DOI: http://dx.doi.org/10.1590/1678-4499.160

Reaction of cabbage lines reveals resistance to infection by *Cucumber mosaic virus*

Mehmet Ali Sevik[1]*, Ilyas Deligoz[2]

1. Ondokuz Mayis University - Faculty of Agriculture - Department of Plant Protection - Samsun (Samsun), Turkey.
2. The Black Sea Agricultural Research Institute - Plant Health Services - Samsun (Samsun), Turkey.

ABSTRACT: *Cucumber mosaic virus* (CMV) is one of the most economically damaging viral pathogens affecting vegetable crops around the world. In the present research, 50 cabbage (*Brassica oleracea* L.) lines chosen from the Black Sea Agricultural Research Institute (BSARI, Turkey) were evaluated for their reaction to CMV. The level of resistance to CMV was determined based on biological assays, symptom expression, disease severity rating, and viral titer, as determined by ELISA. Eight cabbage lines were identified as moderately susceptible and 3 were susceptible to the CMV-BA isolate. These plants exhibited various symptoms and accumulated high levels of virus titer. However, the results showed that 17 lines had high resistance, 12 were found to be resistant and 10 were found to be moderately resistant to the CMV. The lines that showed high levels of resistance to the virus in this study could be used as sources of CMV resistance in cabbage breeding programs.

Key words: *Brassica oleracea*, breeding, CMV, reaction.

*Corresponding author: malis@omu.edu.tr

Received: May 5, 2016 – Accepted: Jun. 7, 2016

Cabbage (*Brassica oleracea* L.) is an important vegetable crop that is widely cultivated around the world. According to the Food and Agriculture Organization of the United Nations (FAO), records of 2011, Turkey annual cabbage production was 710.000 tons and was ranked 11th in the world. Cabbage is mainly used as a green vegetable, widely grown in the Black Sea Region of Turkey (Balkaya and Karaagac 2006). Samsun province has 222.161 tons of cabbage production in a year, which accounts for 32% of Turkey production (TurkStat 2012).

Species of the genus Brassica may be infected by various viruses such as *Cucumber mosaic virus* (CMV), *Turnip mosaic virus* (TuMV), *Cauliflower mosaic virus* (CaMV), *Beet western yellows virus* (BWYV), and *Turnip yellow mosaic virus* (TYMV) (Raybould et al. 1999; Chen et al. 2000; Moreno et al. 2004). CMV is among the most economically damaging pathogens in Brassica crops (Moreno et al. 2004).

CMV is the type species of the genus Cucumovirus in the family Bromoviridae (Palukaitis and Garcia-Arenal 2003). CMV particles are isometric and composed of a coat protein shell which encapsidates the single-stranded, plus-sense RNA genome (Roossink 2001). It has the broadest host range among the known plant viruses, infecting more than 1,200 species of plants from monocotyledons to dicotyledons (Zitter and Murphy 2009). The CMV spreads through the sap of infected plants by leaf contact, through seeds of 19 plant species and by dodder. The large population of aphid vectors is one reason for the widespread nature of CMV (Zitikaitė and Urbanavičienė 2010; Saruhan et al. 2015). Although resistance is generally pathogen-specific, the use of disease-resistant crop varieties is regarded as an economical and durable method for controlling plant diseases, especially those caused by viruses (Ashfaq et al. 2014). The development of disease-resistant cultivars can provide a simple and cheap approach to reduce the economic losses caused by plant viruses. In this method, resistance is detected by inoculating accessions from a germplasm collection with a virus and screening the reactions of each accession (Balci 2005).

The objective of this study was to assess the reactions to CMV of cabbage lines from the Black Sea Agricultural Research Institute (BSARI). Fifty cabbage lines were screened under greenhouse conditions by sap inoculation method. The grade of reaction to CMV in cabbage tissues was evaluated using a combination of biological and serological assays.

A selected set of 50 advanced lines of *B. oleracea* var. *capitata* subvar. *alba* (Turkish origin) utilized in the breeding project white head cabbage was obtained from BSARI, Turkey, and used for screening purpose (Table 1). The CMV isolate obtained from field-collected samples in Samsun province of Turkey was used in this study. The samples were examined for the most widespread cabbage-infecting viruses of that growing area to detect any mixtures with other viruses. Only samples infected with CMV were studied. The CMV-BA, which is a severe isolate, was propagated on the laboratory host Yolo Wonder, a CMV-susceptible cultivar, in an insect-free controlled environment, and symptomatic leaves were harvested for use as inoculum sources. The samples used as inoculum source were free from tested viruses, such as TuMV, CaMV, TYMV, and BWYV. The presence of only CMV was confirmed by the ELISA and polymerase chain reaction (PCR) methods (Oliveira et al. 2012).

Fresh symptomatic leaves of plants were harvested for use as inoculum sources, and these leaf tissues were used as inoculums and homogenized (1/5 w/v) in 0.05 M phosphate buffer, pH 7.2 (Ashfaq et al. 2014), containing 1% Na_2SO_3. The seedlings of each cabbage line, at the 2- to 4-leaf stage, were lightly dusted with carborundum (600 mesh), and the extracts were rubbed onto carborundum-dusted leaves of 50 plants of each cabbage line and grown for 6 weeks.

Forty-five days post-inoculation, the cabbage lines were visually assessed for symptom severity according to a 5-point rating scale proposed by Shin et al. (2013) and Ntui et al. (2014) with some modifications. A modified version of the scale was adopted for the study, and the lines were categorized in a 5-degree scale as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) on the basis of biological assay, symptoms expression, disease rating scale, and serological tests (Shin et al. 2013). All plants in each line were scored; the ratings totaled and were divided by the number of plants to give a disease index for the line.

CMV virions in plant tissue were analyzed by DAS-ELISA using polystyrene 96-well plates and a Bioreba (AG, Switzerland) phytodiagnostic ELISA kit according to the manufacturers' guidelines. The plates were incubated at room temperature after pipetting the substrate buffer, and the absorbance values were read at 30, 60, and 120 min →

following the addition of substrate at 405 nm using a microplate reader (Tecan Spectra). It was also confirmed visually after incubation for 2 h at room temperature. All samples were tested in 2 replicate wells, and the absorbance value greater than 3 times that of non-inoculated negative control plants held in a growth chamber was rated as positive (Ashfaq et al. 2014).

The data were analyzed using the statistical software package SPSS v. 21.0 (SPSS, release V.21.0 for Windows; SPSS, Chicago, Illinois, USA). The results on the reaction of the 50 white head cabbage lines to the isolate of CMV under controlled conditions are given in Table 1. All lines were rated with disease severity indexes of 1 – 5 at the time of final observation. The appearance of the symptoms →

Table 1. Cabbage lines used in the current study and summarized results of reactions of the lines against CMV.

	Lines	Infection index (1 – 5)	Average of ELISA reading, 1 h after substrate addition	Observed symptoms	Types of reaction
1	W-1	1.0	0.122	---	Highly resistant
2	W-31	1.3	0.122	---	Highly resistant
3	W-33	1.2	0.111	---	Highly resistant
4	W-34	1.0	0.112	---	Highly resistant
5	W-35	1.3	0.110	---	Highly resistant
6	W-39	1.0	0.120	---	Highly resistant
7	W-41	1.0	0.120	---	Highly resistant
8	W-42	1.0	0.119	---	Highly resistant
9	W-43	1.0	0.112	---	Highly resistant
10	P-62/1	1.0	0.123	---	Highly resistant
11	P-83	1.0	0.109	---	Highly resistant
12	P-88	1.0	0.124	---	Highly resistant
13	P-93	1.0	0.114	---	Highly resistant
14	BY-27/2	1.0	0.084	---	Highly resistant
15	180	1.0	0.099	---	Highly resistant
16	SEP	1.0	0.080	---	Highly resistant
17	522	1.0	0.107	---	Highly resistant
18	W-24	1.3	0.113	Mosaic	Resistant
19	W-29	1.3	0.099	Mosaic	Resistant
20	W-32	1.2	0.126	Mosaic	Resistant
21	W-36	1.0	0.134	Mosaic	Resistant
22	W-37	1.2	0.132	Mosaic	Resistant
23	W-44	1.2	0.129	Mosaic	Resistant
24	P-63	1.0	0.130	Mosaic	Resistant
25	BY-31	1.0	0.115	Mosaic	Resistant
26	102-1	1.2	0.113	Mosaic	Resistant
27	541	1.2	0.137	Mosaic	Resistant
28	4	1.2	0.086	Mosaic	Resistant
29	145-SA	1.2	0.089	Leaf curling	Resistant
30	W-7	1.3	0.167	Mosaic	Moderately resistant
31	W-8	1.6	0.123	Mosaic	Moderately resistant
32	W-13	1.3	0.120	Mosaic	Moderately resistant
33	W-46	1.3	0.141	Mosaic	Moderately resistant

...continue

Table 1. Continuation...

	Lines	Infection index (1 – 5)	Average of ELISA reading, 1 h after substrate addition	Observed symptoms	Types of reaction
34	P-27	1.0	0.155	Chlorotic local lesions	Moderately resistant
35	YBB-36/2	1.3	0.117	Mosaic, Chlorotic local lesions	Moderately resistant
36	44-F3	1.2	0.140	Mosaic	Moderately resistant
37	508-T	1.3	0.129	Mosaic	Moderately resistant
38	530-2	1.0	0.133	Mosaic	Moderately resistant
39	145-4	1.2	0.122	Mosaic	Moderately resistant
40	W-38	1.3	0.154	Mosaic	Moderately susceptible
41	W-45	1.3	0.157	Mosaic	Moderately susceptible
42	P-43/1	1.3	0.143	Mosaic	Moderately susceptible
43	P-87	1.7	0.139	Mosaic	Moderately susceptible
44	P-92	1.6	0.193	Mosaic	Moderately susceptible
45	HBF-4/2	1.6	0.124	Necrotic local lesions	Moderately susceptible
46	23-1	1.7	0.139	Necrotic local lesions	Moderately susceptible
47	140 M	1.4	0.092	Yellowing, LC	Moderately susceptible
48	W-5	1.7	0.174	Severe mosaic	Susceptible
49	W-40	3.0	0.219	Severe mosaic	Susceptible
50	P-19/2	2.0	0.204	Chlorotic local lesions	Susceptible

varied with the lines. The average intensity of symptoms expressed in the 5-degree scale is presented in Table 1.

Twenty-nine of the 50 *B. olerecea* lines tested showed symptoms of CMV including mosaic, severe mosaic, yellowing, leaf curling, chlorotic local lesions, and necrotic local lesions. Twenty-one lines did not exhibit symptoms and were found virus-free after testing with DAS-ELISA against CMV.

On the basis of host reactions and ELISA results, the lines were grouped as highly resistant, resistant, moderately resistant, moderately susceptible, and susceptible. Out of 50 lines screened for CMV resistance, 39 showed different levels of resistance. About 34% of total lines investigated showed high levels of resistance to CMV. Of the lines screened for CMV reactions, 12 (24%) were found to be resistant, 10 (20%) were found to be moderately resistant whereas 8 (16%) were moderately susceptible and 3 (6%) were susceptible (Table 1).

The reaction of these cabbage lines to CMV isolate has been summarized in Table 1. Based on both disease rating scale and ELISA, the results indicated that the lines W-1, W-31, W-33, W-34, W-35, W-39, W-41, W-42, W-43, P-62/1, P-83, P-88, P-93, BY-27/2, 180, SEP, and 522 were highly resistant; W-24, W-29, W-32, W-36, W-37, W-44, P-63, BY-31, 102-1, 541, 4, and 145-SA were resistant; W-7, W-8, W-13, W-46, P-27, YBB-36/2, 44-F3, 508-T, 530-2, and 145-4 were moderately resistant. Eight lines, i.e. W-38, W-45, P-43/1, P-87, P-92, HBF-4/2, 23/1, and 140 M were moderately susceptible whereas W-5, W-40, and P-19/2 were found to be susceptible against CMV.

There were significant differences ($p < 0.05$) among the lines with respect to the degree of susceptibility to CMV using the Tukey's Studentized Range Test. Multiple linear regression analyses showed that there was a positive correlation between infection index and the degree of reaction against CMV.

Species of the family Brassicaceae (Cruciferae) may be infected by several viruses (Moreno et al. 2004). In a previous study in Turkey, CMV was newly reported by Erkan et al. (2013) on cabbage plants in Izmir province, Turkey. In the present investigation, 50 lines of *B. oleracea* were evaluated in the greenhouses of the BSARI, located in Samsun province. It has got the 1st rank of Turkey's cabbage production capacity for the reaction of resistance to CMV in 2013 – 2014. Forty-five days after inoculation, symptoms of infection were observed in approximately 58% of the tested lines. Similar observations were also reported by other researchers in cabbage plants. →

In *B. oleracea* subsp. *capitata* f. *alba* 'Amager', TuMV presence was detected in 50% of plants inoculated with CAR39 isolate and in 42.5% of plants inoculated with CAR37A isolate. For the 'Langedijker' cultivar, the percentage of infected plants was 35 and 50, respectively, for CAR37A and CAR39 isolates (Gładysz and Hanus-Fajerska 2009).

The majority of tested lines were resistant to CMV. Seventeen of the 50 white cabbage lines tested showed highly resistance to CMV. These cabbage lines remained symptomless throughout the period of the study. Twelve and 10 lines were regarded as resistant and moderately resistant, respectively, based on both disease rating scale and ELISA tests. Similarly, 40 Chilli pepper genotypes were evaluated by mechanical inoculation, and resistance to CMV Chilli isolate was examined by visual observations and DAS-ELISA. Nine genotypes, C-2, CV-2, CV-5, BSS-269, PGRI, M-2001, CM-2001, M-97, and CP-328, were remained free of infection and catalogued as highly resistant. Among these genotypes, 5 were categorized as resistant, 7 as moderately resistant, 8 as moderately susceptible, and 11 as susceptible (Ashfaq et al. 2014). These results are in agreement with Rashid et al. (2007), who did not observe any infection by ELISA in the lines C-1, C-2, C-5, C-7, C-9, and C-11, which did show negative reaction to CMV.

In the present study, 8 cabbage lines tested were detected as moderately susceptible and 3 (W-5, W-40, and P-19/2) as susceptible against CMV. Similar results were reported by Pink and Walkey (1990) with 'Polinius F1' cultivar of white cabbage, and 'Amager' was also recognized as susceptible to TuMV isolates. The majority of tested lines were resistant to CMV, and the majority of highly resistant and resistant lines showed no reaction under mechanical inoculation. The present findings suggest that the lines showing resistance to CMV local isolate should be maintained for further studies for breeding purpose (Ashfaq et al. 2014).

In conclusion, the reactions of the cabbage lines evaluated in this study to CMV isolate ranged between highly resistant and susceptible. The tested lines showed different levels of susceptibility to CMV isolate. However, the majority of the lines were screened by mechanical inoculation in the present study, and certain amount of resistance was evident. Hence, they could be used for resistance breeding programs.

ACKNOWLEDGEMENTS

The authors would like to thank Ondokuz Mayis University and the Scientific and Technological Research Council of Turkey (TUBITAK) (112O578) for their financial support. We thank S. Sari for kindly providing the isolate used in the study.

REFERENCES

Ashfaq, M., Iqbal, S., Mukhtar, T. and Shah, H. (2014). Screening for resistance to *Cucumber mosaic cucumovirus* in chilli pepper. Journal of Animal and Plant Sciences, 24, 791-795.

Balci, E. (2005). Genetic characterization of *Cucumber mosaic virus* (CMV) resistance in tomato and pepper (Master's thesis). Izmir: Izmir Institute of Technology.

Balkaya, A. and Karaagac, O. (2006). Vegetable genetic resources of Turkey. Journal of Vegetable Science, 11, 81-102. http://dx.doi.org/10.1300/J484v11n04_08.

Chen, T. H., Wu, S. F., Chen, T. J. and Chen, H. L. (2000). Occurrence and serodiagnosis of virus diseases of crucifers in Taiwan. Plant Pathology Bulletin, 9, 39-46.

Erkan, S., Gumus, M., Paylan, I. C., Duman, I. and Ergun, M. (2013). The determination of viral agents in certain cold-season vegetables in İzmir province and its around. Ege University Faculty of Agriculture, 50, 311-322.

Gładysz, K. and Hanus-Fajerska, E. (2009). Evaluation of the infectivity of selected *Turnip mosaic virus* isolates towards white cabbage cultivars. Folia Horticulturae, 21, 129-138.

Moreno, A., De Blas, C., Biurrun, R., Nebreda, M., Palacios, I., Duque, M. and Fereres, A. (2004). The incidence and distribution of viruses infecting lettuce, cultivated *Brassica* and associated natural vegetation in Spain. Annals of Applied Biology, 144, 339-346. http://dx.doi.org/10.1111/j.1744-7348.2004.tb00349.x.

Ntui, V. O., Kong, K., Azadi, P., Khan, R. S., Chin, D. P., Igawa, T., Mii, M. and Nakamura, I. (2014). RNAi-mediated resistance to *Cucumber mosaic virus* (CMV) in genetically engineered tomato. American Journal of Plant Sciences, 5, 554-572. http://dx.doi.org/10.4236/ajps.2014.55071.

Oliveira, C. R. R., Freire Filho, F. R., Nogueira, M. S. R., Barros, G. B., Eiras, M., Ribeiro, V. Q. and Lopes, A. C. A. (2012). Reação de genótipos de feijão-caupi revela resistência às coinfecções pelo *Cucumber mosaic virus*, *Cowpea aphid-borne mosaic virus* e *Cowpea severe mosaic virus*. Bragantia, 71, 59-66. http://dx.doi.org/10.1590/S0006-87052012005000007.

Palukaitis, P. and Garcia-Arenal, F. (2003). Cucumoviruses. Advances in Virus Research, 62, 242-325.

Pink, D. A. C. and Walkey, D. G. A. (1990). Resistance to *Turnip mosaic virus* in white cabbage. Euphytica, 51, 101-107. http://dx.doi.org/10.1007/BF00022440.

Rashid, M. H., Khalequzzaman, K. M., Alam, M. S., Uddin, S. A. and Green, K. (2007). Screening of different sweet pepper lines against *Cucumber mosaic virus* and *Chili veinal mottle virus*. International Journal of Sustainable Crop Production, 2, 1-4.

Raybould, A. F., Maskell, L. C., Edwards, M. L., Cooper, J. I. and Gray, A. J. (1999). The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. New Phytologist, 141, 265-275. http://dx.doi.org/10.1046/j.1469-8137.1999.00339.x.

Roossink, M. J. (2001). *Cucumber mosaic virus*, a model for RNA virus evolution. Molecular Plant Pathology, 2, 59-63. http://dx.doi.org/10.1046/j.1364-3703.2001.00058.x.

Saruhan, I., Senyer, N., Ayvaz, T., Kayhan, G., Ergun, E., Odabas, M. S. and Akca, I. (2015). The estimation of adult and nymph stages of *Aphis fabae* (Hem: Aphididae) using artificial neural network. Entomological News, 125, 12-19.

Shin, J., Xu, S., Kim, J. Y., Woo, J., Kim, H. G., Park, Y. J., Hong, S. J. and Kim, B. S. (2013). CMV-P1 resistance evaluation using enzyme-linked immunosorbent assay of pepper genetic sources (*Capsicum* spp.). Korean Journal of Horticultural Science and Technology, 31, 764-771. http://dx.doi.org/10.7235/hort.2013.13021.

TurkStat (2012). Crop production statistics. Turkish Statistical Institute; [accessed 2012 Jul. 11]. http://tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul

Zitikaitė, I. and Urbanavičienė, L. (2010). Detection of natural infection by *Cucumber mosaic virus* in vegetable crops. Biologija, 56, 14-19. http://dx.doi.org/10.2478/v10054-010-0005-4.

Zitter, T. A. and Murphy, J. F. (2009). *Cucumber mosaic virus*. The Plant Health Instructor. http://dx.doi.org/10.1094/PHI-I-2009-0518-01; [accessed 2016 Oct. 18]. http://www.apsnet.org/edcenter/intropp/lessons/viruses/Pages/Cucumbermosaic.aspx