jotaf
Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Eylül/September 2024, 21(4)
Başvuru/Received: 14/04/23
Kabul/Accepted: 21/08/24
DOI: 10.33462/jotaf.1283160

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Assessment of Different Cucurbit Genotypes for Resistance to Zucchini Yellow Mosaic Virus (ZYMV)

Farklı Kabakgil Genotiplerinin Kabak Sarı Mozaik Virüsü (Zucchini Yellow Mosaic Virus-ZYMV)'ne Karşı Duyarlılıklarının Belirlenmesi

Akife DALDA-SEKERCI[1], Cemile TEMUR-CINAR[2*], Emel ÜNLÜ[3], Hakan FIDAN[4], Halit YETISIR[5]

Abstract

Members of the *Cucurbitaceae* family, which includes species with quite different characteristics, have been used for food, medicine, and ornamental purposes for a long time. However, most plant diseases and pests cause yield and quality losses in cucurbits, and one of the most important of these diseases is zucchini yellow mosaic virus (ZYMV), which one of the most common potyviruses worldwide and causes serious yield losses in cucurbit production worldwide. Zucchini Yellow Mosaic Virus shows symptoms such as yellowing, mottling, curling, deformation, mosaic, shortening and thickening of the internodes, and may also cause loss of yield and quality. As widely known, there is no effective chemical control of viral diseases, and the use of resistant or tolerant varieties is the most effective solution. In this study, 92 watermelon genotypes, 14 zucchinis (pumpkin seeds) and 29 ornamental pumpkins collected from different parts of Türkiye were tested against ZYMV. Symptoms of ZYMV in different watermelon genotypes and pumpkins were observed for 21 days. Genotypes showing systemic infection after inoculation were evaluated on a scale of 0-5. Also, RT-PCR studies were carried out on selecting nine symptomless control plants, seven ZYMV-sensitive genotypes showing 5-scale value, one genotype with 1-scale value considered tolerant, and one genotype belongs to *C. lanatus* var. *citroides*. According to the results, it was determined that some watermelon and ornamental pumpkin genotypes could be considered as tolerant. Watermelon, which was having accession number PI560016, was found resistant to Turkish local strain of ZYMV. Although different susceptibility levels were detected between watermelon genotypes, all pumpkin genotypes were discovered to be susceptible to the Turkish local strain of ZYMV.

Keywords: Cucurbitaceae, Watermelon, Pumpkin, ZYMV, RT-PCR

[1]Akife Dalda-Sekerci, Department of Horticulture, Faculty of Agriculture, Erciyes University, 38030, Kayseri, Türkiye. E-mail: akifedalda@erciyes.edu.tr OrcID: 0000-0001-8554-6501

[2]***Sorumlu Yazar/Corresponding Author:** Cemile Temur-Cinar, Department of Plant Protection, Faculty of Agriculture, Erciyes University, 38030, Kayseri, Türkiye. E-mail: ctemur@erciyes.edu.tr OrcID:0000-0002-0248-1835

[3]Emel Ünlü, Department of Horticulture, Faculty of Agriculture, Erciyes University, 38030, Kayseri, Türkiye. E-mail: emelunlu1995@gmail.com OrcID: 0000-0002-1047-7828

[4]Hakan Fidan, Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Antalya, Türkiye. E-mail: hakanfidan@akdeniz.edu.tr OrcID: 0000-0002-0384-9486

[5]Halit Yetisir, Department of Horticulture, Faculty of Agriculture, Erciyes University, 38030, Kayseri, Türkiye. E-mail: yetisir1@yahoo.com OrcID: 0000-0001-6955-9513

Atıf: Dalda-Sekerci, A., Temur-Cinar, C., Ünlü, E., Fidan, H., Yetisir, H. (2024). Farklı Kabakgil Genotiplerinin Kabak Sarı Mozaik Virüsü (Zucchini Yellow Mosaic Virus-ZYMV)'ne Karşı Duyarlılıklarının Belirlenmesi. *Tekirdağ Ziraat Fakültesi Dergisi,* 21(4): 880-891.
Citation: Dalda-Sekerci, A., Temur-Cinar, C., Ünlü, E., Fidan, H., Yetisir, H. (2024). Assessment of Different Cucurbit Genotypes for Resistance to Zucchini Yellow Mosaic Virus (ZYMV). *Journal of Tekirdag Agricultural Faculty,* 21(4): 880-891.

©Bu çalışma Tekirdağ Namık Kemal Üniversitesi tarafından Creative Commons Lisansı (https://creativecommons.org/licenses/by-nc/4.0/) kapsamında yayınlanmıştır. Tekirdağ 2024

Öz

Oldukça farklı özelliklere sahip türlerin yer aldığı *Cucurbitaceae* familyasının üyeleri gıda, ilaç ve süs amaçlı kullanılmaktadır. Ancak çoğu bitki hastalığı ve zararlısı kabakgillerde verim ve kalite kayıplarına neden olmaktadır. Kabakgil üretiminde ciddi verim kayıplarına neden olan bu hastalıkların en önemlilerinden biri dünya çapında en yaygın görülen potyvirüslerden biri olan kabak sarı mozaik virüsüdür (ZYMV). Kabak sarı mozaik virüsü, sararma, beneklenme, kıvrılma, deformasyon, mozaikleşme, boğum aralarının kısalması ve kalınlaşması gibi belirtiler göstermekle birlikte verim ve kalite kaybına da neden olabilmektedir. Bilindiği gibi viral hastalıklara karşı etkili bir kimyasal mücadele mevcut olmayıp, dirençli veya toleranslı çeşitlerin kullanılması en etkili çözümdür. Bu çalışmada Türkiye'nin farklı yerlerinden toplanan 92 karpuz, 14 çekirdeklik kabak ve 29 süs kabağı genotipi, ZYMV'ye karşı test edilmiştir. Farklı karpuz genotipleri ve kabaklarda ZYMV belirtileri 21 gün boyunca gözlemlenmiştir. İnokulasyon sonrası sistemik enfeksiyon gösteren genotipler 0-5 hastalık skalasına göre değerlendirilmiştir. Ayrıca RT-PCR testiyle, dokuz simptomsuz kontrol bitkisi, 5 skala değerine giren ZYMV'ye duyarlı yedi genotip, toleranslı kabul edilen 1 skala değerine giren bir genotip ve *C. lanatus* var. *sitroidler* testlenmiştir. Sonuçlara göre bazı karpuz ve süs kabağı genotiplerinin toleranslı olarak değerlendirilebileceği belirlendi. PI560016 erişim numarasına sahip karpuzun Türkiye'deki yerel ZYMV türüne karşı dirençli olduğu belirlendi. Karpuz genotipleri arasında farklı duyarlılık düzeyleri tespit edilmesine rağmen tüm kabak genotiplerinin Türkiye'nin yerel ZYMV türüne karşı duyarlı olduğu tespit edilmiştir.

Anahtar Kelimeler: Cucurbitaceae, Karpuz, Balkabağı, ZYMV, RT-PCR

1. Introduction

The *Cucurbitaceae* family, commonly identified as cucurbits (cucumber, melon, watermelon, pumpkins, gourds, and squashes), consists of several economically and nutritionally important vegetable crops that are cultivated worldwide (Yanmaz and Düzeltir, 2003; Gáspár et al., 2022). Virus diseases are one of the important factors limiting production, regardless of where cucurbits are grown. At least 59 viruses belonging to major plant virus groups have been reported to infect cucurbits worldwide and cause significant losses (Lecoq and Desbiez, 2012). Diseases of pumpkin plants are caused by various viruses, such as papaya ringspot virus (PRSV), watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) which belongs to the Potyvirus genus (Simmons et al., 2013). Virus infections in cucurbit cultivation areas cause serious economic losses in many parts of the world. ZYMV is one of the main viruses that damage cucurbit crops in Turkey, which is an important cucurbit producer (Yılmaz et al., 1992)

ZYMV is also one of the most common potyvirus worldwide and has been reported to cause yield losses of up to 100% (Moradi et al., 2019). ZYMV is a member of the *Potyvirus* genus of the *Potyviridae* family. The virus is filamentous and flexible in shape, with a 750x13 nm dimension in size, and the genome consists of single-stranded RNA (Balint et al., 1990). ZYMV was first reported in 1973 in Italy (Lisa et al., 1981) and France (Lecoq et al., 1981). After being infected with ZYMV, zucchini plants exhibit symptoms such as a mosaic pattern and yellowing on their leaves. In more severe cases of infection, the leaves may show narrowing and a filamentous appearance. The photosynthetic pigment of leaves (Chl a, Chl b, and carotenoids) is significantly reduced during ZYMV infection (Radwan et al., 2007). In addition, a decrease of approximately 48% in the total pigment content was determined under ZYMV infection, while studies reported that leaf protein, carbohydrate, and proline content increased in infected plants (Radwan et al. 2007). In addition, serious deformations, deterioration in fruit shape and color changes are observed in fruits harvested from diseased plants, which makes them unmarketable. The yield losses can vary between 50-100% because of the virus infection depending on the severity of the infection and the conditions (Blua and Perring, 1992; Massumi et al., 2011).

There are 26 different races of the agent according to its biological, serological, molecular, and epidemiological characteristics. The most common races are ZYMV-MZ (Malaysia), ZYMV-TW (Taiwan), ZYMV-FL (Florida), ZYMV-CT (Connecticut), ZYMV-CH (China), and ZYMV-WK (France) (Wang et al., 1992; Sidek et al., 1999). While the spread of ZYMV in long distance occurs through the transfer of infected seeds from one region to another, while short distance spread by aphids (*Myzus persicae*) in short distances (Gal-On, 2007; Simmons et al., 2011). Plant diseases caused by viruses are among the most important factors limiting the production of watermelons, cucumber, melon, pumpkins, and ornamental pumpkins. The control of ZYMV largely depends on the suppression and control of the insect vector (aphid) population using insecticides; however, this practice may sometimes be ineffective, especially under high aphid population pressure (Shrestha et al., 2021). The development and usage of virus-resistant Cucurbita crops is the most effective strategy to reduce yield losses caused by ZYMV and is therefore a major target for pumpkin growers (Shrestha et al., 2021). Different watermelon, zucchini and ornamental pumpkin, genotypes of Turkiye were collected by our study group, self-pollinated severally to increase homozygosity of genetic structure, and these populations were characterized in terms of some morphological, molecular, and biochemical features (Dalda-Şekerci et al., 2017; Dalda-Şekerci et al., 2020; Morilipınar et al., 2021). However, no screening study for viral diseases has been conducted in these populations. The aim of this study was to determine the susceptibility level of ornamental pumpkin (29 genotypes), watermelon (92 genotypes) and seed pumpkin (14 genotypes) from Turkiye, collected from the main Cucurbit crops growing region of Turkiye, to ZYMV isolate. Identifying potential sources of disease resistance of these genotypes is very important for future breeding studies.

2. Materials and Methods

2.1. Plant Materials

In the study, pumpkin (*Cucurbita pepo*-14, seed pumpkin), ornamental pumpkin (*Cucurbita pepo* var. *ovifera*- 29) and watermelon (*Citrullus lanatus* var. *lanatus*- 92) plants collected from different regions of Turkiye were used as plant material. These genotypes used in the study have been selected from a population whose morphological, genetic, and biochemical characteristics have been determined, and have superior agronomic characteristics previous studies (Solmaz and Sarı, 2009; Solmaz et al., 2016; Dalda-Şekerci et al.,

2017; Coskun, 2019; Dalda-Şekerci et al., 2020; Morilipınar et al., 2021). In addition, one fusarium (F2) resistant accession (*Citrullus lanatus* var. *citroides*) (Wechter et al., 2016), six nematode-resistant watermelon accessions (N1, N3, N4, N5, N6, N7, F2) (*Citrullus amarus*) (Thies et al., 2016) and five ZYMV resistant *C. lanatus* var. *citroides* (Guner et al. 2019) obtained from the Gene Bank were used as the outgroup (Seeds of PI 595203 were obtained from Provvidenti, Cornell University, New York). One ZYMV resistant and one ZYMV susceptible *Lagenaria siceraria* (LS-R and LS-S (Ünlü et al., 2020), and ZYMV susceptible watermelon cultivars Sugar baby and Dixie Lee were used as a positive control. Seedlings were grown under greenhouse conditions in May and when the seedlings reached the 2-3 leaf stage. The experiment was designed with 5 replications of each genotype, they were transplanted into 15 cm diameter plastic pots in peat perlite mixture. The seedlings were kept at +22-24 °C temperature, 60-70% relative humidity and 16/8 (light/dark) hour light period (Agrios, 2005; Fidan et al., 2012; Ünlü et al., 2020).

2.2. Transmission of the Virus by Biological Methods

Virus isolate was obtained from the Akdeniz University Plant Virology Laboratory. In mechanical inoculation, a ZYMV isolate was used (NCBI accession number JF317296.1). The ZYMV isolate used in the study has been tested for all harmful viral diseases in cucurbits, and it has been determined to be a single infection (Helvaci et al., 2019; Nacar et al., 2021). Extractions were prepared on ice in a sterile mortar (to prevent inactivation) and the mixture was filtered with filter paper and smeared on the leaves of the plants with a sponge pad. Inoculations were carried out manually. When the plants were at the 2-3 true leaf stage, all leaves including the cotyledon leaves were inoculated. In addition, a second inoculation was made at the 5-6 leaf stage. Symptoms were observed and recorded daily.

2.3. Symptom Observations

After virus inoculation in genotypes of pumpkin (*Cucurbita pepo*-14), ornamental pumpkin (*Cucurbita pepo* var. *ovifera*- 29) and watermelon (*Citrullus lanatus*-92) plants used in the study, the symptoms of the plants were observed for 21 days, and the symptom types were recorded. Genotypes showing systemic infection after inoculation were evaluated on a scale of 0-5 (*Figures 1* and *2*). As a result of the evaluation, those with a value of 1 or higher were recorded as susceptible (S), and genotypes with a scale value of 0 that did not show systemic infection were recorded as resistant (R). The following 0-5 scale was used to determine the severity of virus symptoms (Aliyu et al., 2013).

0 = Plants without any symptom development

1 = 1 - 20% (very light); Plants showing very slight discoloration in leaf veins.

2 = 21 - 40% (light); Moderate mosaic plants along with slight discoloration in leaf veins.

3 = 41% - 60% (severe); Plants showing moderate to severe mosaic and yellowing in leaves.

4 = 61-80% (very severe); Severe mosaic symptoms de deformation in the leaves and stunted in the plant.

5 = 81-100% (almost dead); Severe mosaic in leaves, speckling, shortening of plant height, shoestring symptom, and deformation of leaves**.**

2.4. ***Nucleic Acid Isolation***

A limited number of samples were randomly selected for nucleic acid isolation from inoculated plants. Sampling was carried out by selecting 9 symptomless control plants, 7 ZYMV-sensitive and 5-scale symptomatic genotypes, 1 1-scale genotype (56) considered tolerant, six genotypes (PI244019, PI560016, PI595200, PI595201, PI 595203, USVL252) obtained from the Gene Bank (Guner et al., 2019) and controls (negative and positive). Nucleic acid isolation was performed using the modified "Dellaporta Nucleic Acid Extraction" method reported by Presting et al. (1995) and carried out as follows. Leaf samples were crushed in 1.2 ml extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 500 mM NaCl, 10 mM 2-mercaptoethanol). 70 µl of 10% SDS was added and left at 65ºC for 10 minutes. After 200 µl of 5 M potassium acetate was added to the tubes, it was kept on ice for 10-15 minutes, and 600 µl of cold 96% ethanol was added to the pellet and washed. The dried pellet was mixed by adding 400 µl of sterile distilled water. It was incubated at 37°C for 15 minutes.

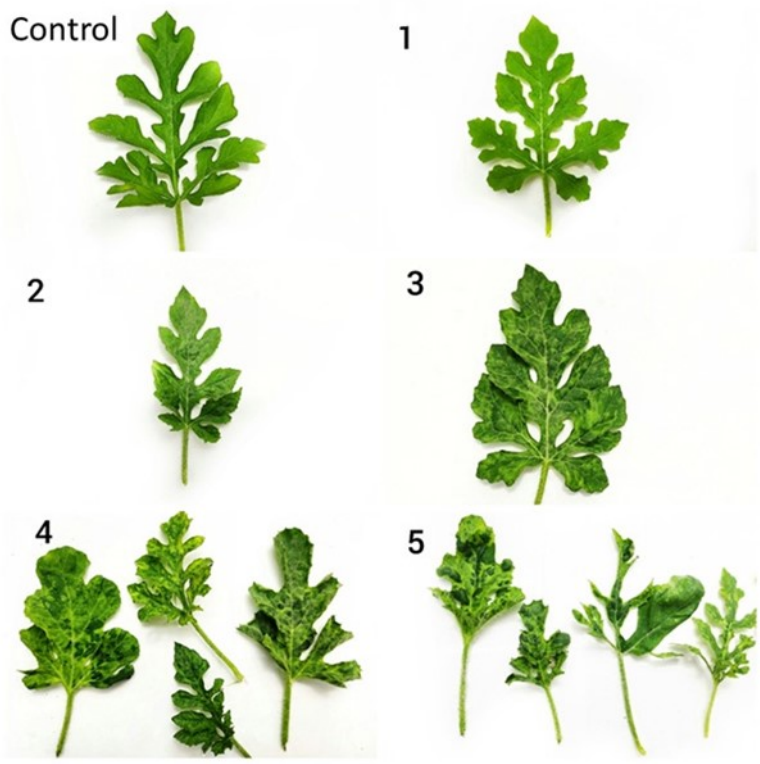

Figure 1. Zucchini Yellow Mosaic Virus symptoms and 0-5 scale in watermelon genotypes

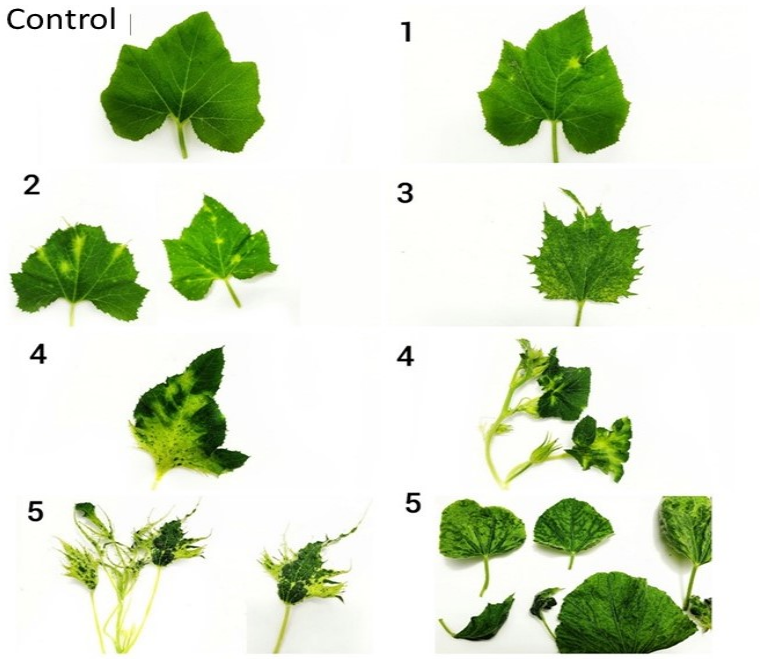

Figure 2. Zucchini Yellow Mosaic Virus symptoms and 0-5 scale in pumpkin genotypes.

2.5. cDNA Synthesis and RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) Analyzes

cDNA was synthesized using total RNAs as templates. After the mixture was prepared with 1 µl of random hexamer primer, 10 µl of RNA and 1 µl of 10mM dNTP, it was incubated at 65°C for 5 minutes and then kept on ice for 5 minutes. 1 µl of M-MLV reverse transcriptase (Moloney Murine Leukaemia virus reverse transcriptase, 200 units/µl, Invitrogen), 4 µl of 5x Reverse Transcriptase buffer (Invitrogen), 2 µl of 0.1M DTT and 1 µl of

RNAse free water were added. After incubation at 25°C for 10 minutes, at 37°C for 50 minutes, and at 70°C for 15 minutes, they were kept on ice. The synthesized cDNAs were stored at -20°C. PCR was performed using cDNAs as templates. RT-PCR analyzes were performed with primers [ZYMVF, (5'–3' ATGCTCCAATCAGGCACYC) ZYMVR, (5'–3' GTGTGCCGTTCAGTGTCTTC)] (Papayiannis et al., 2005).

The content of the 15 µl mixture used in the reaction; 4 µl of cDNA and 2 µl of 10X Taq Buffer, 2 µl of 25 mM MgCl2, 2 µl of 5 mM dNTP, 0.5 µl of Taq polymerase, Forward primer 1µL, Reverse primer 1 µL were prepared and amplified in a Thermal Cycler device. In the amplification program used for RT-PCR analysis, initial denaturation at 95°C for 5mins, 30 sec denaturation at 95°C, 45 sec annealing at 52°C, 1 min extension at 72°C"repeated for 35 cycles and the final extension was performed at 72 °C for 7 minutes (Fidan et al., 2012; Ünlü et al., 2020). RT-PCR products were analyzed in 2% agarose gel electrophoresis. Afterward, the gel was stained with ethidium bromide and visualized in a UV imaging system.

3. Results and Discussion

3.1. Symptom Observation Results

Symptoms of ZYMV in different watermelon genotypes and pumpkins were observed for 21 days. The symptom types depending on the severity of the infection were recorded. Genotypes showing systemic infection after inoculation were evaluated according to the 0-5 scale, and two separate symptom scales were created for watermelon (*Figure 1*), pumpkin and ornamental pumpkin genotypes (*Figure 2*).

All watermelon and pumpkin genotypes were found to be infected with Turkish local isolate of ZYMV, except PI 560016 (*C. lanatus* var. *citroides*), which was reported to be resistant to strain ZYMV-FL by Guner et al. (2019) (*Table 1* and *Figure 4*). All pumpkin genotypes, 93% of ornamental pumpkin genotypes, and 90% of watermelon genotypes scored between 3-5 scale values and were determined to be highly susceptible to ZYMV (*Tables 1* and *2*). Nine watermelon and 2 ornamental pumpkin genotypes with 1-2 scale values were recorded as tolerant. None of the pumpkin and ornamental pumpkin genotypes used in the study were resistant to ZYMV after evaluation of the symptoms. While symptoms such as mosaic formation and chlorosis were observed on the leaves of the genotypes with a scale value of 1-2 (*Figures 1* and *2*), it has been observed that the 3-5 scale genotypes have a narrowing and filamentous appearance in the leaf blades. (*Figures 1* and *2*). According to the results, it was determined that some watermelon and ornamental pumpkin genotypes could be considered as tolerant. By continuing selfing and virus testing, high tolerance individuals can be developed from these genotypes that have not achieved homozygosity. It was observed that 7 of the genotypes obtained from the USDA Gene Bank fusarium and nematode tolerant genotypes showed severe symptoms (*Table 2*). The five watermelon (*C. lanatus* var. *citroides*) genotypes obtained from the gene bank and reported to be resistant to ZYMV (Guner et al. 2019), only PI560016 was found resistant to Turkish ZYMV isolate. Similarly, some PIs reported as resistant to ZYMV-FL by Provvidenti (1991) were found susceptible by Guner et al. (2019). The reason for this was stated that possible resistances may be strain-specific or temperature-dependent. In addition, the fact that most of the tested genotypes are not sufficiently purified may cause different results in the tests. For this reason, resistant genotypes can be found among the genotypes identified as susceptible in this study. It is also possible that genotypes identified as resistant sometimes have susceptible individuals. Therefore, researchers and breeders should continue to self-pollination and select the most resistant individuals to develop resistant inbred lines. Viruses usually change form and mutate very quickly and cause different symptoms in genotypes. Similarly, results from a study conducted by Helvaci et al. (2019) stated that a single melon and pumpkin genotype was found to be resistant to ZYMV in their test study with including 38 pumpkins, 19 melon and 8 watermelon genotypes. In recent studies on the resistance of different local cucurbit genotypes to ZYMV, it has been determined that a significant number of local genotypes were sensitive to ZYMV (Ünlü et al., 2020; Helvaci et al., 2019). As with other given studies, although the majority of genotypes tested are susceptible to ZYMV, resistant genotypes can also be developed by introducing resistance sources in the cucurbit populations.

Table 1 Zucchini Yellow Mosaic Virus symptom observation scores in watermelon (0-5 scale)

Genotype no	Scale value	Genotype no	Scale value	Genotype no	Scale value	Genotype no	Scale value
PI24401*	4	**41**	5	**111**	5	**206**	3
PI482259*	5	**44**	5	**112**	5	**213**	**2**
PI482319*	4	**42**	5	**114**	5	**223**	5
PI189225*	5	**45**	4	**119**	5	**224**	4
PI482324*	5	**46**	5	**122**	5	**225**	**2**
PI482303*	5	**47**	5	**125**	**2**	**229**	5
USVL252**	5	**48**	**2**	**136**	4	**234**	3
PI244019***	5	**50**	4	**137**	3	**241**	5
PI560016***	0	**52**	5	**138**	5	**247**	5
PI595200***	4	**53**	4	**141**	5	**252**	5
PI595201***	3	**56**	**1**	**149**	3	**285**	5
PI595203***	4	**59**	4	**151**	5	**298**	**2**
3	4	**58**	5	**161**	4	**303**	3
5	4	**62**	5	**165**	5	**305**	4
6	5	**63**	5	**168**	4	**322**	4
9	5	**68**	5	**171**	5	**333**	5
11	5	**70**	4	**174**	4	**341**	3
13	5	**71**	5	**183**	**2**	**342**	**2**
18	3	**75**	5	**184**	3	**347**	5
22	4	**77**	4	**187**	4	**350**	5
23	**2**	**78**	5	**190**	5	**354**	5
28	3	**85**	5	**192**	3	**356**	5
35	5	**86**	4	**194**	4	**384**	3
36	4	**89**	4	**195**	5	**LS-R**	**0**
37	5	**90**	3	**199**	5	**LS-S**	**4**
38	5	**91**	4	**200**	4	**Sugar baby**	**5**
40	4	**96**	5	**203**	5	**Dixie Lee**	**5**

*Nematode resistance *Citrullus amarus* accessions (Thies et al. 2016), ***Fusarium oxysporium* f.sp. *niveum*-2 resistance *Citrullus lanatus* var. *citroides* accession (Wechter et al. 2016), *** ZYMV resistance *Citrullus lanatus* var. *citroides* accessions (Guner et al. 2019).

Table 2 Zucchini Yellow Mosaic Virus symptom observation scores in pumpkin genotypes (0.5 scale)

C. pepo		*C. pepo* var. *ovifera*			
Genotype no	**Scale value**	**Genotype no**	**Scale value**	**genotype no**	**Scale value**
12	5	**1**	5	**27**	5
15	5	**3**	4	**29**	4
17	5	**4**	5	**30**	3
19	5	**5**	4	**31**	5
23	5	**6**	5	**32**	5
24	5	**17**	4	**33**	5
27	5	**18**	5	**36**	5
29	5	**19**	**2**	**37**	5
33	5	**21**	5	**39**	5
39	5	**22**	3	**40**	5
44	4	**23**	**2**	**41**	5
45	5	**24**	5	**42**	5
46	5	**25**	4	**43**	4
68	5	**26**	5	**44**	4
				45	4

3.2. *PCR Analysis Results*

The RT-PCR method, which is one of the molecular methods used extensively in current research in the field of plant virology, was used for the detection of ZYMV in infected and negative (symptomless) control plants.

RT-PCR studies were carried out on selecting nine symptomless control plants, seven ZYMV-sensitive genotypes showing 5-scale value, one genotype (56) with 1-scale value considered tolerant, and one genotype (F2) belongs to *C. lanatus* var. *citroides* (*Figure 3*).

In RT-PCR studies, the expected band sizes were obtained after the usage of primers specific to the ZYMV coat protein in the amplification of gene fragments (*Figure 3*). Five *C. lanatus* var. *citroides* accessions reported to be resistant to ZYMV-FL by Guner et al. (2019), positive and negative control plants (watermelon cultivar and bottle gourd accessions) were also tested for the Turkish local strain of ZYMVY. The five accessions reported to be resistant to ZYMV, only PI560016 was found to be resistant to the local Turkish strain of ZYMV (*Table 1* and *Figure 4*). The current results are consistent with the results of previous studies conducted on ZYMV (Fidan et al., 2012; Helvaci et al., 2019; Guner et al., 2019; Ünlü et al., 2020). The researchers detected the presence of ZYMV in plant tissue by RT-PCR process using purified viral RNAs and ZYMV-F1 and ZYMV-R1 primer pairs. In this study, a 791 bp band marker for ZYMV infection was observed in all infected plant samples by agarose gel electrophoresis (*Figures 3* and *4*).

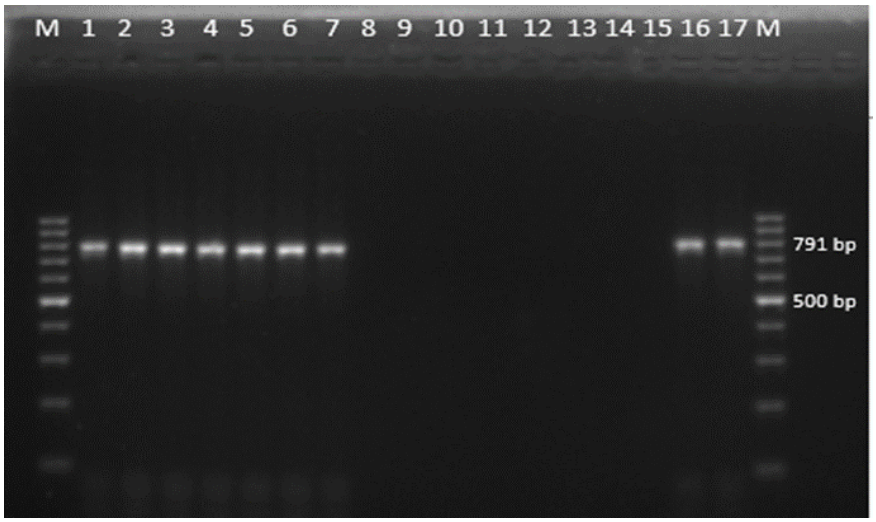

Figure 3 Samples showing a positive and negative reaction after RT-PCR analysis using ZYMV-specific (ZYMVF / ZYMVR) primers.

*(M: DNA ladder, 1: Watermelon 56, 2: Watermelon F2, 3: Watermelon 75, 4: Watermelon 171, 5: Watermelon 199, 6: Watermelon 333, 7: Watermelon 354, 8-9-10-11-12: Watermelon negative control, 13-14-15: Ornamental pumpkin negative control, 16: Ornamental pumpkin 24, 17: Ornamental pumpkin 30).

Figure 4. RT-PCR results of five Citrullus lanatus var citroides accessions found resistant to ZYMV-FL by Guner et al (2019) infected with the Turkish local strain of ZYMV.

Ling and Levi (2007) tested 190 bottle gourd genotypes for ZYMV-FL and they found that 36 of which were fully resistant, 64 genotypes were partially resistant, and 90 genotypes were susceptible. ZYMV-FL resistance was found mostly in *L. siceraria* genotypes collected in India. The 36 resistant genotypes, 33 were from India, one from Indonesia, one from South Africa and one from Zimbabwe, Guner et al. (2019) tested watermelons in the gene bank of USDA against ZYMV-FL strain and they discovered that PI 595203, PI 386015, PI 386016, PI386024, PI 386025, PI 386026, PI 244018, PI 244019, PI 485583, PI 494528, and PI 494529 watermelon genotypes were resistant to ZYMV. In addition, serological tests with Indirect-ELISA in a study conducted in Bali showed that 75% of zucchini plants were infected with ZYMV and up to 8.33% were positive for CMV (Pandawani and Wıdnyana, 2021). In another study, researchers reported that resistance to ZYMV in watermelon is controlled by a recessive gene (Guner et al., 2018). This study demonstrates that ZYMV resistant lines can be developed by producing lines with improved purity from the watermelon populations tested in our study. Many other studies have been carried out to develop new varieties resistant to ZYMV and to reveal the genetic structure of the disease. Many researchers have attempted to analyze the inheritance of ZYMV resistance and identify markers associated with resistance genes (Harris et al., 2009; Pachner et al., 2015; Capuozzo et al., 2017, Guner et al., 2018).

4. *Conclusions*

In this study, 92 local watermelons, 14 zucchinis (seed pumpkins) and 29 ornamental pumpkins genotypes, collected from different parts of Turkiye and 12 *C. lanatus* var. *citroides* genotypes provided form gene bank were tested against local Turkish ZYMV strain. PI560016 was found resistance to ZYMV. Although different susceptibility levels were detected among watermelon genotypes, all pumpkin genotypes were found to be susceptible to the tested Turkish local strain of ZYMV. ZYMV still continues to have a significant threat to the cultivation of cucurbits species both in greenhouses and open fields in Turkiye. Since there is no effective chemical control of viruses, measures to prevent virus infection and spread are still important approaches in crop production. In the control of virus vectors, the use of pesticides is not recommended since there is a very short time between the infection of the virus by the vector and its transmission. The development of varieties resistant to biotic stress factors, especially viruses, is the safest way in terms of sustainable environment and plant production. Breeding studies to develop resistant cultivars to ZYMV are of great importance in Cucurbit species cultivation. Breeding studies related to resistance to ZYMV have been carried out in recent times and some genes responsible for resistance have been identified and reported. The new strategies aimed at producing resistant plants (through conventional breeding programs, biotechnological and molecular breeding techniques or pathogen-derived strategies) should be improved. More tolerant genotypes can be developed by inbreeding and virus testing in each generation from the tolerant watermelon genotypes in the current study. In addition, Guner et al. (2019) reported PIs should be included in Turkish watermelon germplasm and ZYMV-resistant breeding lines should be developed by combination and backcrossing methods. Developing and disseminating virus-resistant cucurbit varieties, preferring ZYMV-free seedlings, regularly observation of the seedlings for ZYMV at regular intervals after planting in the field, eradication of diseased plants and applying control methods to reduce the possibility of ZYMV transmission with aphids are the most effective strategies to reduce yield losses caused by ZYMV. In addition, no resistant or tolerant genotypes were found in the seed pumpkin and ornamental pumpkin tested in this study. However, all seed pumpkin and ornamental pumpkin, except *C. pepo* var. *ovifera* genotypes 19 and 23, showed severe (4-5 scale value) symptoms in a short time. It is thought that some of these genotypes can be used as indicator plants (susceptible control) for ZYMV propagation in further studies.

Acknowledgment

No funding was received for conducting this study

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Akife Dalda-Sekerci Data curation, Formal analysis, Writing – review & editing. Cemile Temur Cinar: Data curation, Writing – review & editing. Emel Ünlü: Data curation, Formal analysis, Writing – review & editing. Hakan Fidan: Review & editing, Supervision. Halit Yetisir: Conceptualization, Methodology, Funding acquisition, Resources, Project administration, Writing – review & editing, Supervision.

References

Agrios, G. (2005). Plant pathology. Elsevier Academic Press. 767p.

Aliyu, T. H., Balogun, O. S. and Uddin, R. O. (2013). Cowpea virus disease occurrence: implication for food security and sustainable development in Kwara State-Nigeria. *Albanian Journal of Agricultural Sciences*, 12:4-633.

Balint, R., Plooy, I. and Steele, C. (1990). The nucleotide sequence of *zucchini yellow mosaic potyvirus*. Abstr. VIIIth *International Congress Virology*. 8:84–107.

Blua, M. J. and Perring, T. M. (1992). Alatae production and population increase of aphid vectors on virus-infected host plants. *Oecologia* 92:65-70.

Capuozzo, C., Formisano, G., Iovieno, P., Andolfo, G., Tomassoli, L., Barbella, M. M. and Ercolano, M. R. (2017). Inheritance analysis and identification of SNP markers associated with ZYMV resistance in *Cucurbita pepo*. *Molecular Breeding*, 37(8):1-12.

Coskun, Ö. F., (2019). *Determination of molecular markers associated with locus controlling some characters affecting yield and quality in watermelon.* (Ph.D. Thesis). Erciyes University, Graduate School of Natural and Applied Sciences. Kayseri. (In Turkish).

Dalda-Şekerci, A., Karaman, K. and Yetişir, H. (2020). Characterization of ornamental pumpkin (*Cucurbita pepo* L. var. *ovifera* (L.) Alef.) genotypes: molecular, morphological and nutritional properties. *Genetic Resources and Crop Evolution*, 67(3):533-547.

Dalda-Şekerci, A., Karaman, K., Yetisir, H. and Sagdic, O. (2017). Change in morphological properties and fatty acid composition of ornamental pumpkin seeds (*Cucurbita pepo* var. *ovifera*) and their classification by chemometric analysis. *Journal of Food Measurement and Characterization*, 11(3):1306-1314.

Fidan, H., Unlu, M. and Unlu, A. (2012). Determination of Batem's melon pure lines for resistance to ZYMV. Xth *EUCARPIA International Meeting on Genetics and Breeding of Cucurbitaceae*, 466-471.

Gal-On, A. (2007). *Zucchini yellow mosaic virus*: Insect transmission and pathogenicity – The tails of two proteins. Molecular Plant Pathology, 8:139–150.

Gáspár, S., Basal, O., Simkó, A., László, K. and Frommer, D., Veres, S. (2022). Production of Hull-less mutant of pumpkin seed under different abiotic conditions. *Journal of Tekirdag Agricultural Faculty*, 19(3), 508-514.

Guner, N., Pesic-VanEsbroeck, Z., Rivera-Burgos, L. A. and Wehner, T. C., (2019). Screening for resistance to *Zucchini Yellow Mosaic Virus* in the watermelon germplasm. *Hortsciıence*, 54(2):206–211.

Guner, N., Rivera-Burgos, L. A. and Wehner, T. C. (2018). Inheritance of resistance to *Zucchini Yellow Mosaic Virus* in watermelon. *HortScience*, 53:1115–1118.

Harris, K. R., Ling, K. S., Wechter, W. P. and Levi, A. (2009). Identification and utility of markers linked to the *zucchini yellow mosaic virus* resistance gene in watermelon. *Journal of the American Society for Horticultural Science*, 134(5):529-534.

Helvaci, M., Yılmaz, N. and Fidan, H. (2019). Evaluation of some cucurbit accessions collected from Northern Cyprus for resistance to *Zucchini Yellow Mosaic Virus* (ZYMV). *Fresenius Environmental Bulletin*, 28(7):5402-5404.

Lecoq, H. and Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. *Advances in Virus Research*, 84:67–126.

Lecoq, H., Pitrat, M. and Clément, M. (1981). Identification et caractérisation d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. *Agronomie,* 1:827–834. (in French).

Ling, K. S. and Levi, A. (2007). Sources of resistance to *Zucchini yellow mosaic virus* in *Lagenaria siceraria* germplasm. *HortScience*, 42(5):1124-1126.

Lisa, V., Boccardo, G., D'Agostino, G., Dellavalle, G. and d'Aquilio, M. (1981). Characterization of a potyvirus that causes *zucchini yellow mosaic virus*. *Phytopathology*, 71:667–672.

Massumi, H., Shaabanian, M., Heydarnejad, J., Hosseini Pour, A.H. and Rahimian, H., (2011). Host range and phylogenetic analysis of Iranian isolates of *Zucchini yellow mosaic virus*. *Journal of Plant Pathology*, 93:187–193.

Moradi, Z., Mehrvar, M. and Nazifi, E. (2019). Population genetic analysis of *Zucchini yellow mosaic virus* based on the CI Gene Sequence. *Journal of Cell and Molecular Research*, 10(2):76-89.

Morilipinar, E. O., Şekerci, A.D., Coşkun, Ö. F. and Gülşen, O. (2021). Genetic analysis of local pumpkin populations: genetic analysis of local pumpkin populations. *International Journal of Agricultural and Natural Sciences*, 14(3):264-272.

Nacar, Ç., Aras, V., Tekin, S. and Fidan, H. (2021). Determination of specific combining ability of ZYMV tolerant lines in summer squash. *Alatarım*, 20(2): 60-69. (In Turkish).

Pachner, M., Paris, H. S., Winkler, J. and Lelley, T. (2015). Phenotypic and marker assisted pyramiding of genes for resistance to *zucchini yellow mosaic virus* in oilseed pumpkin (*Cucurbita pepo*). *Plant Breeding*, 134(1):121-128.

Pandawani, N. P. and Widnyana, I. K. (2021). Identification of virus causes of mosaic diseases in zucchini plants in the Bali Island of Indonesia. *Journal of Tekirdag Agricultural Faculty*, 18(3): 411-418.

Papayiannis, L. C., Ioannou, N., Boubourakas, I. N., Dovas, C. I., Katis, N. I. and Falk, B. W. (2005). Incidence of viruses infecting cucurbits in Cyprus. *Journal of Phytopathology*, 153(9):530-535.

Presting, G. G., Smith, O. P. and Brown, C. R. (1995). Resistance to potato leafroll virus in potato plants transformed with the coat protein gene or with vector control constructs. *Phytopathology*, 85:436-442.

Provvidenti, R. (1991). Inheritance of resistance to the Florida strain of *Zucchini Yellow Mosaic Virus* in watermelon. *Hort Science*, 26:407-408.

Radwan, D. E. M., Fayez, K. A., Mahmoud, S. Y., Hamad, A. and Lu, G. (2007). Physiological and metabolic changes of *Cucurbita pepo* leaves in response to *zucchini yellow mosaic virus* (ZYMV) infection and salicylic acid treatments. *Plant Physiology and Biochemistry*, 45(6-7):480-489.

Shrestha, S., Michael, V. N., Fu, Y. and Meru, G. (2021). Genetic loci associated with resistance to *Zucchini Yellow Mosaic Virus* in squash. *Plants,* 10:1935.

Sidek, Z., Sako, N. and Ohshima, K. (1999). Characterization and coat protein sequence of a Malaysian isolate of Zucchini yellow mosaic virus. *Journal of Plant Protection in Tropics*, 12(2):92-105.

Simmons, H. E., Dunham, J. P., Zinn, K. E., Munkvold, G. P., Holmes, E. C. and Stephenson, A. G. (2013). *Zucchini yellow mosaic virus* (ZYMV, Potyvirus): vertical transmission, seed infection and cryptic infections. *Virus Research*, 176(1-2):259-264.

Simmons, H. E., Holmes, E. C., Gildow, F. E., Bothe-Goralczyk, M. A. and Stephenson, A. G. (2011). Experimental verification of seed transmission of *Zucchini yellow mosaic virus*. *Plant Disease*, 95:751–754.

Solmaz, I. and Sarı, N. (2009). Characterization of watermelon (*Citrullus lanatus*) accessions collected from Turkey for morphological traits. *Genetic Resources and Crop Evolution*, 56(2):173-188.

Solmaz, I., Kaçar, Y., Sari, N. and Şimşek, Ö. (2016). Genetic diversity within Turkish watermelon [*Citrullus lanatus* (Thunb.) Matsumura & Nakai] accessions revealed by SSR and SRAP markers. *Turkish Journal of Agriculture and Forestry*, 40(3):407-419.

Thies, J. A., Ariss, J. J., Kousik, C. S., Hassell, R. L. and Levi, A. (2016). Resistance to southern root-knot nematode in wild watermelon (var.). *Journal of Nematology*, 48(1):14-19.

Ünlü, E., Yetişir, H., Fidan, H. and Denli, N. (2020). Resistance sources to *Zucchini Yellow Mosaic Virus* in Turkish bottle gourd [*Lagenaria siceraria* (Molina) Standl.] Germplasm. *Indian Horticulture Journal*, 10(3/4):45-55.

USDA (2020). USDA-ARS Germplasm Resources Information Network (GRIN) http://www.ars-grin.gov/npgs . (Accessed Date: 13.06.2023).

Wang, H. L., Gonsalves, D., Provvidenti, R. and Zitter, T. A. (1992). Comparative biological and serological properties of four strains of *Zucchini yellow mosaic virus. Plant Disease*, 76 (5):530-535.

Wechter, W. P., McMillan, M. M., Farnham, M. W. and Levi, A. (2016). Watermelon Germplasm Lines USVL246-FR2 and USVL252-FR2 Tolerant to *Fusarium oxysporum* f. sp.*niveum* Race 2. *Hortscience,* 51(8):1065–1067.

Yanmaz, R. and Düzeltir, B. (2003). Cultivation of zucchinis (pumpkin seeds). *Ekin Dergisi*, 7(6):22-24. (In Turkish).

Yılmaz, M. A., Lecoq, H., Abak, K., Baloğlu, S. and Sarı, N. (1992). Viruses Causing Damage to Cucurbit Vegetable Species in Turkey. *Turkey I. National Horticultural Congress*, 13–16 October, İzmir. II, 439-442. (In Turkish).