# Bitki Koruma Bülteni / Plant Protection Bulletin

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Original article

# Serological and molecular detection of *Cauliflower mosaic virus* and its vectors infecting cold-season vegetables in Ankara province

Ankara ili kışlık sebze üretim alanlarında *Cauliflower mosaic virus*'u ve vektörlerinin serolojik ve moleküler olarak tanılanması

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#### ARTICLE INFO

Article history: DOI: 0.16955/bitkorb.694019 Received : 25.02.2020 Accepted : 14.09.2020

Keywords: CaMV, cold season vegetables, ELISA, PCR, Brevicoryne brassicae

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#### ABSTRACT

Ankara province is one of the major planting area of winter vegetables (Brassicaceae), such as cauliflower, broccoli, white cabbage, red cabbage and radish. Periodic surveys were carried out in Centrum, Ayaş, Beypazarı, Nallıhan and Çubuk districts of Ankara province, which are the main winter vegetable production areas in Ankara; thus samples were collected from cabbage, broccoli, radish, and cauliflower plants between February and October 2016 and 2017. In this study, as viral diseases in winter vegetables, Cauliflower mosaic virus (CaMV) and those vectors were detected serologically by DAS-ELISA and molecular by PCR. As a result, among 271 collected plant samples tested by the DAS-ELISA method only 0.03% were positively infected with CaMV, while 15.13% of samples were found to be infected with CaMV; by PCR. Three population of Brevicoryne brassicae aphids were also collected from infected plant samples determined as infected with CaMV. Statistical analysis was made after the determination of infected plants number, then P-value was determined as 0.0001. According to the results of present research, CaMV was present in the Centrum, Ayaş, Beypazarı, Nallıhan districts and not present in Cubuk district of Ankara. This is the first report on the presence of the CaMV, a DNA virus and its vector, B. brassicae in Ankara province.

#### INTRODUCTION

Turkey is one of the leading country for winter vegetable cultivation worldwide, and according to statistical data (TUIK 2019), Ankara as being a part of the central Anatolian region has suitable climate for production of cold season vegetables and all member of *Brassicaceae family*, including cauliflower (*Brassica oleraceae* var. *botrytis*), broccoli (*Brassica oleracea italica*), white cabbage (*Brassica oleracea var. capitata f. alba*), red cabbage (*Brassica oleracea var. capitata f. rubra*) and radish (*Raphanus raphanistrum* subsp. *sativus*) are cultivated. Ankara province is one of the

major planting areas of Brassica plants and they are majorly produced in Ayaş, Beypazarı, Nallıhan and Çubuk districts in Ankara province.

Cauliflower mosaic virus (CaMV), Cucumber mosaic virus (CMV), and Turnip mosaic virus (TuMV) are the main viral agents detected on cold season vegetables plants all over the world, but Lettuce mosaic potyvirus (LMV), Radish mosaic virus (RaMV), Turnip yellow mosaic potyvirus (TYMV), Leek yellow stripe potyvirus (LYSV), Onion yellow dwarf virus (OYDV), and many of the

artichoke infecting viruses were also detected in cabbage plants (Alan 2012, Erkan et al. 2013, Hull 2002, Tuzlalı and Korkmaz 2011).

*Cauliflower mosaic virus* is a virus belonging to *Caulimoviridae* family, *Caulimovirus* genus and it is a pararetrovirus. It contains ds-DNA and infects many plants of the Brassicaceae family, especially radish and cauliflower. Plants infected with the virus show symptoms like systemic mosaic, vein clearing and usually followed by malformation and reduced growth of plants (Shephard 1981). It is transmitted by many species of aphids like as *Myzus persicae* (Shulz, 1776) and *Brevicoryne brassicae* (Linnaeus, 1758) in non-persistent manner (Martiniere et al. 2009). *Brevicoryne brassicae* (winged and wingless form) carries more than 20 different viruses that cause disease in citrus and *Brassicae* plants (Day and Venables 1960).

*Cauliflower mosaic virus* has circular, icosahedral shape and contains a capsid of 52 nm in diameter. The coat protein contains 420 protein subunits. The circular ds-DNA has an 8 kb genome. 35S RNA is responsible for transcription of the entire genome and is used in the transformation of plants (Benfey et al. 1989, Fang et al. 1989, Odell et al. 1985, Prat et al. 1989). It has highly complex structure, 600 nucleotid long and encodes six to eight open reading frames (ORFs). CaMV 35S promotor is the well-known promotor used in plant biotechnology (Hull 2002). The virus causes a high level of gene expression in dicotyledons, but in monocotyledon plants (Fromm et al. 1985, Shephard 1981).

Cold season vegetable viruses are investigated sporadically in Turkey and among them, CaMV is also one of the most prevalent and major viruses which can cause severe vield losses in Brassica crops to tend to become infected wherever they are grown (Spence et al. 2007). The virus is first reported in Turkey by Erkan et al. (1990) on infected cauliflower and cabbage plants, then it was detected as one of the destructive virus infection in Aegean region (Erkan et al. 2013). Alan (2012) has reported as one of the destructive plant viruses is CaMV in Eastern Mediterranean region of Turkey. Tuzlalı and Korkmaz (2014), have reported that in the surveys conducted to cabbage and cauliflower growing areas in Canakkale province, plants bearing symptoms as systemic mosaic, growth retardation, and chlorosis were collected and tested by DAS-ELISA, revealed the most common virus as CaMV which was present in 63 out of 84 leaf samples. Akcura et al. (2015), 235 leaf samples were collected from leaf cabbage production areas. They have used the DAS-ELISA method for detection of present viruses and according to the results of serological tests

revealed that 11.4% of cabbage samples were infected with CaMV and 7.6% with TuMV, while 3.8% of all samples showed CaMV and TuMV mixed infections. No infection with CMV, TYMV, and BWYV was detected in the tested samples.

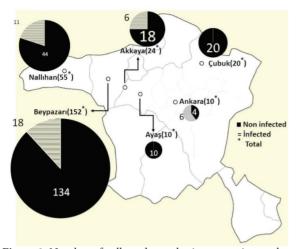
Based on the reports of Sevik (2019), the virus is present in Blacksea region of Turkey. Infected plant samples were collected from Bafra plain region between 2018 and 2017, and then tested with DAS-ELISA against CaMV, *Cucumber mosaic virus* (CMV), *Beet western yellows virus* (BWYV), *Radish mosaic virus* (RaMV), *Turnip mosaic virus* (TuMV), *Turnip yellow mosaic virus* (TYMV), and *Turnip yellows virus* (TuYV), and 2% of the 455 samples were determined as infected with CaMV.

Cold season vegetable viruses are also destructive in Europe. Moreno et al. (2004), in Spain found CMV infections as the most destructive infection in cauliflower and cabbage plants but in England, cabbage plants were infected as 60% with CaMV which was followed by TuMV and TYMV (Raybould et al. 1999). Farzadfar et al. (2007) found CaMV as the prevalent virus infection in cabbage plantation as 57.7% in Iran and the virus was also present in cauliflower, broccoli, and turnip production areas. In order to determine the distribution of winter vegetable infecting viruses in Iran-Golestan, the samples were collected from three main growing areas of the province by Tabarestani et al. (2010) during 2008-2009. The results show that field infection levels in 2008 with TuMV, CaMV, and BWYV are at 4%, 2%, and 6%, respectively. Several types of viruses such as TuMV, CaMV, CMV, and BWYV have been stated to occur in cruciferous crops, including cabbage, Chinese cabbage, radish, and turnip in Japan (Fujisawa 1990, Nguyen et al. 2013).

#### MATERIALS AND METHODS

#### Collection of virus isolates and aphid populations

Periodical surveys were carried out in the districts of Ankara, major winter vegetable production areas in districts, Centrum, Ayaş, Beypazarı, Nallıhan, and Çubuk of Ankara province, and cabbage, broccoli, radish and cauliflower plants were collected between February and October of 2016–2017,especially in winter and fall seasons. Samples (plant and vectors) were collected in proportion to the land size (Figure 1). Symptomatic and asymptomatic plants (Figure 2) were collected, transferred to the laboratory and kept in deep-freezer (-25 °C) until they were analyzed. Aphid samples were places in 70% ethanol solution and send to expert for identification and also applied to DAS-ELISA test and PCR amplification for the investigation of CaMV presence.



**Figure 1.** Number of collected samples in proportion to the land size (Akkaya is a village of Ayaş)



**Figure 2.** Cabbage infected with *Cauliflower mosaic virus* (leaf deformation and systemic mosaic symptom)

#### DAS-ELISA method

DAS-ELISA method (Clark and Adams 1977) was applied to the samples brought to the laboratory. In DAS-ELISA studies, CaMV antiserum were used and IgG and conjugate were prepared at the recommended dilution levels of the company (Bioreba-Switzerland). Plant extracts diluted with extraction buffer at rate of 1:10 (w/v), centrifuged at 5000 rpm for 10 min and used in DAS-ELISA tests. The DAS-ELISA was studied with double replications. CaMV infected cabbage was used as positive control, kindly supplied by Prof. Dr. Savaş Korkmaz (Çanakkale Onsekiz Mart University) and buffer control healthy cabbage leaf extract was used as negative controls. Double of negative controls were accepted as positive.

#### Total DNA isolation

Total DNA isolation was carried out according to the protocol of Somma (2006) with some modifications. 100 mg of frezeed leaf samples were used DNA purification. The pellet was dried and dissolved in 100  $\mu$ l of sterile deionized water. The DNA solution kept in a deep-freezer at –25 °C. Concentrations of DNAs were determined by Nanodrop (Thermo Scientific, USA).

#### PCR amplification of CaMV

PCR amplification was performed with CaMV 35S promotor gene primer set. The composition of PCR mix was 2.5  $\mu$ l of 10 × PCR buffer, 50 ng genomic DNA, 0.1-0.5  $\mu$ M primers, 200  $\mu$ M dNTP mix, and 0.2 M Taq DNA polymerase were used for total 25  $\mu$ l reaction solution.

The DNA was analyzed with PCR for presence of the CaMV 35S promotor and the primers used were: 35S, F: 5<sup>-</sup>-GCTCCTACAAATGCCATCA-3<sup>-</sup>, R:5<sup>-</sup>-GATAGTGGGATTGTGCGTCA-3; PCR product, 195 bp, Khadye et al. (2012). Reaction was started by a denaturation step, 5 min at 95 °C, followed by 35-cycle program, with each cycle consisting of denaturation at 95 °C for 30 s, annealing at 60 °C for 60 s, and extension at 72 °C for 45 s; a final extension step (72 °C for 2 min) was also used. In each series of experiments, at least one negative control and a positive control were processed in parallel (Khadye et al. 2012).

#### RESULTS

Periodical surveys were conducted in 2016-2017 to main winter-vegetable cultivation areas of Ankara and 271 Brassica plant samples (Figure 2) were collected, including cabbage, broccoli, radish, and cauliflower plants. The symptoms were systemic mosaic, growth retention and severe deformation of leaves. On some of the collected plant samples, vector of CaMV, *B. brassicae* colonies were also present. The samples collected were subjected to the DAS-ELISA test in order determine the CaMV presence. DAS-ELISA test resulted in 43 suspected and only 1 positive isolate infected with CaMV. In the ELISA studies, there is only one positive CaMV, isolates while a total of 41 plants were detected as contaminated with CaMV in the following PCR assays.

As a result of the ELISA test, it was found that 0.03% of 271 plants were infected by CaMV where as according to the results of PCR tests, infection rate of the infected plants were increased to 15.13%.

In the samples collected from different regions, the most infected regions were Centrum of Ankara (60%), Ayaş (25%), Nallıhan (25%) and Beypazarı (11.9%), and no CaMV infection was detected in Çubuk (0%) although the collected

plants were showing similar symptoms. They may be caused by the other Brassica virus infections. The plants collected from the fields were cauliflower, cabbage, red cabbage, broccoli and radish. The number of infected plants with the CaMV in the ELISA test was only one, but this number increased to 41 in the PCR assays and remaining 230 plants were free of CaMV. The most infected plant was cabbage by 7.38% and it was followed by broccoli (4.05%), cauliflower (1.84%), and radish (1.84%). Locally the most infected region was Ankara-Centrum, where the most infectious plant was cauliflower by 40% that is followed by broccoli

**Table 1.** Regional distribution of CaMV detected in Ankara and their frequency in cold-season vegetables frequency (number of positive samples)

Region	Cabbage	Cauliflower	Broccoli	Radish	Red cabbage	Total
Beypazarı	55(7)	13(0)	12(9)	58(2)	14(0)	152(18)
Nallıhan	24(7)	14(1)	NP	12(3)	5(0)	55(11)
Ankara	NP	5(4)	5(2)	NP	NP	10(6)
Çubuk	20(0)	NP	NP	NP	NP	20(0)
Ayaş	28(6)	6(0)	NP	NP	NP	34(6)
Total	127(20)	38(5)	17(11)	70(5)	19(0)	271(41)

NP: Not present

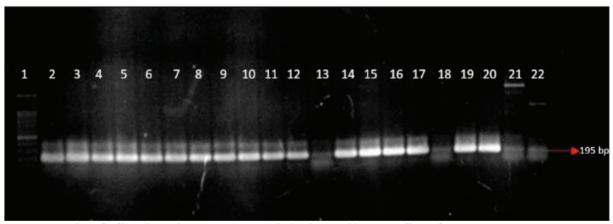


Figure 3. Number of collected samples in proportion to the land size (Akkaya is a village of Ayaş)

Parameter	Frequency	Positive N	samples %	Negative N	samples %	P-value
Region						0.0001
Beypazarı	152	18	11.8	134	88.2	
Nallıhan	55	11	20	44	80	
Ankara	10	6	60	4	40	
Çubuk	20	0	0	20	100	
Ayaş	34	6	17.6	28	82.4	
Crop						0.0001
white cabbage	127	20	15.7	107	84.3	
cauliflower	38	5	13.2	33	86.8	
broccoli	17	11	64.7	6	35.3	
radish	70	5	7.1	65	92.9	
red cabbage	19	0	0	19	100	

<b>Table 2.</b> Fisher's exact test. Examination results for P-	value
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(20%), and the virus was not present on the other plants cultivated in this region. The infection in Ayaş (Akkaya) region was present only on cabbage plants at the rate of 25% and Nallıhan, the most infected plant was cabbage by 12.72% followed by radish (5.45%) and cauliflower (1.82%), and, least infection was found in Beypazarı; where the most infectious plant for CaMV was broccoli (5.92%) followed by cabbage (4.6%) and radish (1.31%) and there is not any cauliflower plant found to be infected by CaMV. In none of the regions, no red cabbage was found to be infected by CaMV (Table 1).

Aphids on infected plants (3 populations) were collected from the Centrum and identified by experts from Ankara Central Plant Protection Institute and it was confirmed to be as *Brevicoryne brassicae* (Linnaeus, 1758) (Hemiptera, Aphididae,). No *Myzus persicae* adults were present on the collected plants or in the area surveyed. In ELISA tests, the results obtained by aphids were lower comparing to the results of infected plants but in PCR tests, amplification were quite visible as seen in Figure 3.

For statistical analysis, all of the data were entered into SPSS (Chicago, IL, USA Ver. 18) by using Fisher's Exact Test. Examination results showed a significant difference between the samples in terms of the region (P-value = 0.0001). Besides, there is the same result for samples in terms of the crop (P-value = 0.0001) (Table 2).

So far to our knowledge, this is the first report on the presence of CaMV and its vector *Brevicoryne brassicae* in Ankara province.

#### DISCUSSION

Different viruses occur on the Brassica plants worldwide and some of the most widespread and well-studied, those are TuMV, CaMV, and CMV. Ankara is one of the regions where the winter vegetables are widely grown. According to the results we have obtained, CaMV present on cold season vegetable crops at a rate of 15.13%. Although the infection rate is low but its vector Brevicoryne brassicae is also present and detected, which indicates that the virus can be epidemic in future. CaMV are reported previously in Turkey. Based on the report of Sevik (2019), the samples were collected from Bafra plain region between 2018 and 2017, and then tested with DAS-ELISA against 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), and 2% of the 455 samples (including cabbage, white and red cabbage, broccoli, kale, radish, rocket salad, garden cress, and turnip) were determined as infected with CaMV. Comparing our results with these results shows

that the incidence of the virus in Ankara region is quite high. Korkmaz et al. (2008), reported that in the survey directed to Çanakkale, Balıkesir and Bursa provinces, cabbage plants showing symptoms such as systemic mosaic, necrotic rings, growth retardation, and chlorosis were collected and tested by DAS-ELISA method, revealed the most common virus as TuMV which was present in 60 leaf samples. The virus was also reported from Çanakkale province by Tuzlalı and Korkmaz (2011) on infected cabbages. Akcura et al. (2015), 235 leaf samples were collected from leaf cabbage production areas. They have used the DAS-ELISA method for detection of present viruses and according to the results of serological tests revealed that 11.4% of cabbage samples were infected with CaMV and 7.6% with TuMV, while 3.8% of all samples showed CaMV and TuMV mixed infections. No infection with CMV, TYMV, and BWYV was detected in the tested samples.

In order to determine the distribution of winter vegetable infecting viruses in Iran-Golestan, the samples were collected from three main growing areas of the province by Tabarestani et al. (2010) during 2008-2009. The results show that field infection levels in 2008 with TuMV, CaMV, and BWYV are at 4%, 2%, and 6%, respectively. Several types of viruses such as TuMV, CaMV, CMV, and BWYV have been stated to occur in cruciferous crops, including cabbage, Chinese cabbage, radish, and turnip in Japan. Besides, TuMV is the most predominant and geographically widespread in Japan (Fujisawa 1990, Nguyen et al. 2013). CaMV is also one of the most prevalent and major viruses which can cause severe yield losses in Brassica crops to tend to become infected wherever they are grown (Spence et al. 2007). Farzadfar et al. (2007) found CaMV as the prevalent virus infection in cabbage plantation as 57.7% in Iran and the virus was also present in cauliflower, broccoli, and turnip production areas. Moreno et al. (2004), in their studies of different regions of Spain and found CMV infections as the most destructive infection in cauliflower and cabbage plants but in England, cabbage plants were infected as 60% with CaMV which was followed by TuMV and TYMV (Raybould et al. 1999).

Presence of CaMV at a range of 15,13% together with its vector in our region indicates that the virus could be epidemic and widespread in near future, according to climatic conditions of Ankara.

### ACKNOWLEDGEMENTS

The authors are grateful to Ankara University BAP Coordination for funding the Project coded 17LO447009. The authors are gratefull to Prof. Dr. Savas Korkmaz (Çanakkale Onsekiz Mart University) for providing positive samples of CaMV and Dr. Isil Özdemir (Ankara Plant Protection Central Research Institute) for the identification of the vectors collected in the research area.

## ÖZET

Ankara ilinde kışlık Brassicaceae familyasına ait sebzelerin, özellikle karnabahar, brokoli, beyaz lahana , kırmızı lahana ve turp üretimi yapılmaktadır. Örnekler, Ankara'nın başlıca kış sebzeleri üretim alanları olan Merkez, Ayaş, Beypazarı, Nallıhan ve Çubuk ilçelerinden, kırmızı ve beyaz lahana, brokoli, turp ve karnabahar bitkilerinden, 2016 ve 2017 yılı şubat-ekim ayları arasında, düzenli olarak toplanmıştır. Bu örneklerde, Karnabahar mozaik virüsü (CaMV) ve vektörleri serolojik olarak DAS-ELISA yöntemi ve moleküler olarak PCR yöntemi ile tespit edilmiştir. Sonuç olarak, DAS-ELISA yöntemi ile testlenen toplam 271 bitki örneğinin %0.03 CaMV ile bulaşık saptanırken, PCR yöntemi ile örneklerin %15.13'ü CaMV ile enfekteli bulunmuştur. Ayrıca, enfekteli bitkiler üzerinden toplanan 3 Brevicoryne brassicae popülasyonunun CaMV ile bulaşık olduğu belirlenmiştir. Enfekte bitki sayısının saptanmasından sonra istatistiksel analiz yapılmış ve P değeri 0.0001 olarak belirlenmiştir. Sürvey yapılan Ankara'nın Merkez, Ayaş, Beypazarı, Nallıhan ilçelerinde CaMV'sünün mevcut olduğu ancak Çubuk ilçesinde bulunmadığı yapılan bu calısma ile ortaya çıkarılmıştır. Bu arastırma Ankara ilinde bir DNA virüsü olan CaMV'ün ve vektörü B. brassicae'nin varlığı konusunda yapılan ilk arastırmadır.

Anahtar kelimeler: CaMV, kış sebzeleri, ELISA, PCR, *Brevicoryne brassicae* 

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Cite this article: Hekmat Kashtiban, A, Ertunç, F. (2020). Serological and molecular detection of *Cauliflower mosaic virus* and its vectors infecting cold-season vegetables in Ankara province. Plant Protection Bulletin, 60-3. DOI: 10.16955/bitkorb.694019 Atıf için: Hekmat Kashtiban, A, Ertunç, F. (2020). Ankara ili kışlık sebze üretim alanlarında *Cauliflower mosaic virus*'u ve vektörlerinin serolojik ve moleküler olarak tanılanması. Bitki Koruma Bülteni, 60-3. DOI: 10.16955/bitkorb.694019