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Resistance Sources to *Zucchini Yellow Mosaic Virus* in Turkish Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Germplasm

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A B S T R A C T

This study was carried out to screen Turkish bottle gourd (BG) [*Lagenaria siceraria* (Mol.) Standl.] germplasm against *Zucchini yellow mosaic virus* (ZYMV) disease. In 2015, the response of bottle gourd accessions to disease was determined by mechanical inoculation in a greenhouse (101) and by natural infection in a field (158). Plant leaf samples collected from the field were first tested by DAS-ELISA and RT-PCR methods and resistant, sensitive, and tolerant accessions were determined. In the natural infection study in an open field, 47-02, 63-12, 63-05, PI442368, and PI381822 were determined to be resistant. The first viral symptoms were observed one week later on the plants that were mechanically inoculated under greenhouse conditions. In the mechanical inoculation study, the accessions 63-04, 66-02, 42-07, and PI442368 were found to be resistant. 63-04 and PI442368 were found resistant to ZYMV in both natural infection and mechanical inoculation experiments. As a result of the DAS-ELISA tests, *Watermelon mosaic virus* (WMV) was also determined in the BG population and the population was found clean in terms of other viruses in the open field study. Seeds were extracted from 14 sensitive accessions with viral symptoms and resistant accessions PI442368. The seeds were germinated on Whatman filter paper and the presence of ZYMV in each organ was tested by RT-PCR. According to the RT-PCR results, one accession in embryo, seven accessions in cotyledons and six accessions in true leaf produced 791 bp band showing the presence of the ZYMV in tested tissue. This shows that a certain percentage of the virus can be transported via seed in BG.

Key words: *Lagenaria siceraria*, ZYMV, WMV, ELISA, Mechanical inoculation, RT-PCR

Conservation and evaluation of genetic resources are two basic crucial issues in sustainable plant production. For this purpose, the characterization and preservation of these resources are among the important issues. Determination of susceptibility or resistance of the available plant germplasm to biotic and abiotic stresses is one of the most important stages in terms of sustainability of plant breeding and thus of plant production [1].

Viruses are one of the most common causes of cucurbit diseases worldwide. While these viruses cause a significant decline in plant growth and decrease in

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yield up to 94% [2], they decrease the marketable product by causing distortion and mottling of fruit. The most important viruses are *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus*-1(WMV-1), *Watermelon mosaic virus-2* (WMV-2), *Cucumber green mottle mosaic virus* (CGMMV) and *Zucchini yellow mosaic virus* (ZYMV). Potyviruses are transmitted in a non-persistent way by more than 200 species of aphids. As indicated by their wide host range, worldwide distribution and diversity of their vectors, potyviruses have an outstanding capacity to adapt to new hosts and environments. ZYMV, a member of the genus Potyvirus in the family *Potyviridae*, is one of the viruses that cause significant damage in cucurbit crops [3]. The WMV, ZYMV and CMV are the most economically important viral agents cause severe mosaic and deformity on cucurbits. The WMV, ZYMV and PRSV are members in Potyvirus genus with filamentous particles. These viruses easily transports from a plant to other plants mechanically and non-persistent manner with aphid species. The ZYMV has also transported with infected seeds and pollens where their ratio is low. In Mersin region, several samples were collected with a dense population of aphids was also found in the region which has not only suitable environmental conditions but also growing wide host varieties [4]. ZYMV was first reported in 1981 in squash grown area in northern Italy [5] and France [6], where it was named *Muskme lon Yellow Stunt Virus* [7, 8]. Although varieties of summer squash (*C. pepo*), melon (*Cucumis melo*), cucumber (*C. sativum*) and watermelon (*Citrullus lanatus*) are particularly affected, all other cucurbit species, including BG are vulnerable to ZYMV infection and is considered the most destructive virus in major cucurbit species grown worldwide [9]. Although ZYMV is predominantly transmitted in a nonpersistent manner by several aphid species (e.g., *Aphis gossypii*, and *Myzus persicae*), it can be transmitted both horizontally by aphids and vertically by seeds [10, 3]. ZYMV, a single strand RNA virus, has about 75 nm long flexuous particles [8]. At least 25 strains of ZYMV have been described [10]. Natural ZYMV infection in bottle gourd has been reported in Hawaii [11], India [12], Serbia [13], USA [14, 15]) and Turkey [16]. Two major ZYMV strain (ZYMVFL and ZYMV-CT) were reported by Provvidenti [17] and reported that although ZYMV-CT causes more severe disease symptoms, its distribution is limited to the northeast of the USA. ZYMV-FL is the most common strain in cucurbit plants in North America. Provvidenti also recorded a new ZYMV strain infecting cucurbit fields around Beijing, China, *Zucchini yellow mosaic virus-China Strain* (ZYMV-CH) [14, 1]. In regions where cucurbit species are not constantly grown, the virus winters in the wild species. The natural infection seems limited to Cucurbitaceae species, but members of the 11 dicotyledon families are considered diagnostic hosts [7, 3]. The plant infected with ZYMV shows reduced photosynthetic capacity, stunting growth, yellow mosaics, malformed and blistered leaves, laminar reduction on leaves and deformed, knobbed, and mottled fruits [9]. The viruses which have been reported in cucurbits in Turkey, one of the major cucurbit producer country, are *Cucumber mosaic virus*, (CMV), *Watermelon mosaic virus-1* (WMV-1), *Watermelon mosaic virus-2* (WMV-2) and *Cucurbit aphid-borne yellows luteovirus* (CABYV), and ZYMV [18, 19, 20, 16, 3].

Bottle gourd or calabash which can be infected by ZYMV belongs to the *Cucurbitaceae* family and its genetic origin is Africa [21]. Cultivated BG known as the white-flowered gourd is an annual, monoecious, vigorous climber species and five wild perennial dioecious *Lagenaria* species were reported from Africa [22]. *L. siceraria* has been utilized as a vegetable, music instrument, decoration tools, pipe, and multipurpose containers, corresponding to properties of the raw and dry ripe fruits [23]. *L. siceraria* is one of the widely used species as commercial rootstock for watermelon and it shows a high compatibility rate with watermelon [24, 25, 26]. It is important to identify the accessions of BG resistant to virus diseases when developing varieties or rootstocks for their intended use. As in many plant diseases, the development of resistant or tolerant varieties in virus diseases is the most appropriate way of solution. Several cucurbit species germplasm were screened for resistance to ZYMV, and the resistance sources were reported in watermelon, cucumber, melon, squash, and bottle gourd. ZYMV resistance is inherited by a single recessive gene in cucumber [27], a single dominant gene in melon [28], and a single dominant gene in squash [29], a single recessive gene in watermelon [10], single dominant gene (*Zym-0*) in *Cucurbita moschata* Duch cv. Minina [30] and a possible single dominant gene in bottle gourd [14]. Resistance to ZYMV in BG in germplasm collected throughout the world was reported in the USA [31, 32, 33, 34]. However, while previous studies were performed on a limited number of accessions, a comprehensive ZYMV test for USDA BG germplasm with a sufficient number of BG accessions was performed by Lingh and Levi [14], and a significant number of resistant accessions were reported.

As noted above, BGs are used for different purposes in Turkey and have an important genetic diversity. Turkey's BG genetic germplasm was collected and morphologically and molecularly characterized. However, no screening studies for viral diseases have been conducted. Therefore, it is necessary to screen Turkish BG germplasm to identify potential sources of disease resistance that may be useful in breeding BG lines for different usage purposes (vegetables, rootstocks, and others). The objective of this study was to evaluate the Turkish BG collection (177 accessions) against ZYMV isolate (ZYMV-Ad) from the main cucurbit growing region of Turkey.

MATERIALS AND METHODS

The study was conducted at Alata Horticultural Research Institute of the Ministry of Food, Agriculture and Livestock. The experimental site is located on 36° 36' 39'' N, 34° 19' 28'' E and elevation above sea level is 8 m. This study conducted to determine resistance sources in Turkish BG germplasm against ZYMV was carried out in two stages. In the first experiment, 158 BG accessions were tested under natural infection conditions in an open field and 101 BG accessions were tested by mechanical inoculation. The BG accessions used in the experiment were selected to ensure maximum diversity according to the morphological characterization previously done and collection regions [35].

Materials

Plant materials: A total of 177 BG accessions from Turkish BG germplasm (Table 2) were used. Perlette and Alata Yeşili (*Cucurbita pepo* L.) were used as susceptible controls.

Virus isolate: ZYMV isolates (ZYMV-Ad) [36] provided from Adana Biological Control Research Institute was used in mechanical inoculation study. Naturally infected samples which have been exhibiting typical ZYMV symptoms were individually photographed, placed in a separate plastic bags and brought to our laboratory in ice boxes for further studies. The collected samples were stored in a-20 °C freezer.

Table 1 Virus-specific primer pairs used in RT-PCR studies and molecular size of the amplified region

Primer	Sequences	Band size (bp)	References
ZYMVF	ATGCTCCAATCAGGCACYC	791	[43]
ZYMVR	GTGTGCCGTTCAGTGTCTTC		
CMVF	TAACCTCCCAGTTCTCACCGT	513	[44]
CMVR	CCATCACCTTAGCTTCCATGT		
CVYVF	AGCTAGCGCGTATGGGGTGAC	450	[43]
CVYVR	GCGCCGCAAGTGCAAATAAAT		
CGMMVF	TTGCGTTTAGTGCTTCTTATGT	440	[45]
CGMMVR	GAGGTGGTAGCCTCTGACCAGA		
WMVF	GGCTTCTGAGCAAAGATG	408	[46]
WMVR	CCCAYCAACTGTYGGAAG		

Methods

Natural infection

In the natural infection study, 158 gourd accessions chosen according to morphological characteristics were tested (Table 2). Twenty-five seeds from each accessions were planted in multipots containing 1: 1 (v/v) peat-perlite mixture and 10 seedlings with two true leaf from each accession were planted in open fields with at 3 x 0.5 m spacing on May 5, 2015. The plants were fertilized with 100 kg N; 100 kg P; 100 kg K/ha by fertigation methods [23]. Virus symptoms were detected by observation in July and August of 2015.

Mechanical inoculation

Mechanical inoculation was carried out in a greenhouse where the ventilation openings were covered with insect-proof mesh. 101 selected BG accessions were used in the mechanical inoculation study (Fig 1). The virus was propagated and maintained on Seyden F1 zucchini squash (*C. pepo* L.). Virus inoculum was prepared by macerating virus-infected leaves (1:5 w/v) in 0.02 M phosphate-buffered saline (%1 K_2HPO_4, %0,1 Na_2SO_3, %0,01 Mercapto ethanol pH:7.5) with a mortar and pestle. Seedlings in 2 liters pots filled with Peat:Perlite mixture (1v:1v) and were inoculated by lightly dusting the leaves with carborundum. Then, the virus was inoculated with with "Softspongypad" method to the first true leaves of the accessions grown with 10 replications (plants). The

soft sponge soaked in virus inoculation was rubbed several times until all the surface of the leaf was covered with the inoculum. The excess carborundum on the leaf surface was rinsed with tap water and the infected plants were kept in a shaded place for several hours to prevent sunlight damage on newly inoculated leaves. After virus inoculation, the symptoms of the plants were evaluated for two weeks after the appearance of ZYMV symptoms in the sensitive controls Perlette and Alata Yeşili. Varieties showing infection after inoculation were evaluated according to the 0-5 scale. Accessions with a scale value of 0 were evaluated as resistant,1-2 were tolerant and 3-5 were susceptible. In both experiments, 10 plants from each accessions were evaluated separately and the average scale value was calculated as the disease index.



Fig 1 Mechanical inoculation of ZYMV

The following scale 0-5 was used to determine the severity of virus symptoms in both experiments (Fig 2) [16].

0. Plants without any symptom development.
1. Plants showing very slight discoloration in leaf veins.
2. Moderate mosaic plants along with slight discoloration in leaf veins.
3. Plants showing moderate to severe mosaic and yellowing in leaves.
4. Severe mosaic symptoms de deformation in the leaves and stunted in the plant.
5. Severe mosaic in leaves, speckling, shortening of plant height, shoestring symptom, and deformation of leaves.

All plants in an accession did not show ZYMV symptoms and negative ELISA was defined as resistance. Partial resistance was designated when only some of the seedlings tested in a accession were symptom-free and the ELISA result was negative. The susceptibility of all plants tested in one attendance was defined as susceptibility [14, 15].

Systemic infections of ZYMV after mechanical inoculation were determined using both serological and molecular diagnostic methods in three plants from each accessions. The tested plants were scored using a

scale of 0-5 as specified, and whether the non-symptom plants were infected with ZYMV was checked by the ELISA test. ELISA-plate and AGDIA-ZYMV polyclonal antiserum reagent set (Catalog number: SRA 77700/0500) was used for the DAS-ELISA test. Total RNA isolation was isolated from leaf samples with Thermo Scientific-RNA purification kit (Catalog number: K0802) to use in RT-PCR studies. The RNAs extracted were optimized in the spectrophotometer (A260/280 1.8-2.0) and their concentrations were adjusted to 200 ng/µl. After RNA optimization, Single Stage RT-PCR (One Step RT-PCR) studies were conducted using Thermo Scientific Verso 1-Step RT-PCR Kit Reddy Mix (Catalog number: AB1454LDB). RT-PCR solution for each sample, Verso Enzyme Mix 0.5 µL, 2X-1- Step PCR ReddMix 12.5 µL, RT Enhancer 1.25 µL, Forward primer µL1, Reverse primer 1 µL, RNA 2 µL, ddH_2O 6.25 µL. It was adjusted to be 25 µL. Amplification for each virus proceeded through a cycle cDNA synthesis 50 ^oC 15 min Verso inactivation 95 ^oC (2 min 1 cycle) after that denaturation at 95 ^oC (45 sec), annealing at 55 ^oC (1 min) and extension at 72 ^oC (1 min) for a total of 35 cycles in Techne Genius thermal cycler. Primers used in this study and the expected length of each amplicon are presented in (Table 1) [16].

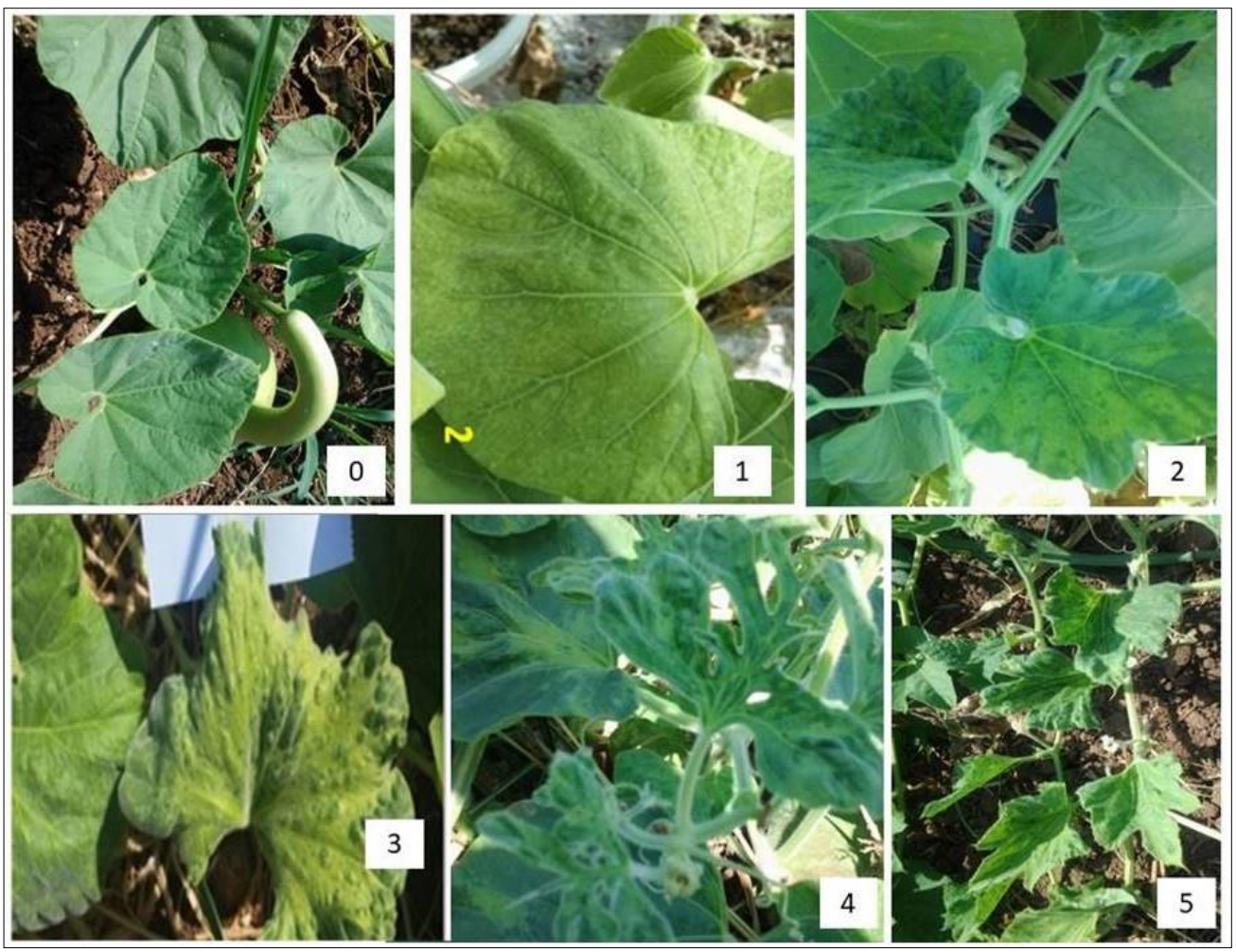


Fig 2 Scale (0-5) used to assess disease severity

Since there are different reports about the transmission of ZYMV with seeds, seedlings were grown from 20 seeds of accessions that are resistant (PI442368) and susceptible (14) in the study. It was tested with RT-PCR with marker specific for the coat protein of the virus. It was determined whether ZYMV was present in the total nucleic acids extracted from the seed coat, embryo, cotyledons, and true leaves of 14 susceptible and one resistant accession.

RESULTS AND DISCUSSION

Natural infection test

The results generated from the natural infection of the 158 BG accessions for ZYMV resistance were

classified into three distinct groups: 1) resistant (5 accessions); 2) partially resistant (106 accessions), and 3) susceptible (47 accessions) (Table 2). The control *C. pepo* cv. Perlette and Alata Yeşili were highly susceptible. Three BG accessions (47-02, 63-04 and 63-12) collected from different regions of Turkey and two BG accessions from USDA (PI381822 and PI442368) were found resistant without showing any disease symptom. While 106 accessions were in the partially resistant group with 1-2 scale values, 46 BG accessions were in the susceptible group with 3 and above scale values (Table 2). While PI381822, which is provided from USDA and found to be resistant [14], is of Indian origin, and PI442368 is of Mexican origin.

ZYMV and *Watermelon mosaic virus* (WMV) were detected in plant samples taken from the experimental area by ELISA test. While ZYMV was detected in all samples showing the viral symptom for virus presence, WMV was also detected in 10 accessions (34-02, 42-09, 45-01, 47-02, 48-09, 63-05, 63-17, 66-02, 80- 01 and VIR 1247) (Table 3). The DAS-ELISA results with mechanically inoculated plants are interpreted as resistant (e.g.0,103) where their reads are much lower than the negative control (e.g. 0.124), and the obtained absorbance value (e.g. 0.138) is higher than negative control accepted as moderate resistant respectively. The DAS-ELISA tests resulted in typical ZYMV symptoms showing plants were examined at 405 nm wave length, a segregation has observed between 0.102 and 0.213 absorbance values. Among the accessions found to be resistant to ZYMV, WMV was detected in accessions other than PI442368 (Table 3). The 791 bp band (Table 1, for ZYMV) developed for the isolate used in the RT-PCR study was determined in the diseased plant samples (Fig 3).

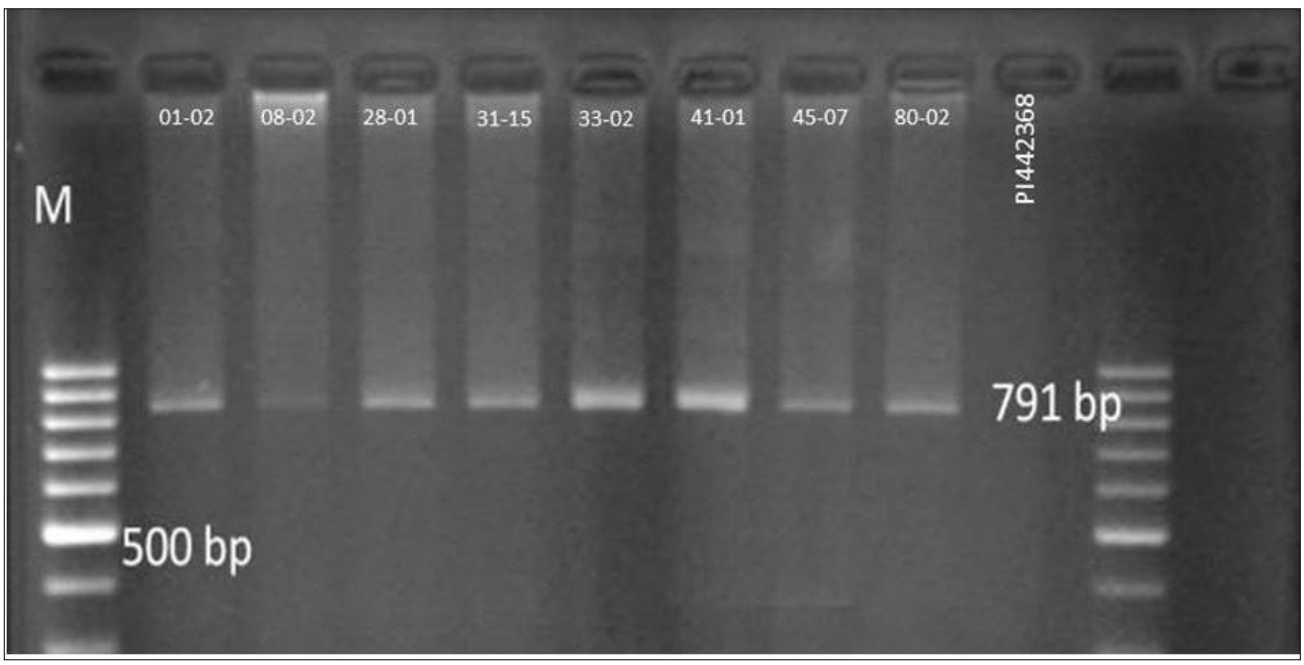


Fig 3 RT-PCR results for plants showing ZYMV symptoms and resistant PI442368 in natural infection

Table 2 Results of natural infection and mechanical inoculation

Resistant (5/158)	Partially Resistant (106/158)					Susceptible (47/158)		
47-02	01-17	27-01	34-04*	50-01*	73-03	01-02	33-23*	53-02
63-04*	03-01*	27-03*	35-06*	50-04*	73-05	07-42*	33-26	59-05
63-12*	07-03*	27-13*	35-07	54-01*	77-01*	08-01*	33-27*	60-04
15-01*	07-10	27-14*	35-10*	55-01*	80-04	08-02*	33-50	63-05
PI381822*[b]	07-15	27-15	37-03	55-02	VIR-1210[c]	09-03*	34-02*	64-08*
PI442368*[b]	07-17*	31-06	38-02	55-03	PI-1*a	20-05*	38-07	66-03*
	07-23*	31-09	38-06	55-06*	PI-3a	28-01	41-01	79-03
	07-25*	31-18*	39-01*	56-01	PI-4*a	28-04*	42-01*	80-01*
	07-31	32-01	42-01	57-01	PI-8a	31-15*	42-09*	80-02*

07-45	33-03*	42-06*	59-03*	PI-381831*[b]	31-32	42-10*	VIR-1247[c]
07-47*	33-04	43-02	59-07	47765*	31-37*	43-04	PI642043*[b]
09-01	33-07*	43-06*	59-11	47745*	33-01*	45-01	TR-28
10-01	33-11*	45-02*	60-02	PI500814*[b]	33-02	45-07*	TR-50*
15-01*	33-13*	45-04*	60-06*	PI5345534*[b]	33-06	47-04	Perlette
16-09*	33-25*	46-14	62-01	PI548736b	33-15*	48-06	Alata Yeşili
17-02*	33-29	46-17	62-03	PI491334b	33-18	51-07*	
19-02	33-35*	47-03	63-11	PI381846*[b]			
20-01*	33-40	48-07*	63-13	PI379367[b]			
20-02	33-42*	48-09*	66-02*	PI470260[b]			
21-01	33-49*	48-11	70-07	PI491349*[b]			
22-01*	34-03	48-13	72-01	PI368640[b]			
				PI287534*[b]			

Mechanical inoculation

Resistant (5/101)	Partially Resistant (38/101)			Susceptible (58/101)				
	07-25	42-10	PI381330[b]	03-01	27-03	33-27	60-06	39-01
	15-01	43-06	PI381831[b]	07-03	27-14	42-05	61-11	80-01
	16-09	45-02	PI500814[b]	07-17	28-04	42-09	64-08	80-02
	21-03	48-06	PI534553[b]	07-20	31-15	45-02	66-03	80-04
07-42	22-01	48-09	PI491252[b]	07-23	31-18	45-04	77-07	47765
63-04	23-04	50-01	Grif11931[b]	07-47	33-01	45-07	33-42	TR-12
66-02	27-13	51-01	PI381822[b]	08-01	33-07	48-07	33-49	TR-22
15-01	31-37	51-07	PI642043[b]	08-02	33-11	50-04	34-02	TR-29
PI442368[b]	33-03	63-12	PI381846[b]	09-03	33-13	54-01	34-04	TR-32
	33-35	77-01	PI491349[b]	17-02	33-15	55-01	35-05	TR-50
	33-42	47745	PI287534[b]	20-01	33-23	55-06	35-06	Perlette
	42-06	PI-1[a]	TR-38	20-05	33-25	59-03	35-10	Alata Yeşili
	42-01	PI-4[a]						

*Accessions commonly used in both experiments.

[a]Bottle gourd accessions provided by Dr. Narinder P.S. Dhillon from India.

[b]Bootle gourd accessions from Plant Genetic Resources Conservation Unit of USA.

[c]Bootle gourd accessions from Senpethersburg Gen Bank of Russia

Mechanical Inoculation Test

The ZYMV symptoms began to appear two weeks after inoculation. The response of the BG accessions was evaluated four weeks after inoculation. While four accessions (07-42, 63-04, 66-02,15-01 and PI442368) were found to be resistant, 38 accessions were partially resistance (2 scale value) and 58 accessions were sensitive (3> scale values) (Table 2). As in the natural infection test, the control *C. pepo* cvs. Perlette and Alata Yeşili were highly susceptible. 66-12 and PI381822, which are found to be resistant in the natural infection test, were included in the partially resistance group with 2 scale value in mechanical inoculation. The overlap ratio of the results of both trials was determined to be 90%. As seen in (Fig 2), dominant symptoms observed in both experiments were moderate to severe mosaic (2. 2-3), deformation in the leaves (2. 4-5), speckling (2. 4) and stunted in the plant (2. 5).

In transmission study, while viruses were not detected in the organs of seedlings produced from the seeds of accessions determined to be resistant to ZYMV, the presence of viruses in different organs (embryo, cotyledon and true leaves) of the seedlings of sensitive accessions was detected. According to the RT-PCR results, one accesion in the embryo (77-01), seven accessions (07-47, 27-13, 31-15, 31-37, TR-28, TR50 and 80-04) in cotyledons and six accessions (09-03, 07-45, 54-01, 55-06, 20-05 and 33-50) in true leaf produced 931 bp band showing the presence of the ZYMV in tested tissue. This shows that a certain percentage of the virus can be transmitted via seed.

Table 3 DAS-ELISA results of leaves with virus symptom from natural infection experiment

Genenotip No	ZYMV	WMV	Genotip No	ZYMV	VMV	Genotip No	ZYMV	WMV
01-02	+		33-18	+		53-02	+	
08-01	+		33-26	+		55-01	+	
08-01	+		33-27	+		60-04	+	
08-01	+		33-50	+		60-04	+	
20-05	+		34-02	+	+	63-05		+
28-01	+		34-04	+		63-17	+	+
28-01	+		35-05	+		64-08	+	
28-04	+		38-07	+		66-02		+
31-15	+		41-01	+		66-03	+	
31-16	+		42-01	+		72-02	+	
31-32	+		42-09	+	+	79-03	+	
31-37	+		45-01	+	+	80-01	+	+
31-50	+		47-02		+	80-02	+	
33-01	+		47-04	+		PI36860	+	
33-02	+		48-09	+	+	PI442368		
33-09	+		51-07	+		Vır1274	+	

In this study, 158 accessions from Turkish BG germplasm under open field conditions were testes against ZYMV by natural infection and 101 accessions were tested by mechanical inoculation. As a result of testing, a significant resistance source (07-42, 63-04, 47-02, 66-02, PI442368, and PI 381822) for ZYMV was determined in Turkish BG germplasm. Accessions PI 381822 and PI442368, which are among the accessions found to be resistant to ZYMV, are found to be resistant in the natural infection experiment, but PI381822 was determined as partially resistant in the mechanical inoculation study. Similar to the current study, Ling and Levi [14] determined that ZYMV resistance was present in USA BG genetic resources. PI 381822 and PI442368, which were found resistant in the natural infection study, were reported by Lingh and Levi [14] as partially resistant and susceptible, respectively. While eight (57%) of the accessions used in common with Lingh and Levi [14] gave similar results, six (43%) accessions gave different results. Similarly, BG accessions PI271353 and PI482261, a *Citrullus lanatus* var. *citroides* genotype, reported being resistant to ZYMV by Provvidenti *et al*. [37] were found partially resistant by Ling and Levi [14]. Similarly, PI482261 watermelon accession, which was reported as resistant to ZYMV by the Providenti [37], was found sensitive in the screening study conducted by Guner *et al*. [10]. This has been attributed to the aggressiveness of the pathogen used by researchers. Guner *et al*. [38] reported ZYMV resistant 31 watermelon accessions. However, they stated that it is possible that resistant accessions reported in their study may not have resistance to other isolates in other regions where ZYMV is found. The reason for the different results obtained by two different research groups in six of the accessions can be explained by the use of different disease factors and the BG accessions used do not have a sufficiently pure genetic structure. This is an expected result in species that are collected from nature and maintained by sib-mating or open pollination. Therefore, accessions evaluated as susceptible in this study may have a low frequency of resistant individuals and could be determined by screening many plants from each accession. It is also possible that susceptible plants are occasionally found in resistant accessions. Lingh and Levi [14] reported that of the 36 BG accessions resistant to ZYMV, 33 were reported to be of Indian origin. Similarly, in our study, accessions originating from India such as PI381831, PI381822, PI-1, PI-3, and PI-4 were included in the resistant and partially resistant group.

According to RT-PCR and ELISA test, naturally infected plants have high ZYMV infection, low rate of WMV infection but no other viruses were encountered. Although CGMMV and CMV are common virus diseases in cucurbit, it has not been detected in this study. Since the most common virus [4] in experimental region (Adana, -Mersin) is ZYMV, single ZYMV infection or mixed infection with WMV are possible in the tested samples.

Resistance to ZYMMV in BGs has been determined to be dominant and transferable to susceptible accessions. Partially resistant/tolerant accessions were still segregating for the resistance so additional single plant selections are required to develop stable resistant lines to ZYMV [14]. The severe ZYMV symptoms on susceptible control plants, Perlette and Alata Yeşili (*C. pepo*), in open field and

greenhouse indicate that our inoculation method and isolate were sufficient and effective. The reason for the high density of ZYMV infection in natural infection may be attributed to the high stability of the virus and the attitude of contamination. Nigam *et al*. (201[3] reported that it is transmitted easily and quickly mechanical and non-persistent manner with a wide variety of aphid types from infected plants to healthy plants when appropriate environmental conditions are present.

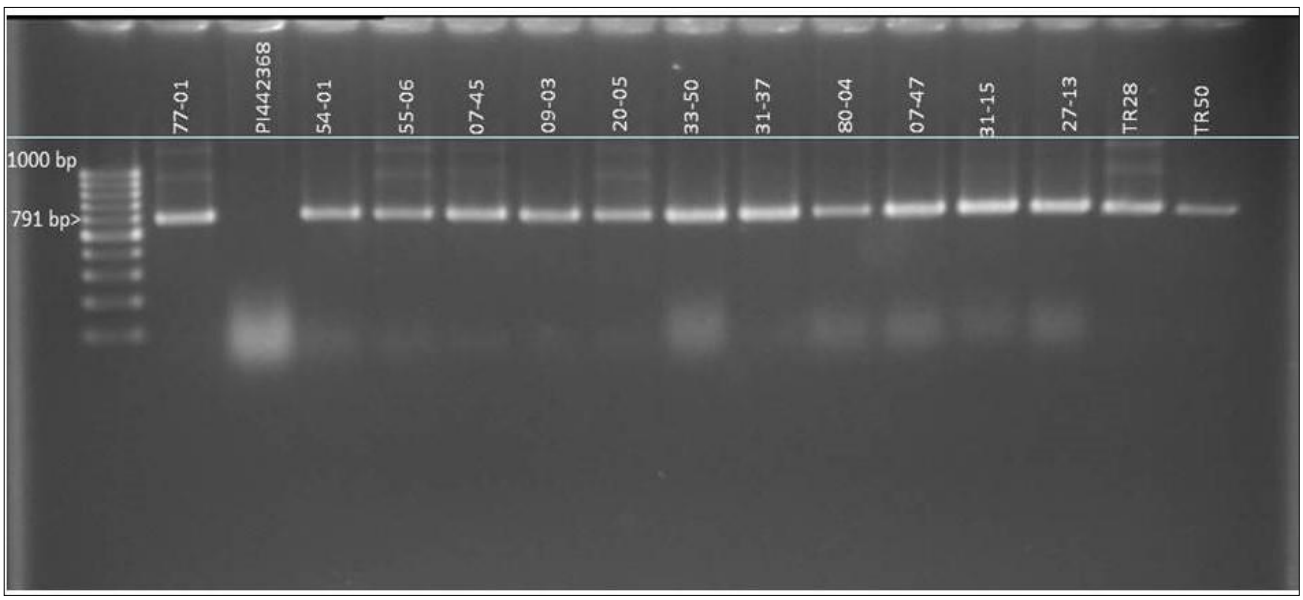


Fig 4 RT-PCR results for ZYMV in the seedlings produced from seed extracted from fruits of resistant and susceptible BG accessions

In this study, it was determined that a certain amount of ZYMV was transmitted vertically with the seeds of the BG (Fig 4). Similarly, the transmission of ZYMV was reported as 13-15, 1.6 and 6.2% in *C. pepo* conv. *citrullinina* var. *styriaca* [39], in *C. pepo* subs. *texana* [2, 40], and in winter squash [41], respectively and they demonstrated that infected seeds have the potential of initiating horizontal ZYMV infection both mechanically and aphids. Simmons et al. [41] stated that the rate of seed infection is much higher (21.9%) than the rate of transmission from seed to seedling, and they reported a decrease in the population at the time of germination and emergence in *C. pepo*. It has been reported that ZYMV is less vertically transmitted with pollen in the squash, and the main route of vertical transmission is the ovüle or the embryo itself [42]. According to our knowledge and the available literature, our study is the first report on the seed transmission of ZYMV in BG. As mentioned in the introduction bottle gourds are used as rootstock for watermelon. Rootstock seeds infected with ZYMV can be a very serious source of transmission in nursery house. Because viruses can be transferred mechanically to virus-free scion by grafting from contaminated seeds, as well as seedlings that have become infected will become sources of contamination in both nursery house and production fields. For this reason, it is important to identify resistant BG accessions and to use them in rootstock breeding programs.

This study showed that there is a sufficient amount of ZYMV resistance in Turkish *L. siceraria* germplasm. While seven BG accessions were found to be resistant, many accessions were found to be partially resistant (Table 2). As Lingh and Levin [14, 15] emphasized that it will be possible to develop new stable resistant inbred lines from the accessions that are partially resistant by single plant selections. For developing ZYMV resistant gourd varieties for different purposes such as rootstocks and vegetables, this resistance source in Turkish BG germplasm can be used easily without facing any hybridization problem. DNA markers against ZYMV can also be developed by molecular studies in segregating populations to be created through resistant and susceptible accessions identified in this study. Identifying and isolating genes responsible for ZYMV resistance may result in the possibility of transferring these genes to other cucurbit species where ZYMV is a problem. Therefore, *L. sicrerari* germplasms should also be taken into consideration during resistance studies of virus diseases in Cucurbit species. Besides, in this study, it

was determined that ZYMV can be transmitted vertically with seed. For this reason, this should be taken into consideration in the production of seeds of gourds that are not ZYMV resistant.

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Ethical approval: This manuscript did not involve any human participants, and/ or animals.

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