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DIVERSITY OF VIRUSES INFECTING *ZEa MAYs* L. IN UKRAINE

Background. *Maize (Zea mays L.)* is an annual plant of the *Poaceae* family, one of the world's most important cereal crops, which is the basis for food supply in many countries. Viral diseases of maize are one of the reasons for its yield losses and grain quality reduction. The aim of the work was to identify and determine the spread of maize viruses in different regions of Ukraine in 2021–2023.

Methods. Double or tripple antibody sandwich (DAS or TAS) enzyme-linked immunosorbent assay (ELISA) was used for detection of 11 viruses including maize dwarf mosaic virus (MDMV), High Plains wheat mosaic virus (HPWMoV), sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV), maize streak virus (MSV), maize mosaic virus (MMV), maize white line mosaic virus (MWLMV), wheat streak mosaic virus (WSMV), barley stripe mosaic virus (BSMV), barley yellow dwarf virus-PAV (BYDV-PAV), and brome mosaic virus (BMV). For ELISA, commercial test systems were used (Loewe Biochemica (Germany) and Agdia (USA)).

Results. ELISA confirmed the presence of SCMV in agrocenoses in Kyiv region (35 % of symptomatic plants) and BYDV-PAV in maize samples collected in Vinnytsia (4.3 %) and Kyiv (8 %) regions. Overall occurrence of SCMV and BYDV-PAV in tested symptomatic maize plants was 197 % and 6 %, correspondingly. It should be noted that the plants infected with SCMV had different symptoms in the form of a mottle or stripe mosaic. Although maize plants sampled in the Kharkiv region showed clear symptoms of virus infection, none of the tested viruses were identified.

Conclusions. The circulation of SCMV and BYDV-PAV in maize plants was confirmed in Ukrainian agrocenoses in 2021–2023, and this is the first report of BYDV-PAV in maize in Ukraine. The necessity of expanding the list of viruses under investigation on *Zea mays* in Ukraine is shown.

Key words: *maize, virus, detection, enzyme-linked immunosorbent assay.*

Background

Maize (*Zea mays* L.) is an annual plant of the *Poaceae* family, one of the world's most important cereal crops, which is the basis for food supply in many countries. Maize is the second most widely grown crop in the world (FAOSTAT, 2013). Ukraine is the leader in maize production and ranks sixth in the world, being outcompeted only by the US, China, Brazil, the EU, and Argentina. In 2018–2022, Ukraine produced about 34 million tons of maize per year. The maize acreage in Ukraine is expected to increase further. Viral diseases of maize are one of the reasons for the yield losses and grain

quality reduction. According to literature, maize is a host plant for viruses from the families *Bromoviridae*, *Fimoviridae*, *Geminiviridae*, *Potyviridae*, *Reoviridae*, *Rhabdoviridae*, *Secoviridae*, *Solemoviridae*, *Tombusviridae*, *Tymoviridae* and *Virgaviridae* (Table 1). In Ukraine, according to available data, only maize dwarf mosaic virus (MDMV, *Potyvirus*, *Potyviridae*) (Naumenko 1973, pp. 468–472), High Plains wheat mosaic virus (HPWMoV, *Emaravirus*, *Fimoviridae*) (Snihur et al., 2020) and sugarcane mosaic virus (SCMV, *Potyvirus*, *Potyviridae*) (Snihur et al., 2021) have been detected on maize so far.

Table 1

Viruses that infect *Zea mays*

Family	Genus	Virus	Acronym	Reference
<i>Bromoviridae</i>	<i>Bromovirus</i>	brome mosaic virus	BMV	Moline, & Ford, 1974
	<i>Cucumovirus</i>	cucumber mosaic virus	CMV	Kim et al., 2011; Wang et al., 2013
<i>Fimoviridae</i>	<i>Emaravirus</i>	High Plains wheat mosaic virus	HPWMoV	Jensen, Lane, & Seifers, 1996; Tatineni, & Hein, 2021
<i>Geminiviridae</i>	<i>Mastrevirus</i>	maize streak reunion virus	MSRV	Pande et al., 2012
		maize streak virus	MSV	Martin et al., 2001
		maize striate mosaic virus	MSMV	Posse et al., 2023
<i>Potyviridae</i>	<i>Potyvirus</i>	johnsongrass mosaic virus	JGMV	Stewart et al., 2017
		maize dwarf mosaic virus	MDMV	Tóbiás, Bakardjieva, & Palkovics, 2007
		pennisetum mosaic virus	PenMV	Fan et al., 2004
		sorghum mosaic virus	SrMV	Zhang et al., 2016
		sugarcane mosaic virus	SCMV	Trzmiel, 2009
	<i>Tritimovirus</i>	wheat streak mosaic virus	WSMV	Tatineni, & French, 2014

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Ending table 1

Family	Genus	Virus	Acronym	Reference
Reoviridae	<i>Fijivirus</i>	maize rough dwarf virus	MRDV	Dovas, Eythymiou, & Katis, 2004
Rhabdoviridae	<i>Gammanucleorhabdovirus</i>	cereal chlorotic mottle virus	CCMoV	Greber, 1979
	<i>Nucleorhabdovirus</i>	maize mosaic virus	MMV	Reed et al., 2005
Secoviridae	<i>Wakavirus</i>	maize chlorotic dwarf virus	MCDV	Pratt et al., 1994
Solemoviridae	<i>Polerovirus</i>	cereal yellow dwarf virus-RPV	CYDV-RPV	Domier, Lukasheva, & D'Arcy, 1994
		maize yellow dwarf virus-RMV	MYDV-RMV	Krueger et al, 2013
		maize yellow mosaic virus	MaYMV	Guadie et al., 2018 Lim et al, 2018
	<i>Sobemovirus</i>	imperata yellow mottle virus	IYMV	Koala et al., 2017
Tombusviridae	<i>Aureusvirus</i>	maize white line mosaic virus	MWLMV	Russo et al, 2008
	<i>Luteovirus</i>	barley yellow dwarf virus-MAV	BYDV-MAV	Haack et al., 1999
		barley yellow dwarf virus-PAV	BYDV-PAV	Ivanović et al., 1995 Haack et al., 1999 Hamdi et al., 2019
	<i>Machlomovirus</i>	maize chlorotic mottle virus	MCMV	Kimani et al., 2021
Tymoviridae	<i>Marafivirus</i>	maize rayado fino virus	MRFV	Vásquez, & Mora, 2006
Virgaviridae	<i>Hordeivirus</i>	barley stripe mosaic virus	BSMV	Jarugula, Willie, & Stewart, 2018
-	<i>Tenuivirus</i>	maize stripe virus	MSpV	Huiet, Tsai, & Falk, 1992

As can be seen from the table, maize viruses belong to different taxonomic groups and therefore have different morphology, structure and routes of transmission. Some viruses are naturally transmitted by only one route, by means of a vector, such as MMV, MCDV, MRDV, MRFV, MSV, CYDV-RPV, BYDV-MAV, BYDV-PAV, etc. Several maize viruses, in addition to vector transmission, can also be transmitted by seed, such as MDMV, SCMV, MCMV, HPWMoV, WSMV and BSMV, which is particularly dangerous and can contribute to the spread of viruses to new areas. The aim of the work was to identify and determine the spread of maize viruses in different regions of Ukraine in 2021–2023.

Methods

Visual diagnostics. In 2021–2023, maize plants in industrial fields and household areas were inspected, and samples with typical viral symptoms, including stripe and mottle mosaic of leaves, spotting, reddening of leaf blades, stunted growth, etc. were sampled in three parts of Ukraine: Vinnytsia, Kyiv and Kharkiv regions. Enzyme-linked immunosorbent assay (ELISA). Double or tripple antibody sandwich (DAS or TAS) enzyme-linked immunosorbent assay (ELISA) was used to identify 11 viruses including maize dwarf mosaic virus (MDMV), High Plains wheat mosaic virus (HPWMoV), sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV), maize streak virus (MSV), maize mosaic virus (MMV), maize white line mosaic virus (MWLMV), wheat streak mosaic virus (WSMV), barley stripe mosaic virus (BSMV), barley yellow dwarf virus-PAV (BYDV-PAV) and brome mosaic virus (BMV). For ELISA, commercial test systems were used (Loewe Biochemica (Germany) and Agdia (USA)). The assay was performed according to the standard guidelines (Clark, & Adams, 1977; Ward et al., 2004) and in accordance with the manufacturers' recommendations. Antibodies conjugated to alkaline phosphatase and p-nitrophenyl phosphate substrate (Loewe Biochemica GmbH, Sauerlach, Germany) were used. Absorption values were measured at the wavelength of 405 nm using

a Thermo Labsystems Osys MR microtiter plate reader (Thermo Fisher Scientific, Waltham, MA, USA) with Dynex Revelation Quicklink software. Absorption values that exceeded the negative controls more than three times and were ≥ 0.2 were considered positive.

Transmission electron microscopy (TEM). Transmission electron microscopy for direct virus indication and studying virus morphology was conducted employing standard techniques of negative contrasting for clarified virus preparations. Extracts obtained from virus-infected maize plants were placed on grids and negatively counterstained with 2 % uranyl acetate. Samples were observed using JEOL (JEM-1400) transmission electron microscopy in the Centre of collective usage NAS of Ukraine at D.K. Zabolotny Institute of Microbiology and Virology NASU (Richert-Pöggeler et al., 2019).

Results

In 2021–2023, a visual inspection of maize crops was carried out in three regions of Ukraine, and plants with typical virus-like symptoms were selected and tested by ELISA. During the visual inspection, plants with signs of various leaf mosaic, reddening of leaf blades were observed, while some plants showed stunted growth. Summarizing the results of serological diagnostics of virus infections in these maize samples, it can be stated that currently SCMV is undoubtedly the main virus threat to maize in Ukraine, as it affected 35 % of tested plants collected in Kyiv region. BYDV-PAV was also detected in maize samples collected in Vinnytsia (4.3 %) and Kyiv (8 %) regions. Overall occurrence of SCMV and BYDV-PAV in tested symptomatic maize plants was 197 % and 6 %, correspondingly (Table 2). It should be noted that plants infected by SCMV showed various symptoms in the form of mottle or stripe mosaic of leaves (Fig. 1), as well as stunted growth and dwarfism (Fig. 2). Plants affected by BYDV-PAV showed symptoms of reddening of the leaf blades (Fig. 3). Although maize plants sampled in Kharkiv region showed clear symptoms of virus infection, none of the viruses tested were identified in this study.

Table 2

ELISA detection of maize viruses causing various symptoms in crops of three regions of Ukraine in 2021–2023

Region	sample quantity	Number of positive samples according to ELISA data										
		MDMV	HPWMoV	SCMV	MCMV	MSV	MMV	MWLMV	WSMV	BSMV	BYDV-PAV	BMV
Vinnytsia	23	0	0	0	0	0	0	0	0	0	1	0
Kyiv	37	0	0	13	0	0	0	0	0	0	3	0
Kharkiv	6	0	0	0	0	0	0	0	0	0	0	0
Total	66	0	0	13	0	0	0	0	0	0	4	0



Fig. 1. Mosaic symptoms on *Zea mays* plants naturally infected with SCMV, Kyiv region

Our results highlight the need to expand the list of tested viruses, as none of the tested viruses were identified in maize plants with clear symptoms of virus infection sampled in Vinnytsia and Kharkiv regions (Fig. 4).



Fig. 2. Mosaic and stunted growth of *Zea mays* plants naturally infected with SCMV, Kyiv region



Fig. 3. Symptoms of reddening of *Zea mays* leaf blades naturally infected with BYDV-PAV, Vinnytsia (A) and Kyiv (B) regions



Fig. 4. Maize plants with clear virus-like symptoms (healthy plant is shown on the right)

The electron microscopic examination of the sap of symptomatic plants that were previously serologically confirmed as SCMV-positive revealed flexible filamentous viral particles with a length of ~750 nm and

a diameter of ~12-13 nm (Fig. 5). Thus, the results of electron microscopy support the results of ELISA detection of the infection of maize plants by a representative of the genus *Potyvirus*.

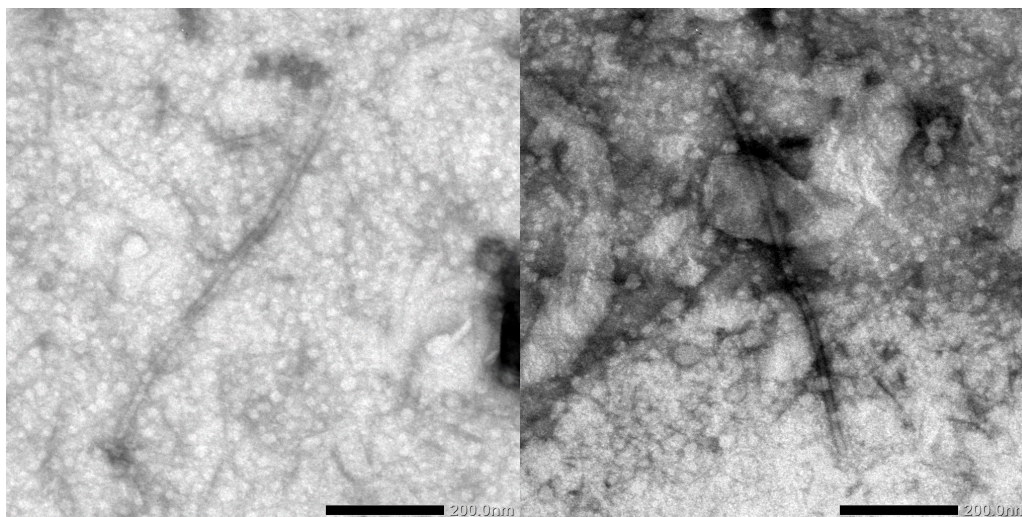


Fig. 5. Morphology of viruses detected in the sap of SCMV-positive maize plants using TEM (bar = 200 nm)

Discussion and conclusions

Summarizing the results of serological virus screening and direct detection (Table 2 and Fig. 5), it can be stated that at least two maize viruses, in particular SCMV and BYDV-PAV, circulated in agroecosystems of Ukraine in 2021–2023. The dominant role of SCMV is shown, with the occurrence of symptomatic plants reaching 35 % in Kyiv region. Considering that SCMV can be naturally transmitted by different aphid species in a non-persistent manner and can also be transmitted by seed in maize with a frequency of up to 0.4 % (Shepherd, & Holdeman, 1965), this virus is an important pathogen in European maize production causing severe grain yield losses in susceptible varieties (Marie-Jeanne et al., 2011; Trzmiel, 2009), and may pose a potential threat to maize production in Ukraine resulting from its subsequent spread to different regions. For the first time, the occurrence of BYDV-PAV in Vinnytsia and Kyiv regions in maize plants was established. Previously, this virus was reported to infect only wheat and other cereal spiked crops in different parts of Ukraine (Pozhylov, & Snihur, 2022; Snihur et al., 2018). Maize clearly plays an

important role in the annual cycle of BYDV-PAV and its aphid vectors. The need to expand the list of viruses studied on *Zea mays* in Ukraine is shown.

Authors' contribution: Halyna Snihur designed the study; Tetiana Vlasova and Halyna Snihur, prepared material for the experiments and collected data. All authors performed the experiments and wrote the paper, discussed the results, commented and approved on the manuscript.

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РІЗНОМАНІТНІСТЬ ВІРУСІВ, ЯКІ ІНФІКУЮТЬ *ZEА MAYS L.* В УКРАЇНІ

Вступ. Кукурудза (*Zea mays L.*) – однорічна рослина родини *Poaceae*, одна з найважливіших у світі злакових культур, яка є основою продовольчого забезпечення багатьох країн. Вірусні захворювання кукурудзи є однією з причин зниження її врожайності та погіршення якості зерна. Метою роботи було ідентифікувати та визначити поширення вірусів кукурудзи в різних регіонах України у період 2021–2023 років.

Методи. Імуноферментний аналіз у модифікації сендвіч (DAS чи TAS) використовували для ідентифікації 11 вірусів: *maize dwarf mosaic virus (MDMV)*, *High Plains wheat mosaic virus (HPWMoV)*, *sugarcane mosaic virus (SCMV)*, *maize chlorotic mottle virus (MCMV)*, *maize streak virus (MSV)*, *maize mosaic virus (MMV)*, *maize white line mosaic virus (MWLMV)*, *wheat streak mosaic virus (WSMV)*, *barley stripe mosaic virus (BSMV)*, *barley yellow dwarf virus-PAV (BYDV-PAV)* and *brome mosaic virus (BMV)*, застосовуючи комерційні тест-системи *Loewe Biochemica* (Німеччина) та *Agdia* (США).

Результати. Результати імуноферментного аналізу (ІФА) підтвердили наявність SCMV в агроценозах Київської області (35 % уражених рослин) і виявили BYDV-PAV у зразках кукурудзи, зібраних у Вінницькій (4.3 %) та Київській (8 %) областях. Частка рослин, уражених SCMV, у загальній кількості досліджених проб становила 19,7 %; BYDV-PAV вразив 6 % зразків кукурудзи. Зазначено, що рослини, уражені SCMV, мали різні симптоми у вигляді штрихуватої або смугастої мозаїки. Хоча зразки рослин кукурудзи, відібрані в Харківській області, показали чіткі симптоми вірусної інфекції, жоден із протестованих вірусів не був ідентифікований.

Висновки. В агроценозах України в 2021–2023 роках підтверджено циркуляцію SCMV і BYDV-PAV на рослинах кукурудзи, слід зауважити, що це перше повідомлення про BYDV-PAV на кукурудзі в Україні. Показано необхідність розширення переліку досліджуваних вірусів на *Zea mays* в Україні.

Ключові слова: кукурудза, вірус, виявлення, імуноферментний аналіз.

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