Anais da Academia Brasileira de Ciências (2019) 91(3): e20180224
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201920180224
www.scielo.br/aabc | www.fb.com/aabcjournal

Viruses infecting cool season crops in the northern Turkey

MEHMET A. SEVIK

Department of Plant Protection, Faculty of Agriculture, University of Ondokuz Mayis, 55139, Atakum, Samsun, Turkey

Manuscript received on March 2, 2018; accepted for publication on August 15, 2018

How to cite: SEVIK MA. 2019. Viruses infecting cool season crops in the northern Turkey. An Acad Bras Cienc 91: e20180224. DOI 10.1590/0001-3765201920180224.

Abstract: Virus diseases of cool season vegetable c*rops (mainl*y cabbage, white and red head cabbage, broccoli, kale, radish, rocket salad, garden cress, and turnip) were surveyed in Bafra Plain, Turkey during winter 2017, and 2018. Leaf samples were collected from different species of the Brassicaceae family showing mosaic, mottling, necrotic spots, malformation, and chlorosis symptoms. These samples were tested for the presence of Cauliflower mosaic virus (CaMV), Cucumber mosaic virus (CMV), Beet western yellows virus (BWYV), Radish mosaic virus (RaMV), Turnip mosaic virus (TuMV), Turnip yellow mosaic virus (TYMV), and Turnip yellows virus (TuYV) by biological and serological methods. A total of 455 samples were collected from cole crop fields and tested for the seven viruses by double-antibody sandwich ELISA using specific polyclonal antibodies. According to the results, out of these, 7 % of the samples were infected by at least one of these viruses. TuMV was the most prevalent virus detected in cole crops. TuMV, CaMV, and CMV were detected in 3 %, 2 %, and 2 % of infected samples, respectively, and the infection rate of these three viruses changed significantly among Brassica species.

Key words: Brassicaceae family, Cole crop, plant disease, occurrence, virus.

INTRODUCTION

Cole crops are generally members of the Brassicaceae family and are natural varieties of the species *Brassica oleracea*. They include white and red head cabbage, cauliflower, broccoli, Brussels sprouts, and kale. The family includes cool season crops such as radish, rocket salad, garden cress, and turnip (Sadowski and Kole 2016). All of these familiar garden crops can trace their history to a common ancestry of wild cabbage originating in the Mediterranean region (Ananda et al. 2017). Cole crops are one of the important contributors to total vegetable production and are most commonly consumed as green and cooked vegetables worldwide (Durairaj et al. 2016). Cole crops are one of the largest horticultural crops in the Northern Region of Turkey. Bafra Plain, Samsun has ranked first in Turkey production capacity (Turkstat 2016).

They are attacked by wide array of diseases which significantly reduce their quality and yield (Durairaj et al. 2016). Plants belonging to the Brassicaceae family may be infected by a number of viruses, i.e., CaMV, CMV, BWYV, RaMV, TuMV, TYMV, and TuYV (Raybould et al. 1999, Moreno et al. 2004). CaMV is one of the important pathogens infecting members of the Brassicaceae family with economic impact. In terms of host range, CaMV ranks second only to TuMV, based on

Correspondence to: Mehmet Ali Sevik
E-mail: malis@omu.edu.tr
ORCid: https://orcid.org/0000-0002-8895-7944

the number of susceptible hosts among vegetable brassicas (Spence et al. 2007). CMV has extremely wide host range, and is one of the main pathogens of cruciferous crops (Zhang et al. 2016). CMV is efficiently transmitted by more than 75 species of aphids in non-persistent manner (Krenz et al. 2015). TuMV, is probably the most widespread and damaging virus that infects cultivated Brassicas worldwide (Nguyen et al. 2013). TuMV is a species of the genus *Potyvirus*, one of the two largest genera of plant viruses (King et al. 2012). TuMV is difficult to control because of its wide host range and non-persistent mode of transmission by aphids (Hughes et al. 2002).

Despite the economic importance of cole crops in Turkey, only limited records and local information are available on the incidence of viral diseases and their impact on production. The objectives of the research were to assess the distribution and frequency of viruses affecting cole crops and to gain information on potentially new or unknown viruses affecting Brassicas in the Northern Region of Turkey.

MATERIALS AND METHODS

SURVEYS AND SAMPLING

Surveys were carried out in Bafra Plain-Samsun, which are the major cole vegetable growing regions of Turkey between Oct and Feb in 2017-2018. The villages in each region were selected randomly. The number of fields visited per village ranged from two to five. Five plants were selected along two diagonals across each field. Samples were collected from cole crops (white and red head cabbage, broccoli, kale, radish, rocket salad, garden cress, and turnip) during winter seasons. The samples were tested by double-antibody sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) and bioassays.

SEROLOGICAL TESTING

The DAS-ELISA was performed according to the manufacturer's instructions (Bioreba AG, Agdia, and Loewe Biochemica GmbH) for all viruses. Polyclonal antisera diluted 1:1000 with coating buffer, were used to coat 96-well polystyrene plates (Nunc MaxiSorp, Denmark). Test wells of a micro titer plates were coated with antibodies specific to viruses (CMV, CaMV, BWYV, RaMV, TuMV, TYMV, and TuYV). The serum samples in were incubated for 4 h at 30 °C. The plates were then emptied and washed three times with PBST. The samples were prepared from leaves by homogenizing 1 g of tissues in extraction buffer (PBST- phosphate-buffered saline, pH 7.2 with 0.05 % Tween 20, 2 % polyvinylprolidone and 0.2% ovalbumin) in a ratio of 1/5. Samples were added to the micro titer plates and incubated at 4°C overnight. After sample incubation, the plates were washed to remove any unbound sample. A virus-specific polyclonal antibody conjugated to alkaline phosphates was added and binds to any captured virus. After incubation the plates were washed to remove any unbound conjugate. PNP substrate was added to the micro titer plates. Color reactions were observed visually and, the absorbance values were read at 405 nm using a spectrophotometer (Tecan Spectra II, Grodig/Salzburg, Austria).

The samples were tested by DAS-ELISA in duplicate. Tests were conducted using two negative controls on each plate for each virus. Sensitivities were deduced from a detection threshold equal to three times the mean value of healthy controls (Nyalugwe et al. 2015). The results of the serological tests were confirmed by biological tests.

BIOLOGICAL TESTING

Confirmation of the presence of the pathogen is obtained by isolation, inoculation and observation of symptoms. Carborandum-dusted five plants of each test species (white head cabbage, kale,

radish, rocket salad, garden cress, and turnip) were inoculated mechanically with extracts from infected leaf tissue of *Brassica* sp. in 0.01 M phosphate buffer (pH: 7.0) (Nguyen et al. 2013). The inoculated plants were maintained in a plant growth room at 24 °C. All indicator host plants producing symptoms were tested by ELISA and confirmed to be positive.

RESULTS

A range of symptoms caused by commonly prevalent viruses were observed on Brassica crops collected from different villages in Bafra Plain, Turkey. For example, mosaic, mottling, necrotic spots, malformation, and chlorosis symptoms were common among the samples collected. A total of 455 leaf samples were collected during the 2017-2018 surveys. The samples were tested for all seven viruses. Only three (CMV, CaMV, and TuMV) viruses and mixed infections of these viruses were detected in screen of infections in the Brassicas populations, whereas BWYV, RaMV, TYMV, and TuYV were not detected in the current study. The occurrence of viruses was reported in Table I.

In the present study, 7 % of the samples were infected by at least one of these viruses. TuMV was the most widely distributed and prevalent virus infecting cool season crops in Bafra Plain, Turkey, followed closely by CaMV (2 %), and CMV (2 %). Mixed infections of viruses only involved 0.7 % of infected plants. However, CaMV was found in mixed infection with both CMV, and TuMV. Triple mixed infection was not detected in this study (Table I). Of the 455 samples collected from white and red head cabbage, broccoli, kale, radish, rocket salad, garden cress, and turnip, 12 were infected singly with TuMV, 10 with CMV, and 9 with CaMV, but none with BWYV, RaMV, TYMV, and TuYV.

The occurrence and distribution of each virus varied according to the species of the Brassicaceae family. In broccoli, CMV was only detected in three samples from Bafra Plain during the 2017-2018 surveys with an average incidence of 4 %, while CaMV (3 %) was only detected in garden cress. The number of virus-infected samples varied from 1 to 3 in kale plants. The predominant virus detected was TuMV, and the virus was detected in 7 % of the kale leaf samples. The CMV and CaMV were the second and third detected viruses. CaMV+TuMV mixed infection was also detected on kale crop in the current study (Table I). Radish crops were found to be common hosts for all three viruses and mixed infections among Brassicas in the current study. As shown in Table I, CaMV was the prevailing virus (4 % of samples analysed),

TABLE I
Occurrence of viruses infecting field-grown species of the Brassicaceae family.

Brassicas	No. of samples	Viruses*				
		CaMV	CMV	TuMV	TuMV+CaMV	CaMV+CMV
Broccoli	65	-	3	-	-	-
Garden cress	60	2	-	-	-	-
Kale	55	1	2	3	1	-
Radish	75	3	2	2	-	1
Red cabbage	25	-	-	-	-	-
Rocket salad	70	-	3	-	-	-
Turnip	35	-	-	-	-	-
White cabbage	75	3	-	7	1	-
Total	**455**	**9**	**10**	**12**	**2**	**1**

***:** TuMV: *Turnip mosaic virus*, CaMV: *Cauliflower mosaic virus*, CMV: *Cucumber mosaic virus*.

followed by the CMV (2 %), TuMV (2 %), and CaMV+CMV (1 %) on radish. No symptoms were observed on red head cabbage plants in any of the regions and no viruses were detected in any of the red cabbage samples analyzed. CMV was detected in only three samples during the two years of the study, indicating a low incidence of the virus (4 %) in rocket salad plants. In the present study, all the viruses tested were not detected in any of the turnip samples analyzed. White cabbage (14 %) was the most infected member of the Brassicaceae family in the current study. TuMV was the most common virus in white head cabbage (9 %), followed by CaMV (4 %), and TuMV+CaMV (1 %), but CMV, BWYV, RaMV, TYMV, and TuYV were not detected in any of the white head cabbage samples analysed in our study.

The results of the serological tests were confirmed by biological assays on Brassicas test plants. The virus isolates were inoculated onto six different plant species of the Brassicaceae family. They were able to systemically infect tested Brassica plants inducing mosaic, mottle, leaf deformation and necrotic local lesions. Based on symptoms, it was however unable to infect turnip plants tested systemically (Table II).

DISCUSSION

The present work illustrates the natural occurrence and distribution of emerging viruses on commercial cole crop fields in the Northern Region of Turkey. Samples were collected from cole crops during winter seasons. The number of samples collected from each Brassica crop varied from 25 to 70. In the whole sample, 7 % of cole crop plants were infected with TuMV, CaMV, and CMV. The previous studies conducted in Brassica crop have reported the existence of TuMV, CaMV, CMV, TYMV, BWYV, and other economically important viruses present worldwide (Shahraeen 2012). In the current study, the predominant virus detected was TuMV. Similarly, viral diseases that affect cabbage in Korea include TuMV, and CMV. TuMV is the most prevalent virus, followed by CMV (Chung et al. 2015).

In the present study, TuMV, CaMV, and CMV were detected in 3 %, 2 %, and 2 % of infected samples, respectively. In order to determine the distribution of viruses in Iran-Golestan, samples from three important growing areas of the province were collected by Tabarestani et al. (2010) during 2008-2009. The results revealed that field infection levels in 2008 with TuMV, CaMV, and BWYV were at 4 %, 2 % and 6 %, respectively. Field infection in 2009 with TuMV, CaMV and BWYV was at the levels of 8 %, 1 % and 6 %, respectively whereas BWYV was detected in any of the cool season crop plants in our study. A virus survey was conducted Moreno et al. (2004) during 2001-2002 to determine the presence, prevalence and

TABLE II
Reaction of host plants to sap inoculation by the viruses and serological results.

Hosts			Tests*	
Family	**Species**	**Common**	**Biological**	**Serological**
Brassicaceae	*B. oleracea* var. *capitata*	cabbage	+	+
	B. oleracea var. *acephala*	kale	+	+
	B. rapa	turnip	-	-
	Lepidium sativum	cress	+	+
	Raphanus sativus	radish	+	+
	Eruca sativa	rocket	+	+

***** += Positive, -: Negative.

distribution in Spain of the viruses that are most commonly found infecting Brassica worldwide. In 2002, CMV and LMV were the most prevalent viruses, while CaMV was the most important virus present in Brassica crops grown in Navarra, followed by CMV and BWYV. Several kinds of viruses such as TuMV, CaMV, CMV, and BWYV have been reported to occur in cruciferous crops, including cabbage, Chinese cabbage, radish, and turnip in Japan. Out of these viruses, TuMV is most predominant and geographically widespread in Japan. TuMV is therefore recognized to be the most important virus in growing cruciferous crops, due to its serious cause for significant yield losses (Fujisawa 1990). The occurrence of TuMV in cole crops (*Brassica* spp.) grown in India was confirmed by the tests (Singh et al. 2015).

Viruses damage Brassica crops worldwide. Some of the most widespread and well studied of these pathogens are TuMV, CaMV, and CMV. TuMV is probably the most widespread and damaging virus that infects cultivated Brassicas worldwide (Nguyen et al. 2013). CaMV is also one of the most prevalent and important viruses which can cause yield losses seriously in Brassica crops tend to become infected wherever they are grown (Spence et al. 2007). In the study, CaMV infections were detected in several cole crops (garden cress, kale, radish, and white head cabbage). Similarly, CaMV was detected in Brassica crops in different provinces of Iran and in three Brassica weeds. Most of the species surveyed, including broccoli, cauliflower, Chinese cabbage, kale (=collard), kohlrabi, small radish, turnip, white cabbage, and white radish were infected (Farzadfar et al. 2007). CMV is one of the three most important global viruses of Brassica crops (Chen et al. 2015). Incidence of the virus, ranged from 3 - 4 % in the current study. Similar results were reported previously by Moreno et al. (2004) and Cai et al. (2017).

In the current study, CaMV has been found in mixed infections with TuMV and CMV in plants of the genus *Brassica*. Park et al. (1998) have also reported that there have been severe outbreaks of mixed infection CMV and TuMV in Chinese cabbage, radish and lettuce. Natural mixed infections of plant viruses are frequent, often leading to unpredictable variations in symptoms, infectivity, accumulation and/or vector transmissibility (Martin and Elena 2009). Interestingly, all of the viruses detected are transmissible by aphids in a non-persistent manner (Whitfield et al. 2015). Moreno et al. (2004) reported that viruses have weeds hosts that provide a reservoir of viruses from which economically important *Brassica* crop plants may become infected.

In the present survey, BWYV, RaMV, TYMV, and TuYV were not detected cool season crop plants in Bafra Plain, Turkey although these viruses were reported in Brassica crops (Raybould et al. 1999). No viruses were detected in any of the red cabbage and turnip samples. Similarly, viral contamination occurred on Brassica crops, whereas no viruses were detected in any of the red cabbage plants (Moreno et al. 2004).

In conclusion, this is report of comprehensive surveys carried out to determine the prevalence and distribution of viruses that affect cool season crops in the major producing areas of Turkey. TuMV, CaMV, and CMV were the viruses detected during the two years study. Thus, future research will be focused on epidemiology, yield loss assessment, and management strategies of these viruses.

ACKNOWLEDGMENTS

The work presented has been funded by Ondokuz Mayis University, Scientific Research Project Funding (O.M.U-PYO.ZRT.1901.17.003). The author gratefully acknowledges the financial support of OMU and N. Cansız for support during the serological and biological tests.

REFERENCES

ANANDA A, DEVI AB, SINGH A, BARIK S AND BAIRWA MK. 2017. Cabbage (*Brassica oleracea* L. var. *capitata*) cv. Rareball introduction with Knol Khol and Broad Bean intercropping: yield efficiency under foot hills of Imphal-West. Phar Inno J 6: 339-341.

CAI L, ZHANG Y, CHEN Y, HONG N AND XU W. 2017. First report of *Cucumber mosaic virus* infecting *Brassica campestris* var. *purpuraria* in China. Plant Dis 101: 1684.

CHEN Q, WANG J, HOU M, LIU S, HUANG J AND CAI L. 2015. BnSGS3 has differential effects on the accumulation of CMV, ORMV, and TuMV in oilseed rape. Viruses 7: 4169-4185.

CHUNG BN, CHOI SK, AHN JJ, JOA JH, DO KS AND PARK KS. 2015. Effects of temperature on systemic infection and symptom expression of *Turnip mosaic virus* in Chinese cabbage (*Brassica campestris*). Plant Path J 31: 363-370.

DURAIRAJ C, SAMBATHKUMAR S AND MOHANKUMAR S. 2016. Integrated pest management of cruciferous vegetables. In: Muniappan R and Heinrichs E (Eds), Integrated pest management of tropical vegetable crops. Springer, Dordrecht, p. 149-165.

FARZADFAR S, AHOONMANESH A, MOSAHEBI GH, POURRAHIM R AND GOLNARAGHI AR. 2007. Occurrence and distribution of *Cauliflower mosaic virus* on cruciferous plants in Iran. Plant Path J 6: 22-29.

FUJISAWA I. 1990. *Turnip mosaic virus* strains in cruciferous crops in Japan. Jap Agri Res Quar 23: 289-293.

HUGHES SL, GREEN SK, LYDIATE DJ AND WALSH JA. 2002. Resistance to *Turnip mosaic virus* in *Brassica rapa* and *B. napus* and the analysis of genetic inheritance in selected lines. Plant Path 51: 567-573.

KING AMQ, ADAMS MJ, CARSTENS EB AND LEFKOWITZ EJ. 2012. Virus taxonomy: Classification and nomenclature of viruses. Ninth report of the international committee on taxonomy of viruses. Elsevier/ Academic Press, San Diego, CA, USA, 1327 p.

KRENZ B, BRONIKOWSKI A, LU X, ZIEBELL H, THOMPSON JR AND PERRY KL. 2015. Visual monitoring of *Cucumber mosaic virus* infection in *Nicotiana benthamiana* following transmission by the aphid vector *Myzus persicae*. J Gen Virol 96: 2904-2912.

MARTIN S AND ELENA SF. 2009. Application of game theory to the interaction between plant viruses during mixed infections. J Gen Virol 90: 2815-2820.

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. Ann App Bio 144: 339-346.

NGUYEN HD, TOMITAKA Y, HO SYW, DUCHENE S, VETTEN HJ, LESEMANN D AND OHSHIMA K. 2013. *Turnip mosaic potyvirus* probably first spread to *Eurasian Brassica* crops from wild orchids about 1000 years ago. PLoS One 8: e55336.

NYALUGWE EP, BARBETTI MJ AND JONES RAC. 2015. Studies on resistance phenotypes to *Turnip mosaic virus*in five species of Brassicaceae, and identification of a virus resistance gene in *Brassica juncea*. Eur J Plant Path 141: 647-666.

PARK WM, CHOI SR, KIM SJ, CHOI SK AND RYU KH. 1998. Characterization and RT-PCR detection of *Turnip mosaic virus* isolated from Chinese cabbage in Korea. Plant Path J 14: 223-228.

RAYBOULD AF, MASKELL LC, EDWARDS ML, COOPER JI AND GRAY AJ. 1999. The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. New Phytol 141: 265-275.

SADOWSKI J AND KOLE C. 2016. Genetics, genomics and breeding of vegetable brassicas. CRC Press, Boca Raton, FL, USA, 450 p.

SHAHRAEEN N. 2012. An overview of oilseed rape (canola) virus diseases in Iran. I Res J Microbiol 3: 24-28.

SINGH R, BANERJEE A, SHARMA SK, BHAGAWATI R, BARUAH S AND NGACHAN SV. 2015. First report of *Turnip mosaic virus* occurrence in cole crops (*Brassica* spp.) from Arunachal Pradesh, India. Virus Dis 26: 211-213.

SPENCE NJ, PHIRI NA, HUGHES SL, MWANIKI A, SIMONS S, ODUOR G AND MARRIS GC. 2007. Economic impact of *Turnip mosaic virus*, *Cauliflower mosaic virus* and *Beet mosaic virus* in three Kenyan vegetables. Plant Path 56: 317-323.

TABARESTANI AZ, SHAMSBAKHSH M AND SAFAEI N. 2010. Distribution of three important aphid borne canola viruses in Golestan province. Iranian J Plant Protec Sci 41: 251-259.

TURKSTAT. 2016. Turkish Statistical Institute Database. Available from: http://www.tuik.gov.tr/bitkiselapp/bitkisel.zul. Accessed 02.05.2016.

WHITFIELD AE, FALK BW AND ROTENBERG D. 2015. Insect vector-mediated transmission of plant viruses. Virol 479: 278-289.

ZHANG X, PENG Y, WANG Y, ZHANG Z, LI D, YU J AND HAN C. 2016. Simultaneous detection and differentiation of three genotypes of *Brassica yellows virus* by multiplex reverse transcription-polymerase chain reaction. Virol J 13: 2-7.