMMV+CMV, and ZYMV+CGMMV+CABYV mixed infections were detected. The results of this study will be useful in developing management strategies to reduce losses of cucurbit viruses.

Keywords: Cucurbits, Zanjan, Hamedan, Mixed infection, Phylogenetic analysis

Introduction

The Cucurbitaceae family, after Solanaceae, ranks second in terms of economic importance among horticultural plant species worldwide (Renner and Schaefer., 2016). Cucurbits comprise over 800 species of plants in 120 genera (Welbaum, 2015). According to the statistics of the Food and Agriculture Organization (FAO), global production of cucurbits was reported to be around 1,205,679 tons in 2020. Viral diseases are important factors for the limiting of cucurbit production worldwide. More than 70 species of plant viruses have been reported to infect cucurbits around the world (Lecoq and Katis., 2014). Plant viruses are responsible for more than $30 billion in annual losses in crops (Sastry et al., 2019). *Cucumber mosaic virus* (CMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Papaya ringspot virus* (PRSV), *Zucchini yellow mosaic virus* (ZYMV), and *Watermelon mosaic virus* (WMV) are viruses that are widely spread on cucurbits in various regions, especially in the Mediterranean area. These viruses mostly cause mosaic, yellowing, and deformation on

leaves and malformation of fruits (Lecoq and Desbiez., 2012). In Syria, as one of the major areas of cucurbit production, ZYMV, WMV, CMV, CABYV, and *Pepo aphid-borne yellows virus* (PABYV) have been identified using molecular methods (Chikh-Ali *et al*., 2019). A recent study in northern and central Argentina showed high levels of WMV incidence in several species of cucurbits (Pozzi et al., 2020). A general survey in 2017 and 2018 showed that WMV is the most common and widespread virus observed in all regions of cucurbit growth in Argentina, with a relative incidence of 46%, followed by 24% for ZYMV and 20% for *Papaya ringspot virus*. Additionally, CMV and *Squash mosaic virus* (SqMV) have been reported on cucurbits in different regions of Azerbaijan (Huseynova et al., 2017; Verdin *et al*., 2018). Several viruses, including WMV, CABYV, SqMV, CMV, CGMMV, ZYMV, *Cucumber yellow stunting disorder virus* (CYSDV), and OuMV, have been reported in cucurbit plants in Iran (Mehrvar and Zakiaghl, 2021 Vafaei, and Mahmoodi; 2017, Gerami Nooghabi *et al*., 2022, Salehzadeh., 2018, Mohammadi *et al*., 2016, Maghamnia *et al*., 2018, Keshavarz and Izadpanah, 2004, Gholamalizadeh *et al*., 2008). ZYMV and CABYV are among the most common viruses infecting cucurbit plants in Iran and have been reported in several cultivation areas (Bananej *et al*. 2008; Massumi *et al*. 2011; Mohammadi et al. 2016; Sokhandan-Bashir *et al*. 2013; Abou Jawdah *et al*.,2000; Kassem *et al*., 2007). A survey of the prevalence of CMV, ZYMV, CGMMV, CYSDV, and *Cucurbit chlorotic yellows virus* (CCYV) in cucurbit cultivation areas of Lorestan Province showed that cucurbit plants are infected with all tested viruses, and reports suggest that they are present in most cucumber cultivation areas in Iran (Hasanvand and Shams-bakhsh., 2017). CMV, ZYMV, CGMMV, and WMV have been traced in cucumber, pumpkin, and watermelon fields in the northwest and west of Iran using RT-PCR method (Mohammadi *et al*., 2016). The simultaneous infection of two or more viruses in plants is a natural occurrence and may have an impact on the evolution and epidemics of viruses (Edwardson, 1966). In some cases, mixed infection of two unrelated viruses may exacerbate symptoms, and it may affect the replication of the infecting viruses (Hull, 2013). The effect of *Cucumber mosaic virus* and *Tomato mosaic virus* (ToMV) on the growth and performance of tomato plants has been observed. The combination of ToMV + TYLCV + CMV and ToMV + TYLCV in mixed infection shows greater synergistic effects on disease symptoms, while a combination of ToMV + CMV has caused milder symptoms (Mohamed, 2010). The combination of CMV and WMV with ZYMV, *Papaya ringspot virus* (PRSV) and CGMMV led to a decrease in symptoms. The dual mixed of ZYMV+CMV and ZYMV+PRSV-W is a common combination of viruses found in infected pumpkin samples (Nontajak *et al*., 2014). PRSV and ZYMV create various symptoms such as mosaic, stunted growth, distortion of stems and leaves, and shoestring-like tendrils (Fletch *et al*., 2000). The majority of viruses that infect cucurbits are widespread in different geographic regions of the world, and their host range is not limited to one plant species. Accurate diagnosis of pathogens is necessary for plant disease management, and the identification and detecting of viruses are crucial to conduct further research to prevent and control these viruses in the future. The economic value and widespread consumption of products derived from Cucurbitaceae plants make them highly significant. However, viral infections pose a significant threat to these plants, causing estimated losses of 3 to 5 percent of total vegetable production, with the potential for even higher losses in some cases (Caciagli, 2010). So far, no comprehensive information is available on the viruses infecting Cucurbitaceae plants in Zanjan and Hamedan provinces, and this research aims to investigate the level of infection of some Cucurbitaceae farms with important viruses, and to examine the level of mixed virus infection in collected plant samples from the Cucurbitaceae family in different regions of Zanjan and Hamedan provinces using molecular methods and determining the sequence and phylogenetic relationship

2. Materials and Methods

2.1. Sampling

To identify and detect the infection of some important viruses in cucurbit farms, sampling was done from different counties of Zanjan and Hamedan provinces based on symptoms such as general yellowing, leaf yellowing, mosaic, green veins, deformity, lack of symmetry in leaves, and leaf vein complexity from late May 2021 to late September 2022. Sampling was performed from leaves and fruits showing characteristic viral symptoms. Suspicious samples were transferred to separate bags and kept in cold conditions before being transferred to the laboratory. Then, a portion of the samples was frozen in liquid nitrogen and stored in a -70°C freezer.

2.2 *RNA extraction and RT-PCR*

Total RNA extraction from 100 to 200 mg of infected leaf and fruit tissue was performed using the CTAB-based protocol with modifications (Gambino and Gribaudo, 2008). The quantity and quality of extracted RNA were determined using a nanodrop. Random hexamer primers and a cDNA synthesis kit (HyperScript) were used to generate complementary DNA (cDNA) in a final volume of 10 µL. PCR

reactions were performed on cDNA obtained from reverse transcription reactions of extracted RNA from suspicious samples with specific primer pairs for viruses in a final volume of 25 µL. After amplification of the desired DNA fragments by PCR, sequencing of several amplified fragments was performed by BMG (Bio Magic Gene).

2.3 ***Nucleotide data analysis***

After sequencing, the nucleotide sequence results of the PCR products were compared with NCBI-GenBank using the nBLAST program. Once the amplified genomic region was identified and compared with the data available in GenBank, the virus species was identified, and the specific region that was amplified was determined. Subsequently, nucleotide data obtained from the detected viruses, which were previously archived in the NCBI database and originated from various regions worldwide, were utilized for conducting phylogenetic analysis. Each of the sequences belonging to the mentioned viruses was separately aligned using the ClustalW method by the MEGA X software. The distance matrix was calculated based on the Jukes-Cantor model. The phylogenetic tree was generated by the MEGA X program using the best model selected. The number of Bootstraps for drawing the tree was selected as 1000.

Table 1. Primers used in RT-PCR and predicted amplicon size for the detection of ZYMV, CABYV, SqMV, CMV, CGMMV and Ortospovirus

Virus name		Sequence 5´→ 3´	Region and Amplicon size (bp)
CABYV	**R**	GCTAGAAATCAA AAT GCA GGGA	**CP, 392 bp**
	F	AGGGAGCTAA GCTTGCAGTG	
SqMV	**R**	GTGGATCTGCGTTGCAAAC	**Polyprotein, 733 bp**
	F	GTTGCCTTTATGTAAGGAGAACTC	
OuMV	**R**	TCCCAAATGTTGCCTCCCA	**RdRp 650 bp**
	F	GGCGCTACCTCCGTTCTGC	
CMV	**R**	CGGATCCATGGA CAAATCTGAAT	**CP , 650 bp**
	F	GGCGGCCGCTCAGACTGGGAGCACCCCAG	
CGMMV	**R**	ACCCTCGAAAACTAAGCTTTC	**CP, 650 bp**
	F	GAAGAGTCCAGTTCTGTTTC	

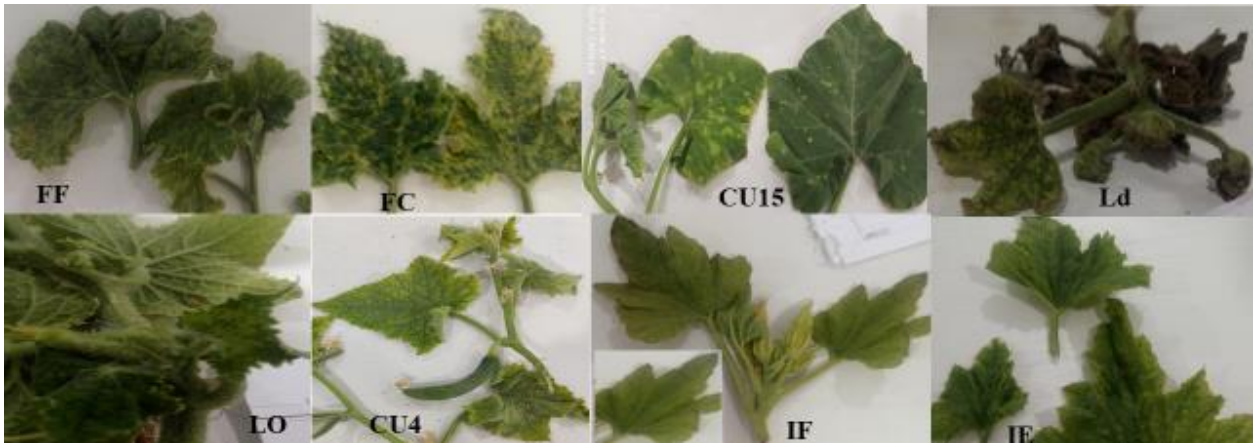

Figure 1. Symptoms in cucurbit plants infected with virus: (FF) showing severe mosaic (FC) chlorosis and mosaic (CU15) chlorosis on leaves (Ld) severe mosaic and leaf deformation (LO) Slight mosaic (CU4) Slight yellowing on leaves and normal growth(IF) Yellowing and slight mosaic on leaves (IF) Yellowing and Slight mosaic

3. Results & Discussion

In this study, a total of 92 plant samples from the Cucurbitaceae family suspected of viral infection were collected, and among the collected samples, 51 representative samples were tested for viral symptomatic signs such as yellowing and severe mosaic using the RT-PCR method in some regions of Zanjan and Hamedan provinces during the agricultural seasons of 1398 to 1400 (spring, summer, and fall). In field observations, most samples showed suspicious viral symptoms such as general yellowing, leaf yellowing, fruit yellowing and drying, thickening of leaves, curling or tube-like shape of leaves, abnormal growth on leaves and stems, stem curling, mosaic, green streaks, leaf malformation and asymmetry, small leaves, yellow leaf mosaic, and plant dwarfing and vein complexity. The highest infection and positive results were observed in pumpkin plants. Viral symptoms in positive samples were mostly severe mosaic and yellowing. In some samples, although severe viral symptoms were observed, none of the viruses mentioned in this study were detected. Using the specific direct and reverse primer pairs of each virus and the RT-PCR method, for the detection and identification of CMV, CGMMV, ZYMV, and CABYV, approximately 650 bp fragments of DNA were amplified in 3, 3, 5, and 7 samples of Cucurbitaceae plants, respectively. However, SqMV, OuMV, and Orthospovirus were not detected in any of the tested samples

Six plant samples showed simultaneous infection with CABYV, ZYMV, CMV, and

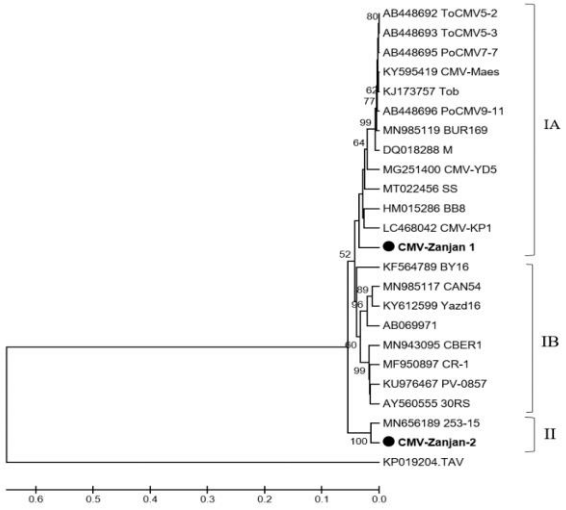

Figure 2. Genetic relationships among the two Zanjan Cucurbit aphid-borne yellows virus (CABYV) isolates analysed in our study with different strains reported from around the world in NCBI for the coat protein gene

CGMMV viruses in the results. According to the results, CABYV was the most common cucurbit virus in Zanjan province, with 41.17% of the samples infected with this virus. The second most important viral disease in the research area was ZYMV, which was detected in 29.41% of the samples. CMV and CGMMV followed, with contamination rates of 17.64% and 17.64%, respectively, in all tested samples. The mixed contamination rate in cucumber and squash plants under study was observed at 11.76% and 23.52%, respectively. The simultaneous viral contamination of CGMMV+CABYV, CMV+CABYV was observed in two cucumber samples showing mosaic symptoms, with a percentage ratio of 11.76%. Triple contamination of ZYMV+CABYV+CMV, YMV+CGMMV+CMV, and ZYMV+CGMMV+CABYV was detected in three squash plant samples showing severe mosaic symptoms accompanied by yellow spots and small leaves, with a detection rate of 17.64%.

In the present study, the sequence fragment of Zanjan strain was compared and blasted against available gene sequences in the gene bank. The amplified fragment belonged to the coat protein gene of CMV Zanjan 1 strain, showing 72.92% to 96.91% and CMV Zanjan 2 strain, showing 93.55 % to 97.56 % similarity with the NCBI strains. The CMV-Zanjan 1 strain had the least nucleotide difference (0.073) with the Syrian strain and the highest nucleotide difference (0.169) with the Sudanese strain. CMV-Zanjan 2 had the least nucleotide difference (0.027) with the Serbian strain and the highest nucleotide difference (0.155) with the Thai strain. The CMV-Zanjan 1 strain newly tracked in this study showed a nucleotide difference of 0.108 with CMV-Zanjan 2, as shown in the table. In the results of phylogenetic analysis, the cucumber mosaic virus strains used for analysis were placed in three separate clusters. In the results of phylogenetic analysis, the cucumber mosaic virus strains were placed in three separate clusters. The CMV-Zanjan 1 strain, newly identified in this study, was placed in group IA along with strains from China and Indonesia, all of which were isolated from different hosts. Group IA consists of

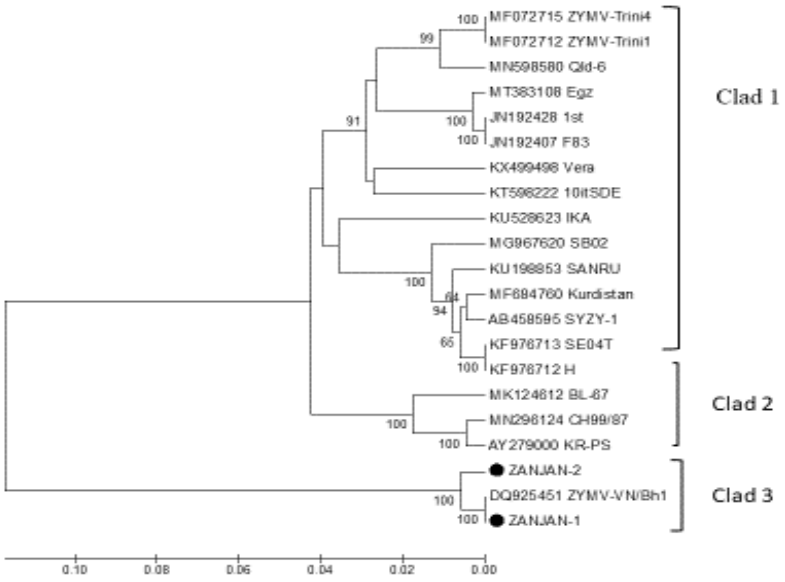

Fig 3. Phylogenetic tree showing the relationship of Zanjan ZYMV isolate (is marked with dot) with 19 ZYMV sequences from worldwide isolates available in GenBank of the coat protein gene. Neighbour-joining tree was built with the program MEGA6, phylogenetic relations based on pdistance method. Bootstrap values of 1000 resembling as percents are indicated at key nodes. Only bootstrap values above or equal 50% are shown. Original tree.

strains from Syria, Iran, Russia, Turkey, Poland, South Korea, and Taiwan. The newly identified CMV-Zanjan 2 strain, along with the Serbia strain, was placed in group II independently of the CMV-Zanjan 1 strain, indicating genetic diversity among newly identified strains. Another strain from Turkey and China, along with strains from India, Sudan, Thailand, Yazd, and Indonesia, was placed in group IB

The Blast results showed that ZYMV Zanjan-1 had a similarity of about 82.15 % to 97.82 % with different strains reported from around the world in NCBI for the coat protein gene. ZYMV Zanjan-2 showed a similarity of about 83.39 % to 96.73 % with different strains. The tracked strains in this study had a similarity of 96% to 99% with strains from Iran, Australia, and the Netherlands. The genetic diversity difference between ZYMV-Zanjan 1 and ZYMV-Zanjan 2 strains with some reported yellow pumpkin mosaic virus strains from other parts of the world was analyzed in the gene bank. ZYMV-Zanjan 1 had zero nucleotide difference with the Vietnam strain and the highest nucleotide difference (0/285) with the IKA strain from Iran. ZYMV-Zanjan 2 had the lowest nucleotide difference (0/012) with ZYMV-Zanjan 1 and Vietnam and the highest nucleotide difference (0/281) with the IKA strain from Iran, and the tracked strains had the least and most common nucleotide difference in terms of strain. Analysis of Yellow Cucumber Mosaic Virus isolates showed that the isolates used for analysis were placed into three independent clusters. The newly traced Zanjan isolates in this study were grouped with the Vietnam isolate in one cluster. Cluster one included isolates from Thailand, Australia, Egypt, the United States, Spain, Argentina, Iran, Kurdistan, India, and Syria. Cluster two included isolates from the United States, China, and Korea.

In the results of the Blast of Cucumber Green Mottle Mosaic Virus, the coat protein of the CGMMV-Zanjan-2 isolate showed 97.93 to 98.76 similarity with the available strains in the NCBI database and 96.09 to-97.33% for CGMMV-Zanjan-2 isolate showed 97.93 to 98.76 similarity with the available strains in the NCBI database and 96.09 to-97.33% for CGMMV-Zanjan-1. Genetic diversity, between CGMMV-Zanjan 1 and CGMMV-Zanjan 2 isolates with some reported strains of Cucumber Green Mottle Mosaic Virus from different regions of the world was evaluated in the gene bank. CGMMV-Zanjan 1 showed the lowest nucleotide difference (0/028) with the Spain isolate and the highest nucleotide difference (0.074) with the Netherlands and Uzbekistan isolates. CGMMV-Zanjan 2

showed the lowest nucleotide difference (0/013) with isolates from Thailand and India and the highest nucleotide difference (0/109) with the Netherlands isolate. The newly traced CGMMV-Zanjan 1 isolate showed a nucleotide difference of (0/052) with CGMMV-Zanjan 2 in the table. The Zanjan isolates were grouped with isolates from India, Pakistan, Uzbekistan, the Netherlands, and Spain in the

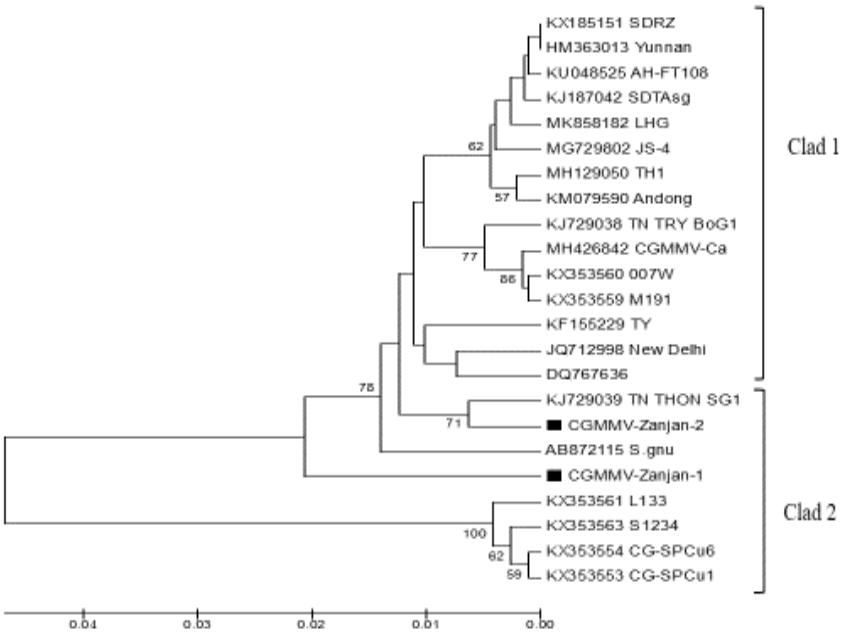

Figure 4. Phylogenetic analysis of the coat protein gene of the CGMMV and its comparison to other Cucumber green mottle mosaic virus isolates based on the nucleotide sequences

second cluster, with each in two separate subgroups, with CGMMV-Zanjan 2 and the Indian isolate in one subgroup, and CGMMV-Zanjan 1 in another separate subgroup. The other isolates in the second cluster were from Pakistan, Uzbekistan, the Netherlands, and Spain. Isolates from China, Israel, Canada, South Korea, and another isolate from India were grouped in the first cluster.

Finally, the results showed that the protein coat gene (CABYV) in OQ351953 strains had similarities of approximately 97.06% to 99.58%, OQ351954 strains had similarities of 98.58% to 100%, OQ351955 strains had similarities of 98.58% to 100%, OQ351956 strains had similarities of 98.11% to 100%, OQ351957 strains had similarities of 97.06% to 99.58%, OQ351958 strains had similarities of 94.34% to 95.28%, and OQ351959 strains had similarities of 96.70% to 98.58% with various reported strains worldwide. The differences and similarities of genetic diversity among the tracked strains in Zanjan were shown. The highest similarities were observed between OQ351954 and OQ351955 strains, as well as the Greek strain, which had 100% similarity. The nucleotide differences of the OQ351958 strain showed differences of 0.62% and 0.85% with OQ351954 and OQ351955 strains, respectively, and 0.85% with the American strain. The analysis of the virus strains showed that the strains used for analysis were placed in three groups: Mediterranean, Asian, and a new group independent of the Mediterranean and Asian groups. The OQ351956, FF, OQ351957, and OQ351953 strains, along with strains from the Czech Republic, Spain, Poland, and Germany, were placed in the Mediterranean group. The OQ351953, OQ351959, OQ351957, and IE strains, along with strains from the Czech Republic, Spain, Germany, and Poland, were placed in subgroup I, while strains from Turkey, Saudi Arabia, Montenegro, and Egypt were placed in subgroup II. The OQ351954 and OQ351955 strains, along with strains from Sistan and Baluchestan in Iran, Montenegro, Uzbekistan, Poland, and South Korea, were placed in the Asian group. The OQ351954 and OQ351955 strains formed two subgroups with the strains available in the gene bank. The American, Polish, South Korean, and Spanish strains were placed in subgroup I, while the OQ351954 and OQ351955 strains, along with another strain from South Korea, Sistan and Baluchestan in Iran, Montenegro, and Uzbekistan, were placed in subgroup II. The OQ351958 strain, along with strains from Taiwan and Indonesia, formed a new independent group from the Mediterranean and Asian groups. This is likely the first strain of the beet mild yellowing virus in this group.

Discussion:

In the present study, a total of 460 samples were collected from different areas of Zanjan province, and most of the samples had various viral symptoms. Some of the viral symptoms were widely observed in many different areas on a wide range of plants, such as vein complexity, yellowing, and mosaic, which are major viral diseases. Based on the characteristics of viral diseases, the main solution for their control is prevention of their occurrence and spread (Holl., 2002). In some areas, some of these symptoms were more prevalent, indicating a specific variant of viruses prevalent in that area. The viral symptoms in the growth stages of different plants in early to mid-summer and late summer to early autumn were different, and viral symptoms were more observed in the late growth season, indicating a higher likelihood of mixed contamination and presence of multiple viruses in samples collected in the late growth season. On the other hand, carriers play an important role in virus transmission, and therefore, they are more active during the mentioned period. Therefore, contamination in the late growth season is one of the cases where we can have the highest incidence rate. Samples collected from various pumpkin farms were confirmed to have viral contamination in later stages using molecular methods, but OuMV, Ortospovirus, and SqMV were not detected in any of the samples. Some of the samples with typical viral symptoms were examined in RT-PCR tests for several different viruses, but no contamination was detected, which could indicate the possibility of the widespread presence of many other different viruses. On the one hand, observing visible symptoms is not enough to ensure the health of a plant. Therefore, genomically-based methods are used to track and identify viruses. The reason for not detecting viruses in some samples may be attributed to the sensitivity stage of RNA extraction, which

requires accuracy and avoidance of the RNase enzyme during extraction. To achieve proper quality of RNA extraction, it is recommended to use RNA extraction kits containing RNase inhibitors. In this study, the RT-PCR method was used to track viruses. Overall, cucumber mosaic virus, zucchini yellow mosaic virus, cucumber green mottle mosaic virus, and Cucurbit aphid-borne yellows virus were scattered in pumpkin fields in Zanjan province, possibly transmitted by aphids. Extensive studies conducted by Lecoq and colleagues showed that Cucurbit aphid-borne yellows virus (CABYV) is one of the most common viruses in pumpkin fields. Based on field observations and host plant studies, the most severe viral symptoms were seen in zucchini plants infected with ZYMV and CMV, indicating co-pathogenicity and severe interactions. This co-pathogenicity and severity of symptoms were also observed in our study, with the mildest symptoms being found in cucumber plants, possibly indicating cultivar sensitivity or virus-host restriction. Viral symptoms had a noticeable percentage in pumpkin fields during the spring, and the weakest symptoms were observed during the summer. However, in the autumn season, viral symptoms were more severe and had a higher percentage, indicating that in low-temperature conditions, viruses are more active. While symptoms provide vital information about viral diseases, sufficient field experience is required when deciding on symptoms alone (Naidu and Hughes, 2001). Simultaneous infection with CABYV+CMV+ZYMV was observed in a sample of squash (IE), which exhibited severe mosaic and malformation symptoms. Mixed infection with ZYMV+CMV+CGMMV was detected in a sample of zucchini (LD) showing severe mosaic and stunting (Figure 2-4). A cucumber sample (CU10) infected with CABYV+CGMMV viruses exhibited mosaic and yellowing symptoms, vein clearing, and a positive PCR result, while a cucumber sample (LO) with mosaic symptoms in young leaves (4-2) was positive for simultaneous infection with two viruses, CABYV+CMV (5-1). Mixed viral infections in plants are very common in nature and can lead to additive, synergistic, or antagonistic interactions that may produce more severe symptoms than single infection with one of the viruses (Mendez-Lozano *et al*., 2003; Renteria-Canett *et al*., 2011). Overall, the results indicate that the highest mixed infection rate in terms of hosts occurred in squash and cucumber, and the geographic location was in Razbin village, followed by Mahneshan city. Mosaic and yellowing symptoms were present in all samples that had mixed symptoms. Identifying the most common and harmful viruses in a location is important for creating comprehensive management strategies that farmers can use to reduce losses caused by these economically important diseases. ZYMV, CABYV, and CMV are transmitted by aphids and are difficult to control. Early infection can significantly reduce pumpkin yield. Infection can significantly reduce the performance of pumpkin. Pest management strategies can minimize damage (Afechtal *et al*., 2019). To prevent viral infection in pumpkins, weeds should be controlled. Precautionary measures are also essential in the production of other plant products, including pumpkins. If all necessary conditions for healthy plant growth are met, the likelihood of phytopathological problems is minimized. Therefore, actions such as soil preparation, planting, fertilization, and irrigation must be performed correctly. Plants displaying viral disease symptoms should be uprooted as soon as they are observed because they act as sources of infection for subsequent infections (YEŞİL., 2019). Plant viral diseases can be pathogens that create various changes and symptoms in plant tissues that may be completely different or similar to each other, and in mixed infections, the effects may be additive or reducing, indicating wide genetic diversity among plant viruses. Among identified viruses, the *Cucurbit aphid-borne yellows virus* virus was the most common virus with the lowest infection rate, which was traced in most regions of Zanjan province. The newly traced isolates of squash mild mottle virus had a very high nucleotide difference and genetic diversity that, in addition to being placed in the Asian and Mediterranean groups, for the first time, an isolate was traced that formed a new independent group with the Taiwan and Indonesia isolates, indicating that *Cucurbit aphid-borne yellows virus* has a very high genetic diversity in the Zanjan province. This virus is limited to the Avand Abkesh region and is transmitted from one plant to another by aphids in a stable (circulatory) manner (Lecoq.and Desbiez *et al*., 2012). Translation:

The Cucumber mosaic virus and the phylogenetic analysis showed a point that these strains had the least nucleotide differences with the Vietnamese strain and were grouped separately in a cluster, while they had the most nucleotide differences with other Iranian strains. These results indicate the existence of different genotypes in Iran. In the phylogenetic analysis, the cucumber strains of the virus were grouped in three independent clusters, IA, IB, and II. The newly identified CMV-Zanjan 1 strain, along with strains from China and Indonesia, were grouped in cluster IA, all of which were separated from different hosts. The newly identified CMV-Zanjan 2 strain, along with the Serbian strain, was grouped in cluster II independently from CMV-Zanjan 1 strain, which indicates genetic diversity among newly identified strains. While cluster IA had a high genetic diversity, cluster II had a much lower genetic diversity, which was one of the fundamental differences between the two subgroups identified in this study. The phylogenetic analysis based on the capsid protein gene of the cucumber mosaic virus strains formed two clusters, but they had diversity within different sub-branches and formed different sub-branches. The Zanjan strains were grouped in cluster II with strains from India, Pakistan, Uzbekistan, the Netherlands, and Spain, each in two separate subgroups, with the CGMMV-Zanjan 2 strain grouped with the Indian strain in one subgroup. The CGMMV-Zanjan 1 strain was placed independently in another subgroup. These results indicate that although the strains were grouped in a cluster, the low nucleotide differences can cause significant differences in subgroups and form different sub-branches. However, low nucleotide differences can create significant differences in subgroups and form various subgroups. Although Cucumber green mottle mosaic virus (CGMMV) is limited to cucurbit plants as hosts, it shows a significant geographical distribution. The global prevalence of CGMMV has become a serious concern for cucumber industries and seed companies worldwide. (Sui *et al*., 2019)**.**

References

1. Abou-Jawdah, Y., Sobh, H., El-Zammar, S., Fayyad, A., & Lecoq, H. (2000). Incidence and management of virus diseases of cucurbits in Lebanon. *Crop Protection*, *19*(4), 217-224.
2. Afechtal, M., Aarabe, A., Chebli, B., & Mounir, M. (2019). The occurrence of major viruses infecting Zucchini Squash (Cucurbita pepo L.) in Morocco. *Eur. Sci. J*, *15*, 188-196.
3. Bananej, K., Desbiez, C., Wipf-Scheibel, C., Vahdat, I., Kheyr-Pour, A., Ahoonmanesh, A., & Lecoq, H. (2006). First report of Cucurbit aphid-borne yellows virus in Iran causing yellows on four cucurbit crops. *Plant Disease*, *90*(4), 526-526.
4. Bananej, K., Keshavarz, T., Vahdat, A., Hosseini Salekdeh, G., & Glasa, M. (2008). Biological and molecular variability of Zucchini yellow mosaic virus in Iran. *Journal of Phytopathology*, *156*(11-12), 654-659.
5. Chikh-Ali, M., Rodriguez-Rodriguez, M., Green, K. J., Kim, D. J., Chung, S. M., Kuhl, J. C., & Karasev, A. V. (2019). Identification and molecular characterization of recombinant Potato virus Y (PVY) in potato from South Korea, PVYNTN strain. Plant disease, 103(1), 137-142.
6. Ebrahim-Nesbat, F. (1974). Distribution of watermelon mosaic viruses 1 and 2 in Iran. *Phytopathologische Zeitschrift*, *79*(4), 352-358.
7. Edwardson, J. R. (1966). Electron microscopy of cytoplasmic inclusions in cells infected with rod-shaped viruses. *American journal of botany*, *53*(4), 359-364.
8. Fletcher, J. D., Wallace, A. R., & Rogers, B. T. (2000). Potyviruses in New Zealand buttercup squash (Cucurbits maxima Duch.): yield and quality effects of ZYMV and WMV 2 virus infections. *New Zealand Journal of Crop and Horticultural Science*, *28*(1), 17-26.
9. Gambino, G., Perrone, I., & Gribaudo, I. (2008). A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. *Phytochemical Analysis*, *19*(6), 520-525.
10. Gerami Nooghabi, M., Mehrvar, M., & Zakiaghl, M. (2022). The Complete Genome Sequences of Two Recombinant Isolates of Squash Mosaic Virus from Iran. *Journal of Agricultural Science and Technology*, *24*(2), 477-490.

11.Gholamalizadeh, R., Vahdat, A., Hossein-Nia, S. V., Elahinia, A., & Bananej, K. (2008). Occurrence of Ourmia melon virus in the Guilan Province of Northern Iran. *Plant Disease*, *92*(7), 1135-1135.
12.Hasanvand, V., & Shams-bakhsh, M. (2017). Identification of viruses infecting cucurbits and determination of genetic diversity of Cucumber mosaic virus in Lorestan province, Iran. Journal of Plant Protection Research, 57(2).
13. Hasanvand, V., & Shams-bakhsh, M. (2017). Identification of viruses infecting cucurbits and determination of genetic diversity of Cucumber mosaic virus in Lorestan province, Iran. Journal of Plant Protection Research, 57(2).
14. Hull, R. (2002). Mathews' Plant Virology,(4thedn) Academic Press, New York, human alimentary tract. *Nature*, *300*, 637-638.
15. Hull, R. (2013). Plant virology. Academic press.
Kassem, M. A., Sempere, R. N., Juárez, M., Aranda, M. A., & Truniger, V. (2007). Cucurbit aphid-borne yellows virus is prevalent in field-grown cucurbit crops of southeastern Spain. *Plant Disease*, *91*(3), 232-238.
16. Keshavarz, T., & Izadpanah, K. (2004). Report of Cucurbit yellow stunting disorder virus (Genus Crinivirus) in Iran. In *Proceedings of the 16th Iranian Plant Protection Congress Tabris, Iran* (Vol. 264).
17. Lecoq, H., & Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. In *Advances in virus research* (Vol. 84, pp. 67-126). Academic Press.
18. Lecoq, H., & Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. In *Advances in virus research* (Vol. 84, pp. 67-126). Academic Press.
19. Lecoq, H., & Katis, N. (2014). Control of cucurbit viruses. In Advances in virus research (Vol. 90, pp. 255-296). Academic Press.
20. Maghamnia, H. R., Hajizadeh, M., & Azizi, A. (2018). Complete genome sequence of Zucchini yellow mosaic virus strain Kurdistan, Iran. *3 Biotech*, *8*, 1-7.
21. Massumi, H., Shaabanian, M., Heydarnejad, J., Pour, A. H., & Rahimian, H. (2011). Host range and phylogenetic analysis of Iranian isolates of Zucchini yellow mosaic virus. *Journal of Plant Pathology*, 187-193.
22. Mehrvar, H. R. A. M., & Zakiaghl, M. (2021). GENETIC DIVERSITY AND BIOLOGICAL CHARACTERIZATION OF WATERMELON MOSAIC VIRUS ISOLATES FROM IRAN. *Plant Archives*, *21*(1), 1195-1203.
23. Mendez-Lozano, J., Torres-Pacheco, I., Fauquet, C. M., & Rivera-Bustamante, R. F. (2003). Interactions between geminiviruses in a naturally occurring mixture: Pepper huasteco virus and Pepper golden mosaic virus. *Phytopathology*, *93*(3), 270-277.
24. Mohammadi, K., Hajizadeh, M., & Koolivand, D. (2016). Detection and identification of four vegetable fruit viruses in west and northwest of Iran. Iranian Journal of Plant Pathology, 52(2).
25. Naidu, R. A. (2001, June). Hughes, JdA Methods for the detection of plant virus diseases. In *Proceedings of the Conference on Plant Virology in Sub Saharan Africa organized by IITA, Ibadan, Nigeria* (pp. 4-8).
26. Nontajak, S., Vulyasevi, S., Jonglaekha, N., & Smitamana, P. (2014). Effect of mixed viruses infection on symptom expression in zucchini (Cucurbita pepo L.). *Journal of Agricultural Technology*, *10*(5), 1329-1341.
27. Pozzi, E., Perotto, M. C., Bertin, S., Manglli, A., Luciani, C., Conci, V. C., & Tomassoli, L. (2020). Molecular variability of watermelon mosaic virus isolates from Argentina. European Journal of Plant Pathology, 156,1-9.
28. Renner, S. S., & Schaefer, H. (2016). Phylogeny and evolution of the Cucurbitaceae. In *Genetics and genomics of Cucurbitaceae* (pp. 13-23). Springer, Cham.
29. Rentería-Canett, I., Xoconostle-Cázares, B., Ruiz-Medrano, R., & Rivera-Bustamante, R. F. (2011). Geminivirus mixed infection on pepper plants: synergistic interaction between PHYVV and PepGMV. *Virology journal*, *8*, 1-14.
30. Salehzadeh, M. (2018). Survey on presence of Cucumber mosaic virus (CMV) in single and mixed infections with potyviruses in North-West of Iran. *Genetic Engineering and Biosafety Journal*, *7*(2), 163-173.

31. Sastry, K. S., Mandal, B., Hammond, J., Scott, S. W., Briddon, R. W., Sastry, S. K., ... & Hammond, J. (2019). Lilium spp.(Lilium longiflorum, L. lancifolium, L. tsingtauense). *Encyclopedia of Plant Viruses and Viroids*, 1376-1385.
32. Sokhandan-Bashir, N., Ghasemzadeh, A., Masoudi, N., Khakvar, R., & Farajzadeh, D. (2013). Degenerate primers facilitate the detection and identification of potyviruses from the northwest region of Iran. *Iranian Journal of Biotechnology*, *11*(2), 115-122.
32. Sui, X., Li, R., Shamimuzzaman, M., Wu, Z., & Ling, K. S. (2019). Understanding the Transmissibility of Cucumber Green Mottle Mosaic Virus in Watermelon Seeds and Seed Health Assays. *Plant disease*, *103*(6), 1126-1131.
33. Vafaei, S. H., & Mahmoodi, M. (2017). Presence of recombinant strain of Cucurbit aphid borne yellows virus in Iran. *Iranian journal of biotechnology*, *15*(4), 289.
34. Welbaum, G. E. (2015). *Vegetable production and practices*. CABI.