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Determination of cabbage (*Brassica oleracea*) virus infection in Niğde region of Turkey

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Abstract

Brassica oleracea is an economically important member of the genus *Brassica* cultivated as an important vegetable crop in Turkey. Field surveys were conducted to determine the virus infection in the various districts of Niğde province during the year 2014-2015. A total of 386 samples were collected from *B. oleracea* fields and screened using double-antibody sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) to detect *Turnip mosaic virus* (TuMV), *Cauliflower mosaic virus* (CaMV), *Tomato spotted wilt virus* (TSWV), *Turnip yellow mosaic virus* (TYMV), *Radish mosaic virus* (RaMV), *Beet western yellow virus* (BWYV) and *Cucumber mosaic virus* (CMV). Average cumulative percentage of these viruses affecting plants was 21.76%. The results revealed that 11.91%, 8.80% and 1.03% of the tested samples were infected with TuMV, CaMV and mixed infection with CaMV+TuMV, respectively. However, no sample was found infected with TSWV, TYMV, RaMV, BWYV and CMV. These results indicate that TuMV and CaMV were the only most prevalent endemic viral pathogens of *B. oleracea* in the dominantly warm humid continental climate province of Niğde.

Keywords: cabbage CaMV, ELISA, TuMV, viruses

1. Introduction

Cabbage (*Brassica oleracea*), genus *Brassica*, family Brassicaceae is an important vegetable crop cultivated worldwide [1, 2]. It possesses both antioxidant and anticarcinogenic properties and has a significant medical importance [3, 4]. Turkey is the fourth largest vegetable and cabbage producing country. Cabbage is cultivated on 31, 000 ha and annual production was 514,344 tons in Turkey. Niğde located in the Central Anatolia Region is the second largest producer of *B. oleracea* of Turkey, after Samsun province located in the Blacksea Region of the country [5]. There are three economically important cabbage varieties, namely *B. oleracea* var. *capitata subvar. rubra* (red head cabbage), *B. oleracea* var. *capitata subvar. alba* (white head cabbage) and *B. oleracea* var. *acephala* (kale = leaf cabbage), that have different climatic adaptations and are widely distributed in agricultural regions of Turkey [6].

The *B. oleracea* may harbor various viral, fungal, bacterial and phytoplasmal diseases that hampers reduction of quality and yield [7, 9].

Cauliflower mosaic virus (CaMV) and *Turnip mosaic virus* (TuMV) are aphid transmitted viruses infecting *B. oleracea* [11] and reported 25% heads as non-marketable and infected with CaMV [12]. Whereas, in case of early stage infection TuMV can reduce yield up to 50% in *B. oleracea*. TuMV is transmitted by at least 89 different aphid species in a non-persistent manner and is considered as a detrimental pathogen of *B. oleracea* [13]. TuMV infects over 318 plant species in 156 genera of 43 plant families, including many important crops and weed plants and is considered among the most economically important viruses of vegetables worldwide [14]. *Radish mosaic virus* (RaMV) is known to infect almost all cruciferous plants such as mustard, rape, arugula and cabbage. The virus produces symptoms such as necrotic lesions along with mosaic and chlorotic areas on the infected crop [15]. *Beet western yellow virus* (BWYV), *Turnip yellow mosaic virus* (TYMV), *Tomato spotted wilt virus* (TSWV) and *Cucumber mosaic virus* (CMV) are common viruses in horticultural and agricultural brassicas that may lead to complete crop failure [16, 17].

The CaMV and TuMV are reported as potential viruses infecting Brassica crops in the Aegean, Marmara and Eastern Mediterranean regions of Turkey. Despite having the economic importance of *B. oleracea* in Turkey, there is no detailed information about relative abundance, distribution and prevalence of viruses infecting this crop in the either dominantly warm humid continental climate province of Niğde or Turkey as whole [18].

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The detection and identification of disease can help in minimizing the severity of losses by developing strategies of integrated management programs. Reliable identification and detection of viruses is crucial for identification and screening virus resistance varieties in certification programs [19, 20]. The objective of the present study was to investigate the prevalence and distribution of the potential viruses infecting cabbage crop in the Niğde province.

2. Materials and Methods

2.1 Field Survey

The surveys were conducted for collecting samples of *B. oleracea* exhibiting virus like symptoms from the districts of Kaynarca, Sazlıca, Kemerhisar, Bor and Ulukışla of Niğde province during the growing season of years 2014-2015.

2.2 Collection and assessment of virus infected samples

A total of 386 virus suspected samples showing symptoms such as discoloration, mosaic patterns, abnormal growth, vein clearing, necrotic spots, malformation, and chlorosis were collected from *B. oleracea* grown fields. The leaf samples were collected, placed in plastic bags and brought to the laboratory by placing in dry ice box and immediately processed.

The leaf samples were subjected to double-antibody sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) using virus specific polyclonal antibodies for TuMV, CaMV, TYMV, RaMV, CMV (Bioreba AG, Switzerland®) and BWYV (Agdia®) to detect the single and mixed infection of these viruses.

2.2.1 Coating IgG

As a first step of DAS-ELISA tests, 96-well micro titer plate was coated with the provided TuMV, CaMV, TYMV, RaMV and CMV specific IgGs. After diluting it with coating buffer in 1:1000 ratio, diluted antibody (200 µl) was added to per microplate well and incubating at 30±2 °C for 2-6 hours.

2.2.2 Washing

The IgG coated plates were washed 4 times with washing buffer provided within used ELISA kit by using Microplate Washer (Microplate Washer Wellwash™).

2.2.3 Adding plant extract

The preparation of plant samples was done by homogenizing the leaf samples in the provided extraction buffer in the ratio 1:20 (w:v) and a volume of 200 µl with two repetitions was added to each IgG coated microtiter well followed by incubation at 4±2 °C overnight. After incubation, plates were washed as described in washing step above.

2.2.4 Adding conjugate

After plant extract incubation and washing, virus specific IgG conjugated with alkaline phosphatase was diluted in 1:1000 ratio in conjugate buffer, added to well and incubated at 30±2 °C for 5 h in a humid box. The plates were washed as described in washing step above.

2.2.5 Adding substrate

Following conjugate incubation step and washing, substrate application was performed by dissolving 1 mg/ml para-nitro phenyl phosphate (pNPP) in substrate buffer followed by the addition of 200 µl to each microplate well. Substrate incubation was done at room temperature under dark conditions.

The development of yellow color was measured spectrophotometrically at 405 nm using ELISA plate reader (Bio-Rad®). The samples having absorbance values two fold greater than those of negative control were considered as virus positive.

3. Results

Various kinds of symptoms included necrotic, local lesion, leaf discoloration, vein clearing and leaf mosaic were observed on the collected plants samples (Figure 1). However, some virus positive samples did not show any disease symptoms. The results obtained from serological tests revealed that tested samples were infected with TuMV and CaMV and some with mixed infection of CaMV+TuMV, whereas no sample was found infected with TYMV, RaMV, BWYV and CMV (Table 1).

Results of tested samples from different localities revealed that TuMV was the most common virus (11.91%). Maximum percent infestation of samples with TuMV were found in Ovacık followed by Bor, Sazlıca, Kemerhisar and Kaynarca in 26.31%, 25%, 16.34%, 7.14% and 5.75% of the samples respectively. The second most common virus was CaMV (8.80%). Occurrence of CaMV was recorded in Sazlıca (13.46%), Ovacık (10.52%), Kaynarca (8.37%) and Bor (4.45%) respectively. Whereas, no sample was found infected with CaMV in Kemerhisar. Furthermore, four samples (1.03%) found infected with mix infection of TuMV+CaMV, three from Sazlıca (2.88%) and one from Kaynarca (0.52%). Whereas no mixed infected sample was recorded in Kemerhisar, Ovacık and Bor. The results obtained from DAS-ELISA revealed that 84 (21.76%) samples were infected with virus infection.

A total of 302 (78.23%) samples were not infected against the tested viruses with maximum of 92.85% frequency at Kemerhisar, and minimum virus free samples of 63.15% at Ovacık (Table 1).

4. Discussion

The present work provides the first records about the occurrence, prevalence and distribution of TuMV, CaMV and TuMV+CaMV infection in *B. oleracea* cultivated in Niğde province of Turkey. Various kinds of symptoms included necrosis, local lesion, leaf discoloration, vein clearing and leaf mosaic were observed on the infected samples [40]. However, some virus positive samples did not show any disease symptoms. The observed symptoms in infected plants confirmed the prevalence of TuMV and CaMV [21]. TuMV, CaMV, TYMV, CMV and BWYV are among the economically important viruses infecting members of Brassica family worldwide [14, 41]. The serological based results confirmed the presence of TuMV, CaMV, whereas, TYMV, CMV and BWYV were not detected. Furthermore, considerable number of the surveyed samples confirmed the presence of TuMV+CaMV mixed infection as already reported from the Samsun province of Turkey [18]. According to various reports TuMV followed by CaMV are among the potential viruses infecting *B. oleracea* in Turkey and worldwide [16, 18, 22]. CaMV and TuMV are stylet-borne, non-persistent aphid transmitted viruses infecting *B. oleracea* [11]. Furthermore; colonizing aphids have been reported in the cabbage cultivated regions of Turkey [23, 24]. Chemical control is commonly practiced methods to control insect pests but frequent use of insecticides may induce problems such as increasing cost of cultivation by decreasing probability of vegetable farming, resurgence of insecticide resistance and

biomagnification of pesticide residues in food [25, 26]. High level of resistance against the used insecticides has been reported in insect vectors of these viruses [31]. The frequent and unchecked application of insecticides to control pests and diseases is also increasing environmental pollution [27-30]. The use of bioinsecticides such as essential oils could be an effective tool for controlling potential insect vectors of *B. oleracea* [32]. Furthermore, for effective combating of viral infection, there is a need to collect reliable information about the impact of virus infection on vegetable crops. This in turn will help in developing the sustainable and alternative disease management practices [12].

Our results showed a proportion of multiple infections in tested samples, same results also reported about multiple virus infections as common phenomenon in *B. oleracea* [16]. It is also important to keep in mind the possible infection source producing mix infection while designing a disease management plan. High proportion of multiple infections was revealed in weeds as compared to other crops while studying the virus severity [11, 42, 43]. Furthermore, exposure of the infection source to a number of virus vectors carrying different viruses for a long period of time may also help to increase the risk of multiplication of infection. Therefore, such infection source host weeds or plants can accumulate multiple infections that could be potential reservoirs of several viruses infecting *Brassica* crops [33]. The eradication of such host plants and weeds must be included as an integral part of management strategies for controlling virus infection.

The difference in virus infections severity was observed among the surveyed regions. The possible variation in virus infection severity may be due to various factors such as selection of cultivated varieties, availability of inoculum source could be host plants, vectors including insect, pests and environmental conditions (Ali *et al.* 2012). The selection and cultivation of resistant varieties are another essential factor towards combating disease and virus infection. According to various survey reports, no virus was detected in the fields where hybrid *B. oleracea* was cultivated. This can be attributed that such developed cultivars are resistant to biotic and abiotic stresses including disease resistance [11, 6].

Plant viruses are difficult to detect and identify, and pose serious threats to crop yield, quality of plants and plant products around the world [34]. Effective management and control of plant diseases relies on the rapid and accurate identification of casual organism. It would be advantageous for making more informed decisions about crop husbandry and disease epidemics, leading to enhanced plant growth and production. Unlikely to other plant pathogens such as bacteria, fungi etc, no direct methods are available for viral detection and identification rather current control measures depend on indirect tactics for disease management. Hence, methods for detection and identification of viruses are inevitable for disease management [35]. The available biological methodologies for diagnosis, detection and

identification of plant viruses are more resource and time consuming but still important [36]. The most common practiced, cheapest and easiest detection of the viruses in plant material and vegetative propagules became possible by the technique introduced by Clark and Adams i.e. enzyme-linked immunosorbent assay (ELISA) but have some limitation, too [37]. ELISA based tests are designed by targeting coat protein region which represents only a small proportion of the genetic information about the virus [38]. In our studies ELISA test was used due to the reason being cost effective, affordable and widely used to detected plant viruses in *B. oleracea* [18, 44, 45].

Recently, certain advancements in the area of molecular detection technology has led to the development of more effective, convenient, precise and specific assays that allows the utilization of these technologies for detecting and diagnosing plant pathogens, including viruses [39]. These assays will assist the crop agronomists, growers and plant pathologists for an early detection of disease infection without relying solely on symptoms of disease. The techniques such as Dot blot hybridization, Polymerase Chain Reaction (PCR), Reverse transcriptase Polymerase Chain Reaction (RT-PCR), Co-operational Polymerase Chain Reaction (Co-operational PCR), Multiplex Polymerase Chain Reaction (PCR Multiplex), Multiplex Reverse transcriptase Polymerase Chain Reaction (Multiplex RT-PCR), Real-time or quantitative Polymerase Chain Reaction (RTq PCR) and RFLP are effective molecular detection tools to be used in parallel with knowledge of the crop, understanding the biology of the pathogen and the ecology of the disease [39]. Although the use to advance technology enabled the research to get verified and authenticated results, the use of such above described advance technologies can be used for the determination of an optimal time, at which the control measures should be implemented for disease management and also for programs aimed at producing virus-free plant propagation materials. There is a need of time that ELISA and molecular diagnostic tools should be coupled with other techniques such as newly emerged proteomic, a promising tool for providing information about virulence factors and pathogenicity, that will open up new possibilities for crop disease detection, diagnosis and crop protection.

5. Conclusion

In this study TuMV and CaMV were recorded as important virus infecting *B. oleracea* crop in the province of Nigde Turkey. These finding could be helpful for the development of integrated management practices in order to decrease the virus infection severity in this crop.

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Table 1: Occurrence of viruses in cabbage (*Brassica oleracea*) samples collected from the province Nigde, Turkey during 2014-2015.

Districts	Sample tested	Viruses Infectivity								Virus free samples
		TuMV	CaMV	TuMV+CaMV	TSWV	TYMV	RaMV	BWYV	CMV	
Kaynarca	191	11 (5.75%) ^e	16 (8.37%) ^c	1 (0.52%) ^b	0	0	0	0	0	163 (85%) ^b
Sazlıca	104	17 (16.34%) ^c	14 (13.46%) ^a	3 (2.88%) ^a	0	0	0	0	0	70 (67.30%) ^d
Kemerhisar	28	2 (7.14%) ^d	0 (0%) ^e	0	0	0	0	0	0	26 (92.85%) ^a
Ovacık	19	5 (26.31%) ^a	2 (10.52%) ^b	0	0	0	0	0	0	12 (63.15%) ^e
Bor	44	11 (25%) ^b	2 (4.45%) ^d	0	0	0	0	0	0	31 (70.45%) ^c
		46 (11.91%)	34 (8.80%)	4 (1.03%)	0	0	0	0	0	
Total	386	84 (21.76%)								302 (78.23)

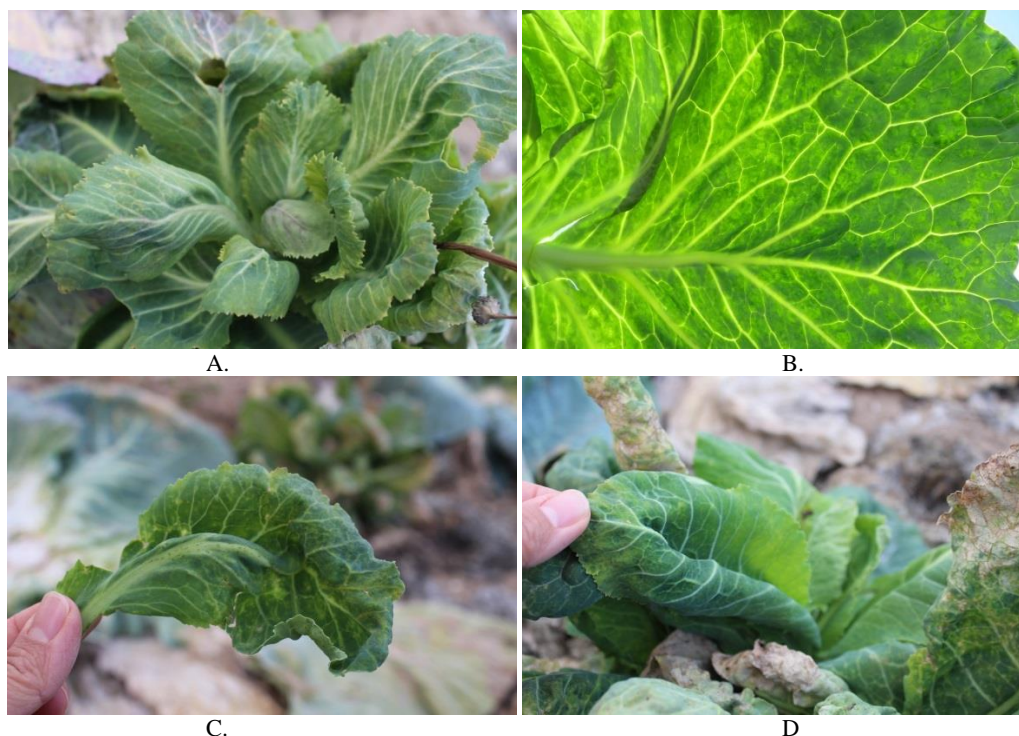


Fig 1: Leaf discoloration, vein clearing and mosaic symptoms on cabbage leaves infected with CaMV (A and B) and TuMV (C and D)

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