Journal of Balkan Science and Technology
journal homepage: www.jbst.nku.edu.tr/

Identification and Incidence of Maize Dwarf Mosaic Virus in Maize Fields in Tekirdağ Province of Trakya

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Research Article

Keywords:
Maize
Virus incidence
ELISA
RT-PCR

Received: 27.08.2024
Accepted: 04.10.2024
Published: 30.12.2024

DOI: 10.55848/jbst.2024.47

ABSTRACT

Maize (*Zea mays* L.) is the third most significant field crop in Tekirdağ province of Trakya, Türkiye, following wheat and sunflower. In addition to seed production, maize has recently been produced as a silage crop for animal husbandry in this region. In June and July 2022, survey studies were performed on eleven districts of Tekirdağ, and a total of 117 symptomatic maize plants were collected to investigate the incidence of *maize dwarf mosaic virus* (MDMV). These maize plants exhibiting streak mosaic, yellowing, chlorotic leaf spot, and leaf deformation symptoms were tested for MDMV using biological, serological, and molecular methods. Serological test results showed that ten out of 117 maize plants were found infected with MDMV. These symptomatic maize plants were inoculated mechanically on seedlings of sweet corn (*Zea mays* saccharata Sturt), and streak mosaic symptoms on the sweet corn after seven, 14-, and 21 days post-inoculation were observed. A total of virus-suspected 30 maize samples were tested by an RT-PCR assay. RT-PCR results revealed that seven maize plants had positive reactions to MDMV. This comprehensive study showed an infection rate of 14.53 % in the maize fields in Tekirdağ province of the Trakya.

1. Introduction

Maize (*Zea mays* L.) is the third crucial cereal species after wheat and rice, and it has been adapted to subtropical climate regions worldwide. Maize is a warm-season cereal species placed into the Poaceae family. It has been classified into seven sub-species depending on its various purposes such as food for humans and farm animals [1]. Maize species originated from Central America and were introduced to North Africa and Türkiye during the Ottoman Empire in the 16th century [2]. Despite being produced as a subtropic cereal species, it can be cultivated in the sunny fields of many countries in between up to 58° North and 40° South latitudes [3]. In field conditions, maize plants develop in four stages, beginning with the seedling and completing with the cob stage. In Tekirdağ, maize was harvested by hand, dried until the reduction of its moisture was under 22 %, and seeds separated from their cobs by hand. Seeds should be dried until moisture reduction is under 13 % for the best storage condition, as reported by Vartanlı and Emeklier [4]. Maize production in most of the fields of Türkiye requires irrigation except in the Eastern Black Sea region of Türkiye. The average annual precipitation rate of 600-1200 mm in the six provinces from Samsun to Artvin, lined up in the Eastern Black Sea Cost of Türkiye, is sufficient to grow maize crops without irrigation. However, seed cultivation and production are exclusively for human feed consumption in the Black Sea region. Depending on the 82 years of precipitation rates between 1940-2022, the average annual precipitation amount measured in Tekirdağ, Edirne, and Kırklareli provinces is just about 580.4 mm [5]. Maize cultivation in terms of seed production needs irrigation in all districts of Tekirdağ. That is why, most of the increasing maize crop cultivation and production in Tekirdağ is to meet the demand for green dry hay or silage for farm animal feeding, as the top maize producer countries are the USA and China. Besides some essential fungal and prokaryotic maize diseases, for the first time, it was a new virus disease, *maize dwarf mosaic virus* (MDMV), that occurred during the 1962 crop production season in the maize fields of Ohio State, USA. Later, at least 12 maize virus diseases were identified and described in the USA by Redinbaugh and Zambrano [6]. Occurrence and the epidemics of those maize virus diseases have been known to be related to the agroecological systems, such as the distances of the initiation of those maize virus epidemics and infestations by their aphid vectors. The high infection rate of MDMV and *sugarcane mosaic virus* (SCMV) in the field conditions, which are transmitted by aphid vectors, was determined by ELISA. The distance of the aphid vector population (*Rhopalosiphum maidis* Fitch.) for both viruses was determined to be about 500 m. However, the mixed infection rate distances of both viruses were measured at 200 m on the perennial weed hosts of MDMV and SCMV. One of the perennial weed species, millet (*Panicum milliaceum* L.), is widespread in Tekirdağ as well as Edirne and Kırklareli provinces all over the Trakya Region. MDMV was identified and reported for the first time as an aggressive plant pathogen in the Ohio state of the USA by Knoke et al. [7]. Kingsland [8] determined that severe epidemics of MDMV may

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have caused a 62 % reduction in maize seed yield. It has also been determined that MDMV and SCMV have usually occurred in maize fields in Germany. In the Çukurova region of Türkiye, Baloğlu et al. [9] identified the MDMV-A strain biologically on indicator plants belonging to ten different families as well as serologically by using MDMV-A polyclonal antisera. Fuchs and Grüntzing [10] determined that SCMV and MDMV infections in maize plants may cause a 16.9 % reduction in plant height and a reduction of 37.1 % in total plant weight. As a result of both virus infections, it was also determined that the maize cob weight reduced by 27.8 %. Achon et al. [11] determined two different isolates of MDMV in tested 33 weed species. For the first time, İlbağı [12] investigated the presence of MDMV in perennial weed hosts in and around the maize fields of the Trakya region of Türkiye and reported the virus diseases as MDMV, SCMV, *barley yellow dwarf virus*-PAV (BYDV-PAV), *cereal yellow dwarf virus* (CYDV-RPV), *johnsongrass mosaic virus* JGMV), *wheat dwarf virus* (WDV) as well as the mix of infections of MDMV, BYDV-PAV, CYDV-RPV, and SCMV. Similarly, Erkan and Kutluk Yılmaz [13] determined that MDMV and wheat spindle streak mosaic virus (WSSMV) by serological tests in and around the wheat fields in Samsun province. Additionally, Ilbağı and Geyik [14] identified MDMV infections at the rate of 30 % individually and 50 % of mixed infections of MDMV+SCMV in the maize fields in Bursa province of Marmara region. Guadie et al. [15] identified eight different viruses and their individual and mixed infections in maize fields in Ethiopia. They reported that the most destructive virus in the maize fields was maize lethal necrosis disease caused by MDMV and SCMV coinfections. Clemente-Orta et al. [16] investigated the agroecological factors and the cobbing design of corn seed production in the presence of both MDMV and SCMV virus infections as well as the virus incidence in maize and alternative grasses. The researchers reported that early planting, the management of edges, and the presence of non-crop habitats are key factors.

Here, we employed biological, serological, and molecular methods to determine the presence and incidence of MDMV in symptomatic maize samples collected from Tekirdağ province by comprehensive survey studies, including eleven districts. Our findings indicate that the MDMV infection rate has been 14.53 % in Tekirdağ maize fields.

2. Material and Method

2.1. Plant sampling

Survey studies were performed in eleven districts, including Süleymanpaşa, Süleymanpaşa Şarköy, Malkara, Çorlu, Çerkezköy, Kapaklı, Muratlı, Marmara Ereğlisi, Saray, Hayrabolu, and Ergene in Tekirdağ province in June and July of 2022. Symptomatic leaf samples exhibiting streak mosaic, yellowing, vein discoloration, chlorotic leaf spot, and dwarfing symptoms were collected as study materials from the maize fields (Fig. 1). During the surveys, symptomatic plants were primarily observed in neglected fields, mixed sowing fields of maize and vegetables, and individual fields such as home gardens.

2.2. Biological assay

A set of indicator test plants, including sweet corn (*Zea mays* L.) and *Nicotiana benthamiana* L., were used for mechanical transmission of the maize samples infected with MDMV. Three replications of each indicator test plant, including five plants and healthy control, were grown into 500 ml pots filled with a sterilized mixture of soil, sand, and compost (1:1:1) and maintained in a growth chamber at 23 °C with a 16 h light/8 h dark photoperiod cycle. Symptomatic maize leaf samples were ground 1:10 (w/v) using mortar and pestle in 0.1 M phosphate buffer (pH:7.2) and were used for inoculating indicator plants. The inoculated plants were observed regularly for local or systemic symptom development over a period of 1-3 weeks post-inoculation.

Fig. 1 Streak mosaic, yellowing, dwarfing, and leaf deformation symptoms in maize fields.

2.3. Serological assay

A total of 117 symptomatic leaf samples (~150 mg/sample) were tested with polyclonal antibodies (manufactured by Bioreba, Switzerland) for the presence of MDMV using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark & Adams [17].

2.4. RT-PCR assay

Total RNA was extracted from symptomatic maize leaf samples using a Qiagen RNeasy plant mini kit (Hilden, Germany). Treatment with DNase I and synthesis of first-strand cDNA with 500 ng RNA and random hexamers were done using a Maxima first-strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, USA). PCR was carried out with specific primer pairs designed by İlbağı et al. [18]. Each of the 20 µL reaction mixtures included 0.5 µM forward primer, 0.5 µM reverse primer, 10 µl DreamTaq DNA polymerase Mastermix, 2 µl cDNA (100 ng/µl), and 7 µl DEPC-treated water. The thermocycling conditions were as follows: initial denaturation step of 95 °C for 3 min; 35 cycles of 98 °C for 10 s, 59 °C for 30 s, 72 °C for 3 min; and a final extension step of 72 °C for 5 min. After conducting RT-PCR, the amplification products were visualized by gel electrophoresis using 1.5 % agarose gels under a UV light system (Marne La Vallee, France).

3. Results and Discussion

Survey studies of Tekirdağ maize fields performed from the beginning of June to the end of July 2022 revealed that sporadic and uneven maize seed germination, seedling deaths, and dwarfing were the common visible symptoms observational in the fields. Mature maize plants in July usually exhibited short in height, dwarf, and small in size. *Maize dwarf mosaic virus* (MDMV) infected maize plants, and their leaves displayed streak mosaic symptoms and leaf deformations. Some infected plant leaves showed yellow and reddish stripes and drying leaves from tips to stems as similar symptoms that occurrence caused by MDMV epidemics as reported by Knoke et al. [7] in the State of Ohio, which is located in the Corn Belt of the USA, between the Northern parallels of 38°27' and 41°58' at Northern Hemisphere of the World. Meanwhile, the Trakya region and Tekirdağ province are also located in almost the same parallels, between 40°36′ and 41°31′. MDMV is one of the most widespread virus diseases that is transmitted mechanically, by seeds and by aphid vectors. The virus causes characteristic viral symptoms in maize plants, such as streak mosaic, dwarfing, and leaf deformations, as reported by Agrios [19]. Our results revealed similar and identical symptoms in the fields, such as streak mosaic, yellowing, dwarfing, chlorotic leaf spot, and leaf deformation (Fig.1). Also, mechanical transmission experiments showed typical streak mosaic symptoms on the indicator plants, as shown in Fig. 2; however, no visible symptoms were observed on *Nicotiana benthamiana* L. indicator plant. These results disclosed that MDMV might readily transmit mechanically. Similarly, Baloğlu et al. [9] reported the host range of MDMV being transmitted by mechanical inoculation experiments on young seedlings of various weed species in eight families. They pointed out susceptible weed species to MDMV in the Poaceae family. Previously, Rao et al. [20] identified MDMV and *sugarcane mosaic virus* (SCMV) in maize, sugarcane (*Saccharum officinarum* L.), and sorghum (*Sorghum bicolor* L.) fields in India, causing widespread infections in these crops. Later on, Williams [21] indicated that the sowing date of maize seed is an important factor for the control strategy of the virus. In this regard, maximum plant height is longer when seed sowing is made in July than in April. However, the total leaf mass did not change with early sowing in April, as Fuchs and Grüntzig [10] reported the synergistic effects of MDMV and SCMV on maize plants caused by high yield losses. They stated that those coinfections reduced plant length by at least 16.9 %, total plant weight by 37.1 %, and total cob weight by 27.8 %. On the other hand, Clementa-Orta et al. [16] cited that MDMV-infected plant density was affected by the sowing date of maize. Also, Achon [11] pointed out the importance of early sowing and identified the high incidence rate in perennial weed species such as *Sorghum halepense* and *Cynadon* spp. and in annual weed *Steria verticillata* (L) P.B. Similarly, İlbağı [22] reported that MDMV and other four cereal viruses were identified in *Phragmites communis* Trin., which is a widespread perennial grass in the wetlands. However, even if our study does not determine the host range of MDMV in weed species, here we report the infection rate of MDMV as 15.39 % by ELISA and RT-PCR test results, as shown in Table 1. Likewise, İlbağı et al. [12] reported that the rates of symptomatic plants were estimated at 3.7 to 63.6 %, depending on locations in the Trakya region. Tokgöz and Kutluk Yılmaz [13] determined the presence of MDMV in maize fields in Samsun and identified MDMV in 4.8 % of the collected 290 symptomatic maize plants. Nevertheless, 111 leaf samples collected from maize fields grown for silage had only 0.9 %. Additionally, our results showed that ten out of 117 maize samples were found to be infected with MDMV in accordance with the DAS-ELISA test. İlbağı and Geyik [14] identified MDMV in 15 out of 50 symptomatic maize samples collected from Bursa province, and mixed infections of MDMV and SCMV were in 10 out of 50 maize samples. In contrast to our results, these results pointed out a higher disease incidence than in Tekirdağ province in terms of the presence of MDMV. Additionally, our results showed that eleven out of 117 maize samples had positive reactions to MDMV by the RT-PCR test (Fig. 3). According to this, five maize samples from the Süleymanpaşa district and two maize samples from the Şarköy and Malkara districts in Tekirdağ were infected with MDMV. In parallel to our results, İlbağı et al. [12] reported the presence and incidence of MDMV and SCMV in maize fields of the Trakya region firstly by DAS-ELISA. The presence of MDMV was confirmed by Western blot analysis and IC-RT-PCR. SCMV was also identified by IC-RT-PCR. As a result of this study, SCMV and JGMV were reported as the first record in Türkiye. Guadie et al. [15] indicated mixed infections of MDMV and SCMV in maize-producing African countries like South Africa and Ethiopia, which caused maize lethal necrosis virus disease; thus, because of this disease, the maize yield and quality were considerably reduced. Maize lethal necrosis disease in Trakya and Türkiye has not been investigated so far. However, since both virus diseases are present in Trakya and Türkiye, the MLND should be investigated in terms of its presence in the maize fields in

Türkiye. In conclusion, virus infections cause considerable yield and quality losses in maize

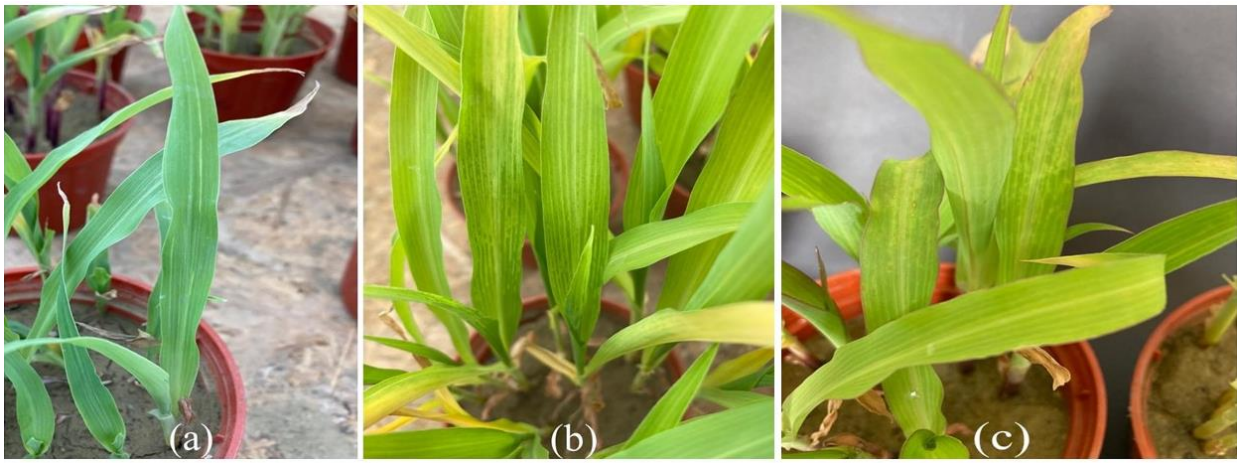

Fig 2. Streak mosaic symptoms on sweet corn after seven (a), 14- (b), and 21 days (c) post inoculation.

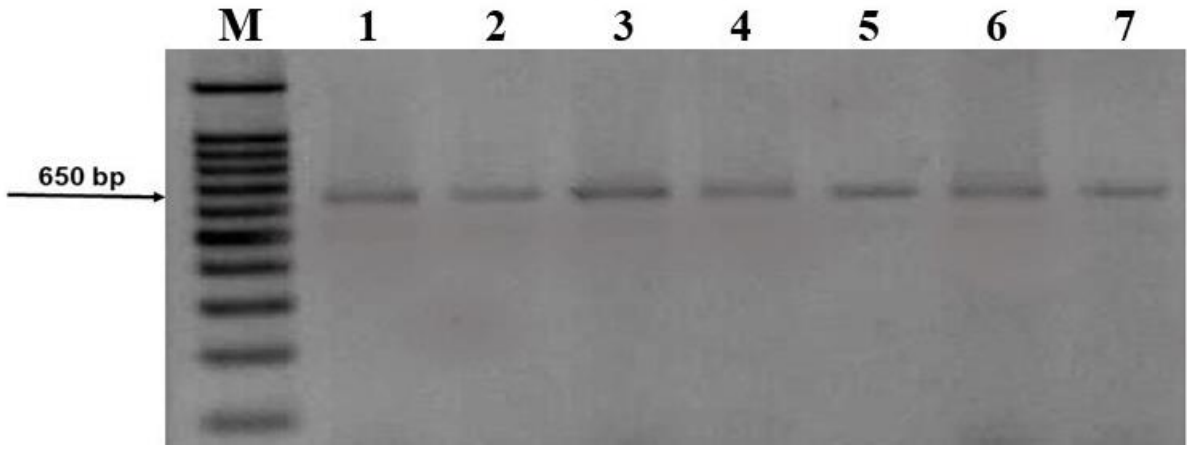

Fig 3. RT-PCR detection of MDMV in maize samples. Lanes 1-7 infected maize samples; M:100 bp molecular weight marker.

Table 1. Distribution of virus-infected maize plants according to ELISA and RT_PCR results.

Districts	Sample number	DAS-ELISA	RT-PCR	Total infection
Süleymanpaşa	66	5	5	10
Şarköy	11	4	1	5
Malkara	17	1	1	2
Çorlu	1	-	-	-
Çerkezköy	3	-	-	-
Kapaklı	3	-	-	-
Muratlı	4	-	-	-
Marmara Ereğlisi	3	-	-	-
Saray	2	-	-	-
Hayrabolu	3	-	-	-
Ergene	4	-	-	-
Total	117	10	7	17

and other cultivated crops. Effective strategies are necessary to manage viral infections in agriculture.

4. Conclusion

Maize dwarf mosaic virus (MDMV) is one of the most important viral pathogens in maize fields worldwide. MDMV is transmitted by aphid vectors, mechanically, and by seeds. At least 15 aphid species can non-persistently transmit MDMV. Its host range is maize, sorghum, and Johnsongrass, as well as alternative hosts such as weed species, which play an important role in virus epidemiology. In this study, we employed biological, serological, and molecular methods in order to determine the incidence of MDMV in the maize fields in Tekirdağ province of Trakya. Mechanical transmission experiments showed typical streak mosaic symptoms on sweet corn indicator plants. DAS-ELISA results revealed that ten out of 117 samples were infected with MDMV. A total of virus-suspected 30 maize samples were tested using an RT-PCR assay, and seven maize plants were found infected with MDMV. This study showed an infection rate of 14.53 % in the maize fields in Tekirdağ province of the Trakya. Declaration

Author Contribution: Conceive– N. Çetin, H. İlbağı; Design– N. Çetin, H. İlbağı; Experimental Performance, Data Collection and Processing– N. Çetin; Analysis and Interpretation– H. İlbağı; Literature Review– N. Çetin, H. İlbağı; Writer– H. İlbağı; Critical Review– H. İlbağı.

Acknowledgment

This study was conducted in the molecular phytopathology and virology laboratory at the Department of Plant Protection of the Faculty of Agriculture at Tekirdağ Namık Kemal University. The first author would like to thank Prof. Dr. Ahmet ÇITIR and Dr. Lerzan ÖZTÜRK for their assistance during the master thesis study.

Conflict of interests: The author(s) have declared no conflict of interest.

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