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MOLECULAR IDENTIFICATION OF MDMV AND ITS EFFECTS ON PHYSIOLOGICAL PROPERTIES OF *ZEA MAYS* L.

Z.SH. SOBIROVA*, K.A. MUTALOV, A.A. TEMIROV, N.I. SHONAZAROVA, G.U. SUYUNOVA, N.B. FAYZIEVA, and N.R. BERDIKULOVA

Department of Natural Sciences, Chirchik State Pedagogical University, Chirchik, Uzbekistan
*Corresponding author's email: z.sobirova@cspi.uz
Email addresses of co-authors: mutalovkarimjan@gmail.com, guljahon_suyunova@mail.ru, nigoraberdiqulova811@gmail.com, nasiba.shonazarova0304@mail.ru, nazirafayziyeva886@gmail.com

SUMMARY

The molecular identification of maize dwarf mosaic virus (MDMV) and its effects on the morphophysiological traits of maize (*Zea mays* L.) was the focus of the presented research, intending to study the activity dynamics of two main types of enzymes, peroxidases, weakly binding to the membrane, and soluble peroxidases. The molecular identification of the virus engaged the use of the RT-PCR method. Results revealed that in the climatic conditions of Uzbekistan, red necrotic spotting, yellow mosaic with a large border on the edge of the leaf, curling of the leaves, yellow striped mosaic, and short stature all appeared in the maize plants. According to previous symptoms, the visual diagnostic methods used determine the maize yellow mosaic virus indications. In the existing study, the gene responsible for the protein coat synthesis (SR) nucleotide sequence served to diagnose the MDMV, and as a result, PCR tests showed yellow streaks on the leaves of maize plants. The mosaic and motility symptoms have been characteristic proofs of MDMV. The MDMV infects the maize plants in the initial growth phase (3–5 leaves), then the symptoms appear after a few days. Through morphological indicators, viral disease identification is possible at subsequent stages (6–7 leaves). Using spectrophotometry, the peroxidase enzyme activity in maize plants receives the virus infection to determine an early level of infectivity. The results confirmed that, in infected maize plants, peroxidase associated with the cell membrane was much more active than in control plants. It proves that contaminated *Zea mays* plants were in a stressful situation due to the virus. The RT-PCR method, widely used in diagnostics, sought to identify the virus species affiliation. PCR proceeded based on the virus coat protein (CP) gene.

Keywords: Maize (*Zea mays* L.), maize dwarf mosaic virus (MDMV), distribution, peroxidase, ascorbate peroxidase (APX), guaiacol peroxidase (GPX), thylakoid

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Key Findings: Given the participation of the peroxidase enzyme in the protection mechanism of the maize plants from MDVM disease, its consideration for use succeeded on local maize cultivars with high peroxidase activity, such as, Sherzod, and acclimatized cultivars, i.e., Extra Early Dightau-209 and Hickax, for the selection of virus-resistant cultivars.

INTRODUCTION

Maize (*Zea mays* L.) is a valuable cereal crop and ranks third after wheat and rice worldwide. Viral diseases are one of the foremost factors negatively affecting corn growth and development and, eventually, grain yield. Therefore, the said research has greater importance in providing significant data, which necessitates considering the seriousness of the problem.

MDMV's first discovery was in 1963 in Iowa, USA, and shortly after, it proved to be a new virus called maize dwarf mosaic virus (MDMV) (Seifers and Hackerott, 1987). Later, it validated that strain B (MDMV-B) is not MDMV. Based on reactions in N-20 and other maize inbred lines, Knoke and Louie differentiated four more strains of MDMV, naming these C, D, E, and F (MDMV-C, -D, -E, and -F). McDaniel and Gordon also described a new strain of MDMV and named it O (MDMV-O) based on its infectivity to all. However, like MDMV-B, later evidence showed MDMV-KSl and MDMV-O were MDMV (McDaniel and Gordon, 1985; Kong and Steinbiss, 1998; Sobirova *et al*., 2020).

To date, more than 50 maize viruses have been well-defined. The most common is the corn dwarf mosaic virus - maize dwarf mosaic potyvirus (Sobirova *et al.*, 2020; Lukuyu *et al*., 2022). This virus has several strains that differ in minor biological features. The MDMV disease exists in all continents; therefore, its study has been sufficient. The dwarf mosaic virus, like other phytopathogenic viruses, penetrates the plant cell through the bite of carriers and actively begins its replication. Virus-infected cells become stressed due to virus influence, thus affecting the physiological processes, i.e., metabolism, membrane transport function, and enzyme activities (Ludmerszki *et al*., 2015). In Uzbekistan, its first occurrence was in 1980, prompting the study of the biological and ecological characteristics of the virus (Davranov, 1984; Sobirova *et al*., 2020). In some regions, scientists have been studying the molecular genetic characterization of this virus (Achon *et al*., 2007; Gell, 2011; Achon *et al*., 2012).

In living cells, peroxidase enzymes are keys to maintaining molecules in a reduced state, which is the basic requirement for the standard existence of living organisms. Peroxidases are among the inducible enzymes, under the influence of a wide range of effects, either the set of their isoforms altered or the activity of already present molecular forms increases (Yermakov *et al*., 1987; Andreeva, 1988; Fryer *et al.*, 1998).

Past findings enunciated that variations occur in the activity of peroxidase enzymes in plants affected by pathogenic microorganisms. The host plant cells' interaction with the pathogen incorporates the whole chain of reactions in the plant tissues. The enhancement in the oxidative metabolism and peroxidase activity results in the action of only one of the spot in a complex chain of processes, and such studies are of greater interest for identifying plant resistance to viruses (Ludmerszki *et al.*, 2015; Kingston-Smith and Foyer, 2000; Sobirova *et al.,* 2020).

Since MDMV destroys the thylakoid, any damage to thylakoid membranes causes the development of highly reactive oxygen species (ROS) (Fryer *et al*., 1998). Compared with other plant species, maize vascular bundle sheath cells are uniquely sensitive to oxidative stress (Ludmerszki *et al.*, 2015; Sobirova *et al*., 2020). Such issues have solutions from the presence of antioxidant enzymes, primarily ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in plant cells, which protect the cell by direct removal of these reactive molecules (Andreeva, 1988).

In studying the effects of viruses on the plant photosynthetic apparatus, it was evident that chlorophyll a and b and carotenoid

contents decreased by 50%, 40%, and 30%, respectively (Fayziev *et al.*, 2020). This outcome reduced photosynthesis, causing a decrease in vital physiological processes for the plant. The resistance level of the crop plants to phytopathogenic viruses depends on their genetic makeup, with the host cultivars' resistance to various stress factors, including drought, salinity, and diseases, is one of the means to solve the problem (Baboev *et al*., 2017; Amanov *et al*., 2022; Buronov and Xamroev*,* 2022; Qulmamatova *et al.,* 2022; Buronov *et al.,* 2023; Muminov *et al*., 2023). Past studies revealed that different corn cultivars differ in terms of resistance to viral diseases (Sobirova *et al*., 2020).

Viral diseases cause disorder symptoms of different intensities depending on the type of host plant, including patchy mosaic (Fayziev *et al*., 2020, 2023), linear mosaic along leaf veins (Sobirova *et al*., 2020), and ring spots (Sattorov *et al*., 2020) affecting negative impacts on various physiological processes of crop plants, requiring further research in this direction. Additionally, the physiological processes in plants have definite influences from the soil-climate conditions, the composition of the soil, and geographical zoning (Ramazonov, 2020; Ramazonov *et al*., 2020). The purpose of this study was the molecular identification of MDMV and its effects on the physiological properties of *Zea mays* L.

MATERIALS AND METHODS

In 2018–2022, the research used genotypes, i.e., Extra Early Dightau-209 (USA), San Pedro LATA (Argentina), Sherzod (Uzbekistan), Hickax (USA), Osnova 209, 205-2 (Uzbekistan), and San Pedro2IMTA (Argentina) from the *Zea mays* collection available at the Institute of Plant Science and Plant Genetic Resources of Uzbekistan and the breeding material collected by the Molecular Biology and Bioinformatics, Chirchik State Pedagogical University, Chirchik, Uzbekistan. The study used primers for molecular identification of MDMV, as shown in Table 1. The virus isolation came from the variety Sherzod.

Given that the object under study has an RNA genome, it warrants the RT-PCR's use. This type of PCR has its advantages in determining the nucleotide sequence in the RNA of genomic viruses. Nucleic acids isolated utilized the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scientific). Using the ITS region as a molecular genetic marker is advantageous, with the marker characterized by relatively high conservatism, good knowledge, and numerous nucleotide sequences in open databases for the studied and related species (Fayziev *et al*., 2023; Makhmudov *et al*., 2023).

Table 1. Primers used for the identification of corn viruses.

No.	Viruses	Name of primers	Nucleotide sequence	Number of homologous isolates
1	Sorghum mosaic virus (SrMV)	SrMV (76)-F	GGNAAYAAYAGYGGNCARCC	54
		SrMV (76)-R	GTNTGYGTNGAYGAYTTYAAYAA	
2	Johnsongrass mosaic virus (JGMV)	JGMV (76)-F	GTTTTCCCAGTCACGACTTT	24
		JGMV (76)-R	GTTTTCCCAGTCACGAC	
3	Sugar cane mosaic virus (SCMV)	SCMV (76)-F	TGGTHTGGTGYATHGARAAYGG	43
		SCMV (76)-R	TGCTGCKGCYTTCATYTG	
4	Maize dwarf mosaic virus (MDMV)	MDMV (76)-F	CAACCAGGGCYGAATTTGATAG	76
		MDMV K (76)-R	GTGCAAGGCTRAAGTCGGTTA	
5	Maize dwarf mosaic virus (MDMV)	MDMV (63)-F	AGCGAAGGAARCYGAGRC	63
		MDMV (63)-R	YAGTGYCCYTGCYGAACT	
6	Maize dwarf mosaic virus (MDMV)	MDMV (14)-F	TGTTGATGCGGGACAGAA	14
		MDMV (14)-R	AAACCTCACCACAATAGCTT	
7	Maize dwarf mosaic virus (MDMV)	MDMV (49)-F	AYRCTGGTGCWAARGTTTC	49
		MDMV (49)-R	AACCTCACCMCAMTAGCTT	

The analysis of bioinformatic data, the calculation of pairwise molecular distances, and the design of the test system began using the MEGA 6 program (Sattorov *et al*., 2020). Nucleotide sequences came from GenBank databases (*http*://*www*.*ncbi*.*nlm*.*nih*.*gov*/*genbank*). Species-specific primers' selection involved the NCBI (National Center Biotechnological Information, USA) bioinformatic database. Analysis of oligonucleotide sequences performed in GenBank used the BLAST program, with alignment of nucleotide sequences running the Clustal W algorithm and primer parameters' manual selection. It resulted in the development of the design of primers (Table 1).

PCR proceeded in a TOUCH.T 960 amplifier. Using two methods detected the PCR product: "by the endpoint" (electrophoretic) and "in real time" (using intercalating dyes). Gel electrophoresis comprised a 1% agarose gel, with ethidium bromide as an intercalator at 110 V for 80 min. Gel documentation used the BioDocAnalyze system (Biometra). In calculating the optimal primer annealing temperature, the gradient PCR method is as follows:

1. 94 °C – 2 min
2. 94 °C – 50 s
3. Gradient 54 °C – 30 s
4. 72°C – 45 s

Then, repeating the steps 2 to 4 for 45 times. Finally,

5. 72 °C – 5 min

In this research, the conducted experiments determined the dynamics of the activity of enzymes that are weakly bound to the membrane and dissolved peroxidases in the leaves of corn cultivars that differ in resistance to MDMV. The study uniquely selected the genotypes recently infected with the virus and control. Under laboratory conditions, peroxidase activity's determination employed spectrophotometry. Enzyme activity assessment in the tissues and organs of the leaves of various corn cultivars ensued, comparing the infected ones with control plants.

The dynamics of the activity of enzymes weakly in association with the membrane and dissolved peroxidases in the samples acquired exposure by Boyarkin's method (Andreeva, 1988). It determines the rate of the benzidine oxidation reaction under the action of an enzyme contained in tissues over a specified time. For the experiment, selected samples came from the experimental field of seven maize cultivars recently infected with the virus.

Statistical analysis

Statistical description of the experiments advanced according to the methods proposed by Ivannikov and Tomilov (Ivannikov and Tomilov, 2000). A significance level of 1% ($P \leq 0.01$) was acceptable for all the data. Running computer programs, Snedecor, Biostat, and Microsoft Excel 2010 helped with the data analysis and compilation.

RESULTS AND DISCUSSION

The experiment had three repetitions with different time frames after infection with a plant virus and the peroxidase activity with formulas, data, and methods, compared with the monitoring of average performance (Table 2). The comparative results of peroxidase activity of control and infected maize samples are available in Table 2. The intensity of infection determined by the enzyme activity showed that the higher the enzyme content, the stronger the resistance of the cell. In the maize cultivar Extra Early Dightau-209, the control peroxidases had weak bonding to the cell membrane at 2.1, and those infected with the virus at 10.8, almost five times higher than the control plants.

In the maize cultivar Sherzod, the dynamics of activity were, for control, 3.6, and for infected, 23.6, which was 6.5 times more than control samples. In the corn cultivar Hickax, the check was 5.9, and the infected was 11.1, almost twice more. In cultivar 205-2, the dynamics of enzyme activity was 2.3 for the control and 3.9 for the infected, with the difference at 1.69. These results prove that

Table 2. Dynamics of activities of peroxidase of *Zea mays* cultivars artificially infected with the virus.

No.	Corn cultivars	Dynamics of peroxidase activity (U/mg)			
		Peroxidases are weakly associated with cell membrane		Soluble peroxidases	
		Control	Infected	Control	infected
1	Osnova 209	1,8±0,011	4,1±0,01	1,5±0,02	3,6±0,013
2	Extra Early Dightau-209	2,1±0,02	10,8±0,02	1,8±0,012	9,6±0,04
3	San Pedro LATA	1,7±0,012	4,9±0,05	1,3±0,011	4,4±0,05
4	Sherzod	3,6±0,015	23,6±0,19	1,8±0,011	17,1±0,07
5	Hickax	5,9±0,02	11,1±0,01	1,9±0,012	6,8±0,012
6	205-2	2,3±0,02	3,9±0,02	1,4±0,02	3,5±0,014
7	San Pedro2IMTA	2,1±0,016	4,7±0,02	1,4±0,01	4,5±0,02

a. b.

Figure 1. a) Mosaic along the edges of the leaves, and b) yellow stripes along the veins of corn leaves.

different cultivars have different indications of enzyme activity based on their resistance to viral infections. For example, in varieties Sherzod, Hickax, and Extra Early Dightau-209, the enzyme activity germinates five to 6.5 times. The action of soluble peroxidase also provided similar results. Thus, according to experiment results, it is clear that out of seven cultivars of sweet *Zea mays*, cultivars Sherzod, Hickax, and Extra Early Dightau-209 are more resistant to stress, and these cultivars can be quickly detectable with viral infections.

In most of the reported cases, the interaction between viruses and host plants negatively affects the morphology and physiology of the host, leading to disease. With few exceptions, acute viruses have a parasitic relationship with the host and cause adverse effects, such as, leaf distortion and discoloration, fruit malformations, stunted plant growth, and low fruit yield (Hill and Benner, 1976).

The average peroxidase activity associated with membrane control was -2.78, with infected samples was -9.1; the movement of dissolved peroxidase was -3.3, while in the virus-infected samples, the value was -7.64. Based on the experiments carried out to identify viral diseases in the corn fields of the Tashkent region, the presence of a striped mosaic, red necrosis along the veins of the leaf tissue, yellow mosaic along the veins of the leaf, and symptoms of plant dwarfism were also prominent (Figure 1).

From the disease symptoms, determining the biologically identified viral particles in the collected samples further established the terms of the contagiousness of diseases. The exact infected samples served as the starting material for advanced virological studies. More research depended on validating the species of the virus and viral identification. The virus was characteristic of a complex of morphological and biochemical features.

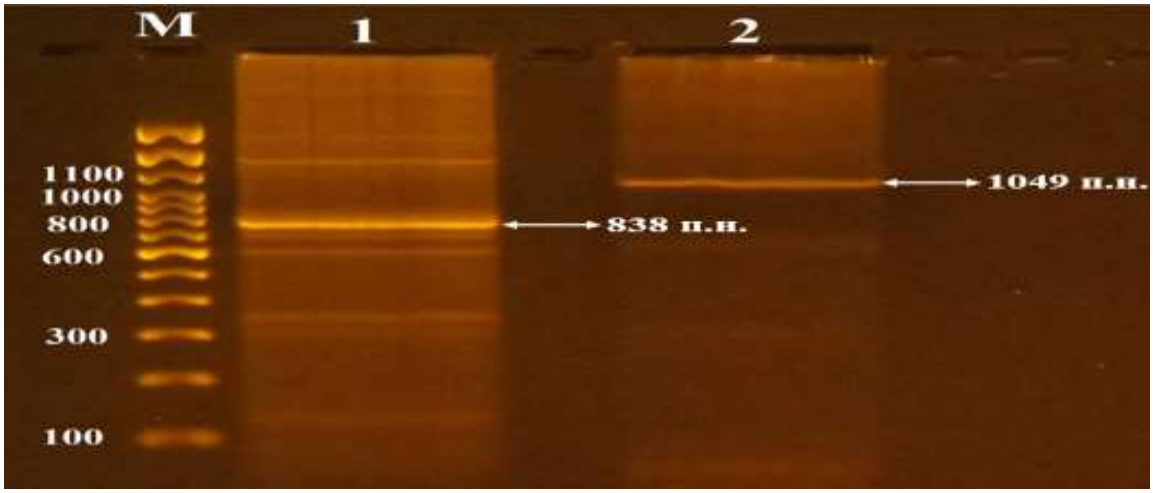

Figure 2. Photograph of the gel electrophoresis of PCR products.
Electrophoresis was performed on a 2% agarose gel. M1 - O'GeneRuler 1 kb DNA ladder (Fermentas); M2 - 100 bp DNA ladder Plus. 1-7 - samples taken from corn plants with various symptoms of the disease, 1, 2 - a sample isolated from a plant - with a yellow striped mosaic along the edges of the leaf blade with a striped yellow mosaic along the leaf veins and a symptom of dwarfing primer for: MDMVF/MDMVR.

Changes in physiological processes during plant stress proved the presence of a virus, making these methods also diagnostics; however, accurate and fast diagnosis can progress using molecular genetic techniques.

Plant growth incurs influences from genetic and environmental components and refers to a quantitative and irreversible increase in dry matter or volume (Hill *et al.*, 1973). Plant growth slows down, causing dwarfism due to the decrease in the photosynthetic area of plants. Usually, plant growth acquires sufficient nutrients. In addition to this, growth regulation involves a hormone. When nutrients are insufficient in the growth cone and the roots, plant growth slows down (Mikel *et al.*, 1981; Abdenaceur *et al.*, 2022).

The proposed method for diagnosing phytopathogens resulted from the polymerase chain reaction (PCR) (Sattorov *et al*., 2020; Lukuyu *et al*., 2022; Makhmudov *et al*., 2023), which has several advantages for diagnostics, as it allows in determining the presence of the pathogen by trace amounts in a relatively short time. The PCR is a way to quickly obtain multiple copies of a specific DNA sequence of bacteria, fungi, and viruses, which might be sufficient for identification by other methods (Sattorov *et al*., 2020; Makhmudov *et al*., 2023).

The indicated method hinged on multiple selective cloning of a specific section of DNA nucleic acid using enzymes under artificial conditions (in vitro). In this case, only the sector that satisfies the specified settings gets copied, and only if present in the test sample (Sattorov *et al.,* 2020). Accounting for these circumstances, recognizing the symptoms detected from different maize fields of the Tashkent region used the PCR method (Figure 2).

As a result of the PCR analysis, it was clear that the maize samples isolated from a plant with a yellow mosaic along the leaf veins and dwarfism with signs of corn is a dwarf corn virus, as confirmed by the PCR diagnostics. Among the primers prepared for diagnostics, the MDMV (63)-F/R primer showed an accurate result. Thus, based on the experiments, plants with yellow mosaic banding along the veins and signs of maize dwarfism were molecularly distinct as maize dwarf mosaic virus and transferred to the "Collection of Unique Objects of Phytopathogenic and Other Microorganisms" at the Institute of Genetics, Academy of Sciences, Uzbekistan.

CONCLUSIONS

Based on the experiments, it was evident that the type of peroxidase weakly bound to the cell membrane increased the enzyme activity by 3.33 times (9.01 ed/mg) compared with the control (2.7 ed/mg) in corn cultivars. The soluble form of peroxidase increased 4.7 times (7.07 ed/mg) in infected maize plants versus the control (1.5 ed/mg), with the activation of this type of enzyme to provide the plant defense mechanism in the initial pathological process can be explicable, with these processes can help in the future to identify plant defense mechanisms against pathogens. Additionally, in the pathological processes, the specific aspects of the plants ensured consideration, including the activity of the type of peroxidase in weak bonding to the cell wall in the maize cultivar Sherzod was 6.5 once (23.6 ± 0.19) compared with the control (3.6 ± 0.015); the soluble form of peroxidase increased by 9.5 once (17.1 ± 0.07) as against the check (1.8 ± 0.011). The red necrotic spots on the corn leaves, mosaic with a yellow border on the edge of the leaf blade, and the symptoms of linear mosaic along the veins of the leaves and dwarf maize plants, according to the PCR method, are typical of MDMV.

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