ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 22(1) 2023, 87–94
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2023.4568
ORIGINAL PAPER
Accepted: 22.08.2022
Published: 24.02.2023

EVALUATION OF CAPSICUM GENOTYPES FOR RESISTANCE TO CUCUMBER MOSAIC VIRUS

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ABSTRACT

Cucumber mosaic virus (CMV) is one of the most destructive viruses that affect pepper all over the world. Using resistant varieties is one of the most effective ways to control the virus. Identification of new resistance sources is essential for the development of resistant cultivars. In the present study, the reactions of 50 Capsicum genotypes (25 *Capsicum baccatum*, 15 *C. annuum* and 10 *C. frutescens*) were determined against CMV-Sa isolate with mechanical inoculation under controlled conditions in 2020. A 0–4 disease rating scale was used to analyze the genotypes, and the genotypes were categorized as resistant, moderately resistant and susceptible based on disease incidence and disease severity scores. The findings revealed that there were significant ($p < 0.01$) differences in disease incidence and severity among genotypes. The study demonstrated that one of the 25 tested *C. baccatum* genotypes (KTB-11) found to be resistant, and three genotypes (KTB-29, KTB-34, KTB-57 and KTB-72) were rated as moderately resistant to CMV. However, it was determined that all tested *C. frutescens* and *C. annuum* genotypes were susceptible to CMV. The genotypes identified as resistant and moderately resistant can be used as a source of resistance in pepper breeding studies.

Key words: CMV, screening, *C. frutescens*, *C. annuum*, *C. baccatum*

INTRODUCTION

Pepper (*Capsicum* spp.) is among the most important vegetable species cultivated around the world due to its economic significance and nutritional value. The genus *Capsicum* includes more than 30 species, five of which are commonly cultivated (*Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens*) for consumption and non-nutritional purposes (such as cosmetics) [Parisi et al. 2020]. During the growth period, pepper is attacked by several viral agents such as *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Potato virus Y* (PVY), and *Tobacco etch virus* (TEV). Among these viruses, CMV is one of the most common and economically damaging pathogens that affects pepper [Moury and Verdin 2012].

CMV, a type member of *Cucumovirus* genus in the family *Bromoviridae*, infects a wide range of plants including more than 1200 species in over 100 families of monocots and dicots and it is transmitted by more than 60 aphid species in a non-persistent manner [Palukaitis et al. 1992, Jacquemond 2012]. The virus can also be transmitted via pepper seeds [Ali and Kobayashi 2010]. CMV symptoms may vary depending on virus strain, host genotype and plant age at the time of infection in pepper [Kenyon et al. 2014]. Generally, infected pepper plants exhibit symptoms such as mottle, mosaic, vein clearing, yellow discoloration, leaf distortion, plant dwarfism and fruit lesions, and the virus may cause significant crop losses in case of severe infection [Palukaitis

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et al. 1992, Chaim et al. 2001, Kang et al. al. 2010, Li et al. 2020].

Cultural measures (e.g., weed removal, eradication of infected plants, and disease-free seed use) and control of vector aphid species are significant in reducing crop losses caused by CMV. However, the broad host range of the virus and the presence of many aphid vectors make it difficult to control of the virus [Palukaitis et al. 1992]. The use of CMV-resistant or tolerant varieties is one of the most effective ways to control CMV [Yao et al. 2013]. Furthermore, genetic resistance is a simple and inexpensive approach to reduce crop losses caused by plant viruses. Studies reported that several *C. annuum* [Chaim et al. 2001, Caranta et al. 2002, Suzuki et al. 2003, Kang et al. 2010, Yao et al. 2013], *C. frutescens* [Grube et al. 2000, Suzuki et al. 2003], and *C. baccatum* [Suzuki et al. 2003] accessions or cultivars were found to be resistant to CMV. Most genetic studies on CMV resistance in pepper demonstrated that the genetic properties of resistance were complex and controlled by multiple loci, and different resistance sources exhibited different inheritance patterns [Grube et al. 2000, Suzuki et al. 2003, Yao et al. 2013].

Turkiye is a significat pepper producer and ranks third after China and Mexico [FAO 2020]. A total of 3,091,295 tons of pepper were produced in 2021 in Turkiye [TUIK 2021]. Although it was reported that CMV infection was prevalent in pepper cultivation fields in Turkiye [Uzunogullari and Gumus 2015, Keleş Öztürk and Baloglu 2019], there are limited studies on CMV resistance in pepper. Başay and Uzunoğlu [2006] tested 14 pepper lines against CMV and found that one line could be tolerant to CMV. Balcı [2005] determined CMV resistant and tolerant *C. frutescens* × *C. annuum* hybrids. Most resistance sources in pepper exhibit partial resistance to CMV and their utilization in pepper breeding studies has been limited [Li et al. 2020]. Furthermore, factors such as climate change and the risk of resistance breakdown could affect the durability of resistance [Parisi et al. 2020]. Therofore, the identification of novel CMV resistance sources and their incorporation into pepper breeding programs are important. In the present study, a total of 50 *Capsicum* genotypes were screened for CMV resistance by mechanical inoculation.

MATERIAL AND METHODS

Plant material. In this study, fifty Capsicum genotypes (Tab. 1) that included *C. baccatum* (25), *C. annuum* (15) and *C. frutescens* (10) were tested against CMV. Yolo Wonder pepper variety was used as the susceptible control and Mertcan variety (Yuksel Seeds) was used as the resistant control. The seeds of Capsicum genotypes were obtained from the Black Sea Agricultural Research Institute (Samsun, Turkiye).

Virus source and plant inoculation process. In the present study, a Turkish isolate CMV-Sa isolated from a pepper plant in Samsun province of Turkiye was used as virus source for mechanical inoculation. The virus was propagated and maintained in *Nicotiana benthamiana* plants. The inoculum of virus was prepared from infected leaves of *N. benthamiana*. Pepper seeds were germinated in a plastic tray with organic substrate, and then each plant was transferred to a 10 cm diameter plastic pot filled with sterile soil and peat mixture (1 : 1). The experiment was conducted with the completely randomized design with 3 replicates, where each replicate included 10 plants. The virus inoculum was prepared by grinding one-gram CMV-Sa infected leaf in 10 ml of 0.1 M phosphate buffer (pH 7). When the pepper seedlings reached the two true leaves stage, the leaves were dusted with 400 mesh carborundum and inoculated by rubbing the leaves with the inoculum [Kang et al. 2010]. Inoculated plants were washed with tap water 5 minutes after the inoculation. Then, the inoculated plants were maintained for four weeks in a climatized room with a temperature of 20–25°C (lightness/darkness cycle) and a photoperiod of 14 hours. To prevent the plants to escape from infection, the inoculation procedure was repeated one week later.

Host response. The reactions of the plants to CMV were assessed 4 weeks after the first inoculation. Each plant was visually examined and analyzed with a 0–4 disease rating scale (0 – no symptom, 1 – mild mosaic, no leaf distortion; 2 – strong mosaic, mild leaf distortion; 3 – severe mosaic and distortion; 4 – severe mosaic, distortion and stunted plants) based on the severity of mosaic and leaf deformation [Monma and Sakata 1997]. Disease incidence and severity were calculated with the method described by Rahman et al. [2016].

Table 1. Capsicum genotypes evaluated in the present study

Genotype	Species	Origin	Type	Genotype	Species	Origin	Type
KTB-11	*C. baccatum*	Costa Rica	Introduced	KTF-28	*C. frutescens*	Mexico	Introduced
KTB-14	*C. baccatum*	Brazil	Introduced	KTF-29	*C. frutescens*	Mexico	Introduced
KTB-16	*C. baccatum*	Ecuador	Introduced	KTF-33	*C. frutescens*	Mexico	Introduced
KTB-19	*C. baccatum*	Peru	Introduced	KTF-36	*C. frutescens*	India	Introduced
KTB-22	*C. baccatum*	Brazil	Introduced	KTF-37	*C. frutescens*	Philippines	Introduced
KTB-23	*C. baccatum*	Guatemala	Introduced	KTF-42	*C. frutescens*	Mexico	Introduced
KTB-24	*C. baccatum*	Paraguay	Introduced	KTF-57	*C. frutescens*	Brazil	Introduced
KTB-25	*C. baccatum*	Peru	Introduced	KTF-68	*C. frutescens*	USA	Introduced
KTB-29	*C. baccatum*	Guatemala	Introduced	KTF-91	*C. frutescens*	USA	Introduced
KTB-30	*C. baccatum*	Kenya	Introduced	KTF-92	*C. frutescens*	Mexico	Introduced
KTB-34	*C. baccatum*	Brazil	Introduced	KTA-20	*C. annuum*	Turkiye	Local
KTB-36	*C. baccatum*	Uruguay	Introduced	KTA-27	*C. annuum*	Turkiye	Local
KTB-37	*C. baccatum*	Peru	Introduced	KTA-29	*C. annuum*	Turkiye	Local
KTB-38	*C. baccatum*	Brazil	Introduced	KTA-32	*C. annuum*	Turkiye	Local
KTB-39	*C. baccatum*	USA	Introduced	KTA-33	*C. annuum*	Turkiye	Local
KTB-41	*C. baccatum*	Brazil	Introduced	KTA-39	*C. annuum*	Turkiye	Local
KTB-43	*C. baccatum*	Brazil	Introduced	KTA-45	*C. annuum*	Turkiye	Local
KTB-48	*C. baccatum*	Colombia	Introduced	KTA-51	*C. annuum*	Turkiye	Local
KTB-54	*C. baccatum*	Peru	Introduced	KTA-63	*C. annuum*	Turkiye	Local
KTB-57	*C. baccatum*	Chile	Introduced	KTA-67	*C. annuum*	Turkiye	Local
KTB-59	*C. baccatum*	Argentina	Introduced	KTA-75	*C. annuum*	Turkiye	Local
KTB-60	*C. baccatum*	Hungary	Introduced	KTA-77	*C. annuum*	Turkiye	Local
KTB-66	*C. baccatum*	Ecuador	Introduced	KTA-81	*C. annuum*	Turkiye	Local
KTB-72	*C. baccatum*	Ecuador	Introduced	KTA-82	*C. annuum*	Turkiye	Local
KTB-73	*C. baccatum*	Uruguay	Introduced	KTA-87	*C. annuum*	Turkiye	Local

Each genotype was scored between 1 and 4 points based on disease incidence and severity. Virus incidence scoring: <20% = 1, 20.1–30% = 2, 30.1–50% = 3 and > 50% = 4, and disease severity scoring: 0.1–1 = 1, 1.1–2.0 = 2, 2.1–3.0 = 3 and > 3 = 4. Then, each genotype was categorized into four groups based on the cumulative disease incidence and severity scores: 0–3= resistant (R), 4–6 = moderately resistant (MR) and 7–8 = susceptible (S) [Waweru et al. 2020].

Testing the lines for virus infection. Four weeks after the first inoculation, uninoculated upper leaves of each plant were tested with double antibody sandwich assay (DAS-ELISA) using CMV-specific antisera (Bioreba, Switzerland), and the method was applied according to the protocols determined by Clark and Adams [1977] and the the manufacturer's instructions. Incubations with polyclonal antibody and conjugate were carried out at 30°C for four hours each. Absorbance values were measured at 405 nm 120 min after the substrate was added with a microtiter plate reader (Tecan Sunrise, Austria). Each sample with an absorbance of more than twice the mean negative control absorbance was considered positive.

Statistical analysis. Data were analyzed by the Scott-Knott test ($p < 0.05$) [Scott and Knott 1974]. Relationships between mean absorbance and degree

of susceptibility were determined using the Pearson correlation analysis.

RESULTS

In the present study, a total of 50 Capsicum genotypes, 25 of which were *C. baccatum*, 15 of which were *C. annuum*, and 10 of which were *C. frutescens*, were tested against CMV in a climatized room. Seven-ten days after the inoculation, plants infected with CMV developed systemic symptoms. The majority of the genotypes exhibited severe infection including symptoms such as mosaic, mottle and distortion in the leaves after inoculation with the virus (Fig. 1).

The incidence and severity of CMV infection in tested Capsicum genotypes are summarized in Table 2. Significant differences ($p < 0.01$) were determined between the tested genotypes based on disease severity and incidence. The mean disease incidence in the tested genotypes was 78.53%, and the mean disease severity was 2.1. Disease incidence and severity varied between 16.7% and 93.3% and 0.2 and 3.2, respectively in the genotypes. The lowest disease incidence was observed in the resistant control variety Mertcan (16.7%), followed by KTB-11 (30%), KTB-57 (46.7%) and KTB-34 (56.7%), respectively. The highest disease incidences were identified in the susceptible control variety Yolo Wonder (93.3%) and KTF-57 (93.3%). The control variety Mertcan exhibited the lowest disease severity (0.2), followed by KTB-11 (0.3), KTB-57 (0.4), KTB-34 (0.6), KTB-72 (0.7) and KTB-29 (0.9). The highest disease severity was identified in the KTF-57 (3.2) genotype. In the susceptible control Yolo Wonder, the disease severity was 3.1. Based on the total disease incidence and severity scores, the lowest total score was recorded in the resistant control Mertcan (2), while the highest scores were identified as 8 in the susceptible control Yolo Wonder, KTB-43, KTB-59, KTF-57 and KTA-32 (Tab. 2).

Capsicum genotypes were classified as resistant (R), moderately resistant (MR) and susceptible (S) based on the total disease severity and incidence scores. Of the 50 genotypes tested with CMV, only five exhibited varying levels of resistance. One genotypes was determined to be resistant to CMV, while 4 of them were moderately resistant. Forty-five genotypes were susceptible to CMV (Tab. 2). Virus pres-

Fig. 1. Mosaic, mottle and leaf distortion symptoms on KTB-43 genotype after inoculation with CMV

Table 2. The reactions of the tested Capsicum genotypes against the *Cucumber mosaic virus*

Genotype	Species	CMV severity index	Severity scores	Incidence (%)	Incidence scores	Cumulative scores	Mean absorbance (405 nm)	Host reaction
KTB-11	C.b.	0.3 ef	1	30 fg	2	3	0.26	R
KTB-14	C.b.	2.3 cd	3	86.7 abc	4	7	0.40	S
KTB-16	C.b.	2.1 d	3	90 ab	4	7	0.40	S
KTB-19	C.b.	2.1 d	3	86.7 abc	4	7	0.52	S
KTB-22	C.b.	2.5 bcd	3	83.3 abc	4	7	0.54	S
KTB-23	C.b.	2.1 d	3	83.3 abc	4	7	0.39	S
KTB-24	C.b.	2.2 d	3	86.7 abc	4	7	0.35	S
KTB-25	C.b.	2.3 cd	3	86.7 abc	4	7	0.37	S
KTB-29	C.b.	0.9 e	1	63.3 cde	4	5	0.31	MR
KTB-30	C.b.	2.3 cd	3	83.3 abc	4	7	0.42	S
KTB-34	C.b.	0.6 ef	1	56.7 de	4	5	0.33	MR
KTB-36	C.b.	2.2 d	3	83.3 abc	4	7	0.42	S
KTB-37	C.b.	2.7 a-d	3	90 ab	4	7	0.46	S
KTB-38	C.b.	2.3 cd	3	83.3 abc	4	7	0.40	S
KTB-39	C.b.	2.2 d	3	83.3 abc	4	7	0.40	S
KTB-41	C.b.	2.6 a-d	3	90 ab	4	7	0.39	S
KTB-43	C.b.	3.1 ab	4	83.3 abc	4	8	0.51	S
KTB-48	C.b.	2.1 d	3	80 a-d	4	7	0.42	S
KTB-54	C.b.	2.3 cd	3	83.3 abc	4	7	0.46	S
KTB-57	C.b.	0.4 ef	1	46.7 ef	3	4	0.28	MR
KTB-59	C.b.	3.1 ab	4	83.3 abc	4	8	0.48	S
KTB-60	C.b.	2.2 d	3	83.3 abc	4	7	0.39	S
KTB-66	C.b.	2.3 cd	3	86.7 abc	4	7	0.50	S
KTB-72	C.b.	0.7 ef	1	66.7 b-e	4	5	0.29	MR
KTB-73	C.b.	2.2 d	3	76.7 a-d	4	7	0.39	S
KTF-28	C.f.	2.1 d	3	80 a-d	4	7	0.38	S
KTF-29	C.f.	2.1 d	3	80 a-d	4	7	0.39	S
KTF-33	C.f.	2.1 d	3	76.7 a-d	4	7	0.38	S
KTF-36	C.f.	2.1 d	3	80 a-d	4	7	0.37	S
KTF-37	C.f.	2.1 d	3	76.7 a-d	4	7	0.40	S
KTF-42	C.f.	2.4 bcd	3	76.7 a-d	4	7	0.40	S
KTF-57	C.f.	3.2 a	4	93.3 a	4	8	0.53	S
KTF-68	C.f.	2.5 bcd	3	90 ab	4	7	0.44	S
KTF-91	C.f.	2.3 cd	3	86.7 abc	4	7	0.43	S
KTF-92	C.f.	2.2 d	3	80 a-d	4	7	0.45	S
KTA-20	C.a.	2.1 d	3	83.3 abc	4	7	0.48	S
KTA-27	C.a.	2.1 d	3	76.7 a-d	4	7	0.46	S
KTA-29	C.a.	2.1 d	3	73.3 a-d	4	7	0.48	S
KTA-32	C.a.	3.1 ab	4	80 a-d	4	8	0.55	S
KTA-33	C.a.	2.1 d	3	73.3 a-d	4	7	0.47	S
KTA-39	C.a.	3.1 ab	3	83.3 abc	4	7	0.54	S
KTA-45	C.a.	2.2 d	3	90 ab	4	7	0.50	S
KTA-51	C.a.	2.1 d	3	86.7 abc	4	7	0.51	S
KTA-63	C.a.	2.1 d	3	86.7 abc	4	7	0.41	S
KTA-67	C.a.	2.9 abc	3	83.3 abc	4	7	0.53	S
KTA-75	C.a.	2.1 d	3	76.7 a-d	4	7	0.43	S
KTA-77	C.a.	2.2 d	3	80 a-d	4	7	0.41	S
KTA-81	C.a.	2.1 d	3	86.7 abc	4	7	0.47	S
KTA-82	C.a.	2.1 d	3	83.3 abc	4	7	0.41	S
KTA-87	C.a.	2.1 d	3	80 a-d	4	7	0.45	S
Y.Wonder	C.a.	3.1 ab	4	93.3 a	4	8	0.60	S
Mertcan	C.a.	0.2 f	1	16.7 g	1	2	0.23	R
Mean		2.1		78.53			0.43	
CV (%)		10,91		13,21				
P value		<.0001		<.0001				

C.b.: *C. baccatum*, C.f.: *C. frutescens*, C.a.: *C. annuum*

Levels not connected by the same letter are significantly different ($P < 0.01$)

Severity scores: 1 = 0.1–1, 2 = 1.1–2, 3 = 2.1–3 and 4 = >3

Incidence scores: 1 = <20%, 2 = 20.1–30%, 3 = 30.1–50% and 4 = >51%

Cumulative scores i.e. incidence + severity indices: 0–3 = resistant (R), 4–6 = moderately resistant (MR) and 7–8 = susceptible (S)

ence was confirmed by ELISA in all tested genotypes. Among the tested genotypes, the total score of the KTB-11 genotype was 3 and it was found to be resistant to CMV. The total scores of the KTB-29, KTB-34 and KTB-72 genotypes were 5 and that of the KTB-57 genotype was 4, and these genotypes were classified as moderately resistant. The total scores of KTB-14, KTB-16, KTB-19, KTB-22, KTB-23, KTB-24, KTB-25, KTB-30, KTB-36, KTB-37, KTB-38, KTB-39, KTB-41, KTB-43, KTB-48, KTB-54, KTB-59, KTB-60, KTB-66, KTB-73, KTF-28, KTF-29, KTF-33, KTF-36, KTF-37, KTF-42, KTF-57, KTF-68, KTF-91, KTF-92, KTA-20, KTA-27, KTA-29, KTA-32, KTA-133, KTA-39, KTA-45, KTA-51, KTA-63, KTA-67, KTA-75, KTA-77, KTA-81, KTA-82 and KTA-87 genotypes varied between 7 and 8, and these genotypes were categorized as susceptible (Tab. 2).

The mean ELISA absorbance of the Capsicum genotypes inoculated with CMV varied between 0.23 and 0.60. The lowest mean absorbance was observed in the resistant control Mertcan (0.23), while the highest was in the susceptible control Yolo Wonder (0.60) (Tab. 2). Pearson correlation analysis revealed a positive and significant correlation between the degree of susceptibility and ELISA absorbance values.

DISCUSSION

It was reported that more than 70 viruses infect the pepper plants in the world [Pernezny et al. 2003]. Among these viruses, CMV is one of the most common viruses that infect pepper, leading to significant crop losses [Li et al. 2020]. The virus could lead to serious epidemics and crop losses in certain years. Genetic resistance is one of the most effective and environmentally friendly virus control methods. Identification of the sources of resistance is significant in the development of resistant and hybrid varieties in CMV control. In the present study, 50 Capsicum genotypes were tested against CMV under controlled conditions for their potential as a source of resistance in pepper breeding programs.

The CMV incidence varied between 30% and 93.3% in the majority of the genotypes (51), while the CMV incidence was lower than 20% only in the resistant control Mertcan (16.7%). Among the tested genotypes, the lowest CMV symptom severity was identified in Mertcan (0.2), followed by KTB-11 (0.3), KTB-57 (0.4), KTB-34 (0.6), KTB-72 (0.7) and KTB-29 (0.9). Most of these genotypes exhibited mild mosaic symptoms after CMV inoculation. The remaining tested genotypes showed characteristic symptoms such as mosaic, mottling, distortion in the leaves, at varying degree (Fig. 1). The present study findings demonstrated that none of the tested genotypes were immune to CMV. Virus infection was confirmed by ELISA in symptomatic plants. Similarly, Rahman et al. [2016] did not report any plant that was immune to CMV among the 30 tested pepper genotypes. On the other hand, Naresh et al. [2016] determined that 18 out of 50 tested Capsicum genotypes were immune to CMV. It was determined that only the control varieties Mertcan and KTB-11 were resistant to CMV, while KTB-29, KTB-34, KTB-72 and KTB-57 were moderately resistant.

In the present study, 25 *C. baccatum* genotypes were tested against CMV, and it was determined that 21 genotypes were susceptible, one was resistant, and three were moderately resistant. Castagnoli et al. [1997] reported that the *C. baccatum* var. *pendulum* 1-15421 genotype was moderately resistant to the Italian CMV isolate under field conditions. Similarly, Suzuki et al. [2003] and Naresh et al. [2016] determined CMV resistance in *C. baccatum* genotypes. Previously, various *C. annuum* [Chaim et al. 2001, Caranta et al. 2002, Suzuki et al. 2003, Kang et al. 2010, Yao et al. 2013, Rahman et al. 2016] and *C. frutescens* [Grube et al. 2000, Suzuki et al. 2003] genotypes exhibited different CMV resistance levels; however, all of *C. annuum* and *C. frutescens* genotypes tested in the current study were susceptible to CMV.

ELISA could be beneficial in the quantitative analysis of viral resistance in pepper breeding programs [Marco and Cohen 1979]. In the present study, a positive correlation was determined between the ELISA absorbance of the tested pepper genotypes and susceptibility to CMV. The lowest mean absorbance was determined in the resistant control Mertcan (0.23), while the highest mean absorbance was recorded in the susceptible control Yolo Wonder (0.60). The mean absorbance of resistant and moderately resistant genotypes (KTB-11, KTB-57, KTB-72, KTB-29 and KTB-34) were 0.26, 0.28, 0.29, 0.31 and 0.33, respectively. Similar results were reported by Rahman et al. [2016]

in a study where *C. annuum* genotypes were tested both in field conditions and with artificial inoculation. However, the studies conducted with different CMV strains and pepper genotypes could report conflicting findings. Lapidot et al. [1996] reported that the ELISA results revealed the same virus titer in the high-resistance lines as the susceptible pepper variety, and there was no correlation between variety resistance and virus accumulation.

CONCLUSIONS

The insecticide control of vector aphid species is very difficult due to non-persistent transmission of CMV. The using of virus-free seeds is important in CMV control. However, resistant varieties remain the most economic and reliable control method. In conclusion, the present study findings demonstrated that *C. baccatum* genotype KTB-11 were resistant to CMV and KTB-29, KTB-34, KTB-72 and KTB-57 genotypes were moderately resistant. Although *C. baccatum* and *C. annuum* are categorized in different gene pools, interspesific hybridization between the two species can be made successfully [Yoon et al. 2006, Manzur et al. 2015].Thus, resistant genotypes can be used as a source of resistance to CMV in pepper breeding programs. Future research are required to determine the inheritance and resistance mechanisms of resistant genotypes.

ACKNOWLEDGEMENTS

The author thanks the Black Sea Agricultural Research Institute for supporting this research. The author is grateful to Dr. Erkan Ozata for helping with statistical analysis.

SOURCE OF FUNDING

This research received no external funding.

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