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I. Pozhylov[1], PhD Stud.,
H. Snihur[1,2], PhD
[1]ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
[2]Laboratory of plant viruses, D. K. Zabolotny Institute of Microbiology and Virology
of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

INCIDENCE AND SPREAD OF CEREALS VIRUSES IN 2020–2021 IN UKRAINE

Cereals play a significant role in the Ukrainian economy and agriculture. Viral diseases can cause a serious reduction in yields. Aim. The aim of the work was to identify and determine the spread of grain viruses in the main cereal-growing regions of Ukraine in the period 2020–2021 using different methods of virus detection. Methods. Enzyme-linked immunosorbent assay was used to identify viruses using commercial test systems by for wheat streak mosaic virus (WSMV), barley stripe mosaic virus (BSMV), brome mosaic virus (BMV), wheat dwarf virus (WDV), High Plainswheat mosaic virus (HPWMoV), barley yellow dwarf virus-PAV (BYDV-PAV), barley yellow dwarf virus-MAV (BYDV-MAV), andcereal yellow dwarf virus-RPV (CYDV-RPV). Transmission electron microscopy was used for direct virus detection. Reverse transcription polymerase chain reaction was performed to identify viruses by molecular methods. Results. Summarizing the results obtained by different methods, we can say about the circulation of WSMV, HPWMoV, BSMV, BYDV-PAV, and BYDV-MAV in the Ukrainian agrocoenoses in 2020–2021. Significant prevalence and the dominant role of WSMV have been shown, with the degree of damage to symptomatic plants reaching 52 %. The spread of HPWMoV in Kyiv and Poltava regions has been established, although previously the virus was detected only in the eastern part of our country. WSMV was more likely to induce monoinfection (70 %). Conclusions. During the testing of symptomatic plants in the agrocenoses of Ukraine in 2020–2021 the most common were WSMV and HPWMoV (52 % and 19 %, respectively), also found BYDV-PAV in winter wheat and BYDV-MAV in oats.

Keywords: cereals, virus, detection, enzyme – linked immunosorbent assay, polymerase chain reaction.

Cereals play a significant role in the Ukrainian economy and agriculture. In particular, 6.5 million hectares of winter wheat had been expected to besownin2022, while spring wheat had less attention from Ukrainian farmers – only 174 thousand hectares in 2021. In 2020, Ukraine exported wheat to 55 countries (mostly in Africa and Asia) for 4.61 billion US dollars, which accounted for 8.76 % of total Ukrainian exports. There are currently 7.9 billion people in the world, but by the end of the 21st century there may be 3 billion more of us, so the demand for wheat is only growing.

Yields of wheat (*Triticumaestivum,* L) in Ukraine are at the world average (about 4 t/ha), while in some Western European countries this figure reaches 10 t/ha. Viral diseases can cause a serious reduction in yields. Examples of this isHPWMoV (High Plains wheat mosaic virus, *Emaravirustritici, Emaravirus, Fimoviridae*), which poses a particular risk of co-infection with other viruses spread by wheat curl mite (*Aceriatosichella,* Keifer), WSMV (wheat streak mosaic virus*, Tritimovirus, Potyviridae*), and TriMV, (Triticummosaicvirus, *Poacevirus, Potyviridae*) together forming the so-called "WSMD complex" [1]. Viruses of this complex lead to a loss of an average of 2–3 % of the crop, and sometimes up to 10 % [2]. Mixed infection can occur from 2 % [3] to 40 % of cases [4].

In Ukraine, according to the literature, it is known about the detection of viruses in cereals that cause mosaic symptoms, in particular, WSMV (*Tritimovirus, Potyviridae*) [5–8, 9], HPWMoV[10, 11], barley stripe mosaic virus (*Hordeivirus, Virgaviridae*) [6, 8, 12], brome mosaic virus (*Bromovirus, Bromoviridae*) [12–14] and barley yellow mosaic virus (*Bymovirus, Potyviridae*) [14, 15]. It is also known about the spread of viruses in the agrocenoses of our country, which cause symptoms of leaf yellowing/reddening and plant stunting: wheat dwarf virus (*Mastrevirus, Geminiviridae*) [17–21] and barley yellow dwarf virus PAV (BYDV-PAV) [14, 15,23-26], barley yellow dwarf virus MAV (BYDV-MAV) [8, 24] and cereal yellow dwarf virus RPV (CYDV-RPV) [8], belonging to the group barley/cereal yellow dwarf viruses (B/CYDVs). It should be noted that in 2021 the taxonomy of viruses that cause yellow dwarf disease has changed, in particular, BYDV-PAV, BYDV-MAV, barley yellow dwarf virus PAS (BYDV-PAS), barley yellow dwarf virus kerII, and barley yellow dwarf virus kerIII belong to the genus *Luteovirus* of the family *Tombusviridae* [27]; cereal yellow dwarf virus RPS (CYDV-RPS) and CYDV-RPV belong to the genus *Polerovirus* of the family *Solemoviridae*; Barley yellow dwarf virus GPV (BYDV-GPV) and barley yellow dwarf virus SGV (BYDV-SGV) are not included in the family*Solemoviridae* [27, 28].

The aim of the work was to identify and determine the spread of cereal viruses in the main grain-growing regions of Ukraine in the period 2020–2021 using different methods of virus detection.

Materials and methods. To detect grain viruses during May-June 2020–2021, a survey of grain crops (*Triticumaestivum* L., *Secalecereale* L., *Avena sativa* L., × *Triticosecale*sp.) for the presence of plants with typical viral symptoms in agrocenoses of major grain-growing regions of Ukraine, in particular, Vinnytsia, Zaporizhzhia, Kyiv, Poltava, Kharkiv, Cherkasy and Chernihiv regions was conducted. Selected plants with symptoms of viral infection were divided into two groups, the first group included plants with symptoms of various mosaics, and the second – plants with symptoms of leaf yellowing/reddeningand plant stunting.

Identification of viruses in selected samples was performed using Enzyme-Linked Immunosorbent Assay (ELISA) in various modifications using commercial test systems. Double Antibody Sandwich-ELISA (DAS-ELISA) was performed using Loewe Biochemica test systems (Germany), Triple Antibody Sandwich-ELISA (TAS-ELISA) was performed using Agdia test systems (USA); two ELISA modifications were performed according to standard procedures following the manufacturer's recommendations [27, 28]. Antibodies conjugated to alkaline phosphatase using p-nitrophenyl phosphate substrate (Loewe Biochemica GmbH, Sauerlach, Germany). Absorption values were measured at 405 nm using a Thermo Lab systems Opsys 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.

Samples were tested for the presence of the most relevant viruses for Ukraine based on literature data. Samples from the first group (with mosaic symptoms) were tested for WSMV, HPWMoV, BSMV, and BMV. Samples from the second group(with symptoms of leaf yellowing/reddening and plant stunting) were tested for WDV, BYDV-PAV, BYDV-MAV, and CYDV-RPV.

Transmission electron microscopy (TEM) was used to detect causative agents of viral diseases in cereals and to determine the morphology and size of viral particles. Preparations were contrasted with 2 % aqueous uranyl acetate for 10 min [31]. Samples were observed under JEOL Ltd., Japan (JEM-1400) transmission electron microscope in the Centre of collective usage NAS of Ukraine at D.K. Zabolotny Institute of Microbiology and Virology NASU.

Reverse transcription-polymerase chain reaction (RT-PCR) was performed to identify viruses by molecular methods. Total RNA was extracted from 0.5 g of symptomatic or healthy leaf tissues using a Gene JET RNA Purification kit (Thermo Fisher Scientific) following the manufacturer's instructions. RT-PCR was performed in two steps. Reverse transcription was performed using RevertAid H Minus Reverse Transcriptase (Thermo Scientific™) with 10 mMdNTP Mix (Thermo Scientific™)and specific primers (Table 1).Total RNA extracted from healthy wheat plants was used as a negative control. PCR was performed using DreamTaq Green PCR Master Mix (2X) (Thermo Scientific™) according to the manufacturer's instructions. Amplification was performed using PTC-150 Mini Cycler (MJ Research).

Table **1. Sequences of oligonucleotide primers used in this work.**

Virus	Region	Name	Sequence	Product size	Reference
WSMV	capsid protein gene	forward WS-8166F	GAGAGCAATACTGCGTGT ACG	750 bp	[32]
		reverse WS-8909R	GCATAATGGCTCGAAGTGATG		
HPWMoV	nucleoprotein gene	forward HPV-F1	TTTATGGCTCTTTGTATTGG	339 bp	[33]
		reverse HPV-R1	TATGTTTCCCCTCTTTGTG		
BSMV	Alpha a protein gene	forward	GTGAGGAGGTGATGGGTAAT	417 bp	[14]
		reverse	TTCCAGTCTTTCAGAATCTCTC		
BYDVs	coat protein gene and the intergenic region	forward BYcpF	CCACTAGAGAGGTGGTGAATG	641 bp	[34]
		reverse BYcpR	CCGGTGTTGAGGAGTCTACC		

Reactions were performed under the following conditions: initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C/53°C/58°C/58°C (for WSMV/HPWMoV/BSMV/BYDVs accordingly) for 30 s, and extension at 72°C for 1 min. The final extension was at 72°C for 7 min. PCR products with DNA markers CSL-MDNA-100BP(Cleaver Scientific), were separated in a 1.5 % agarose gel stained with ethidium bromide and visualized under UV light [35].

Results and discussion. Crops of cereals were visually inspected during May-June 2020–2021 in seven regions of Ukraine and plants with typical virus-like symptoms were selected and examined by ELISA. During the visual inspection, plants with different mosaic symptoms, from moderate striped mosaics to streak mosaics of different widths of light green stripes were observed, and some plants had growth retardation. Summarizing the results of serological diagnosis of viruses in these samples of winter wheat, we can say that WSMV is undoubtedly the main viral threat to cereals in Ukraine, as it affected the largest number of symptomatic samples in 2020 and 2021 (Table 2). WSMV was detected in 6/7 of the surveyed regions, with a total incidence of 52 % in symptomatic plants (27/52). Interestingly, HPWMoV, in addition to the central-eastern regions of Ukraine (Zaporizhzhya and Kharkiv regions), where we detected this virus in previous years [10], was first identified in Kyiv and Poltava regions. HPWMoV was detected in 19 % of collected wheat samples (10/52). Importantly, screening of symptomatic wheat samples for WSMV and HPWMoV allowed to assess the occurrence of mono- and mixed viral infections that induce striped mosaic in winter wheat, as these pathogens have one vector – the mite *Aceriatosichella* Keifer (also known as *Aceriatritici*, *Acarina*: *Eriophyidae*) [36]. It should be noted that WSMV caused symptoms of distinct fine streak mosaic on winter wheat plants, while mosaic-like yellowing was caused by HPWMoV. then, the mixed infection caused the symptoms of streak mosaic, formed by broad yellow and green bands on winter wheat leaves (Fig. 1). In 2020, WSMV monoinfection was recorded for 70 % of symptomatic wheat plants, while 15 % of samples were infected only with HPWMoV and 15 % had co-infection (WSMV + HPWMoV). In 2021, also 70 % of wheat plants were monoinfected with WSMV, 20 % of wheat plants were monoinfected with HPWMoV, while mixed infection was registered in only 10 % of tested plants (Fig. 2). Unlike WSMV and HPWMoV, BSMV was found in only one sample from the Kyiv region in 2020, which is 2 % of the total number of tested samples. Symptoms of BSMV on winter wheat plants were in the form of a distinct striped mosaic (Fig. 1). It should be noted that BMV was not identified in both 2020 and 2021 (Table 2).

Table **2. Detection of wheat viruses that cause mosaic symptomsin agrocenoses of Ukraine in 2020–2021**

Year	Region	District	ELISA*			
			WSMV	HPWMoV	BSMV	BMV
2020	Vinnytsia	Bershad	2 (2)	0 (2)	0 (2)	0 (2)
	Zaporizhzhya	Vasyliv	1 (1)	1 (1)	0 (1)	0 (1)
	Kyiv	Kyiv-Sviatoshyn	4 (7)	1 (7)	1 (7)	0 (7)
	Poltava	Poltava	1 (3)	0 (3)	0 (3)	0 (3)
	Kharkiv	Vovchansk	2 (6)	1 (6)	0 (6)	0 (6)
		Kharkiv	1 (3)	1 (3)	0 (3)	0 (3)
2021	Vinnytsia	Bershad	2 (2)	0 (2)	0 (2)	0 (2)
	Zaporizhzhya	Vasyliv	2 (2)	1 (2)	0 (2)	0 (2)
	Kyiv	Kyiv-Sviatoshyn	3 (5)	0 (5)	0 (5)	0 (5)
	Poltava	Poltava	5 (10)	4 (10)	0 (10)	0 (10)
	Kharkiv	Kharkiv	2 (4)	1 (4)	0 (4)	0 (4)
	Cherkasy	Cherkasy	0 (1)	0 (1)	0 (1)	0 (1)
	Chernihiv	Pryluky	2 (6)	0 (6)	0 (6)	0 (6)
Total percent			27 (52) 52 %	10 (52) 19 %	1 (52) 2 %	0 (52) 0 %

* number of positive samples (number of tested samples).

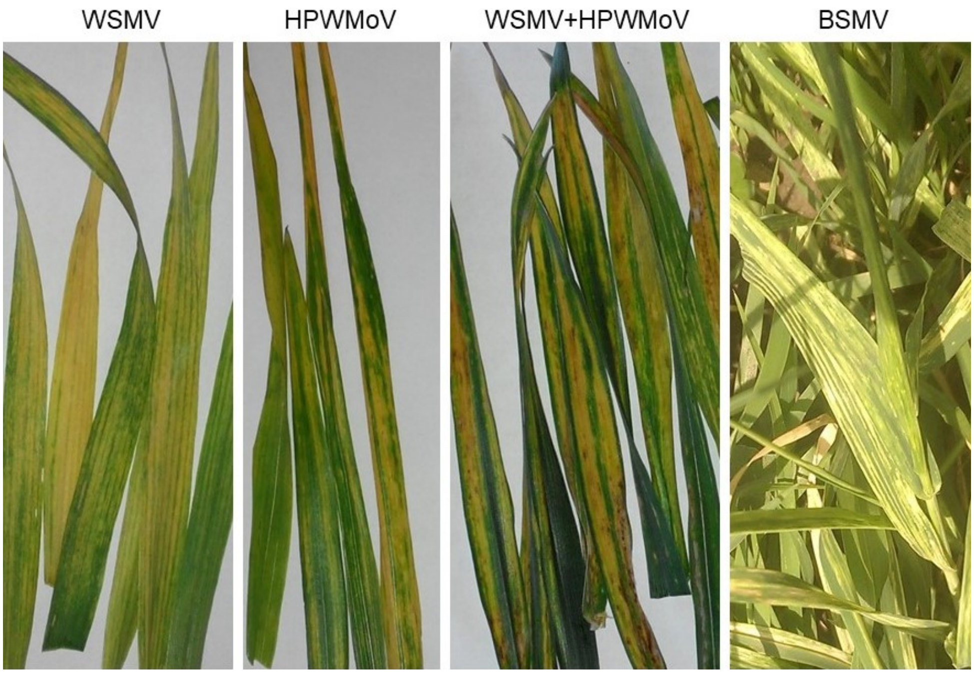

Fig. 1. Symptoms of mosaic on naturally infected winter wheat plants

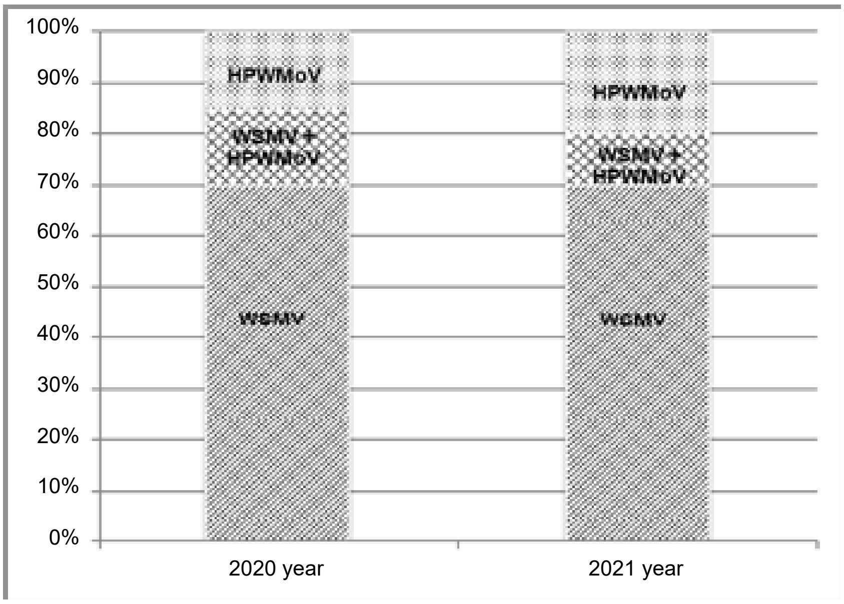

Fig. 2. Incidence of winter wheat WSMV and HPWMoV in mono- and co-infection in 2020–2021

The data obtained on the detection of mosaic pathogens in winter wheat were confirmed by electron microscopy studies. Filamentous viral particles about 700 nm and 12–13 nm in diameter, which is characteristic of the genus *Tritimovirus*, represented by WSMV were found in the sap of diseased plants. Viral particles 120–150 nm long and 20 nm in diameter were also found, which are characteristic for BSMV, a member of the genus *Hordeivirus* (Fig. 3).

When testing samples with symptoms of yellowing/reddening of leaf blades and growth retardation for B/CYDVs and WDV, BYDV-PAV in winter wheat in 2020 and 2021 was detected. In addition, BYDV-MAV in oats in 2020 was detected; it is important that we first identified BYDV-MAV in oats in our country. It should be noted that the polyclonal test system for the detection of BYDV-PAV by Loewe, apart from BYDV-PAV, also detects BYDV-MAV, because these viruses belong to the genus *Luteovirus* of the family *Tombusviridae*. Interestingly, CYDV-RPV and WDV were not detected in plants with typical symptoms for these viruses (Table 3).

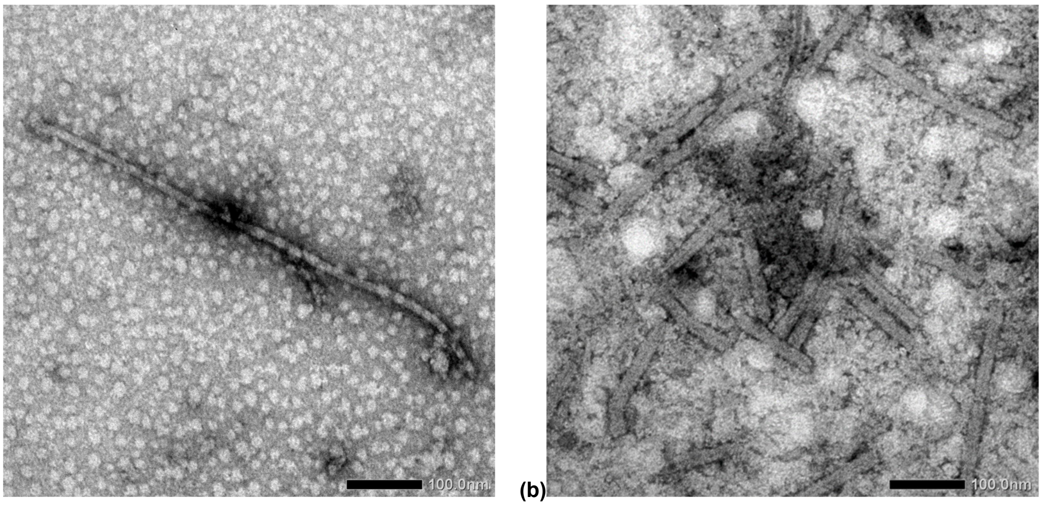

Fig. 3. Morphology of viruses detected in wheat sap with symptoms of striped mosaic using TEM: WSMV (a), BSMV (b). Bars are indicated on images

Table **3. Detection of cereal viruses that cause symptoms of yellowing/redness and growth retardation in crops of Kyiv region in 2020–2021 by ELISA**

Year	Plant	O.D. at 405/630 nm*				
		BYDV-PAV Loewe	BYDV-PAV Agdia	BYDV-MAV Agdia	CYDV-RPV Agdia	WDV Loewe
2020	winter wheat	**3,585**	**0,455**	0,181	0,160	0,154
	winter wheat	**2,146**	**0,364**	0,149	0,154	0,107
	rye	0,164	0,198	0,127	0,094	0,203
	spring wheat	0,114	0,201	0,121	0,176	0,180
	oats	**2,059**	0,143	**0,594**	0,116	0,165
	triticale	0,135	0,106	0,118	0,112	0,129
2021	winter wheat	**2,592**	**1,038**	0,233	0,086	0,092
	winter wheat	0,194	0,173	0,201	0,193	0,093
	oats	0,189	0,204	0,198	0,182	0,194
controls**	k +	**3,276**	**1,365**	**1,967**	**0,794**	**0,861**
	k- 1	0,182	0,141	0,186	0,165	0,137
	k- 2	0,094	0,036	0,065	0,058	0,043

* optical density, average value
** "k +" – positive control; "k-1" – negative control, sap of virus-free plants; "k-2" – negative control, buffer.

As a result of total RNA extractionand RT-PCR with the application of specific primers to the gene fragment of viruses detected in ELISA, their identification by molecular research methods was confirmed. was obtained DNA fragments of the expected size for all viruses that were detected in ELISA: WSMV, HPWMoV, BSMV, BYDV-PAV, and BYDV-MAV (Fig. 4).

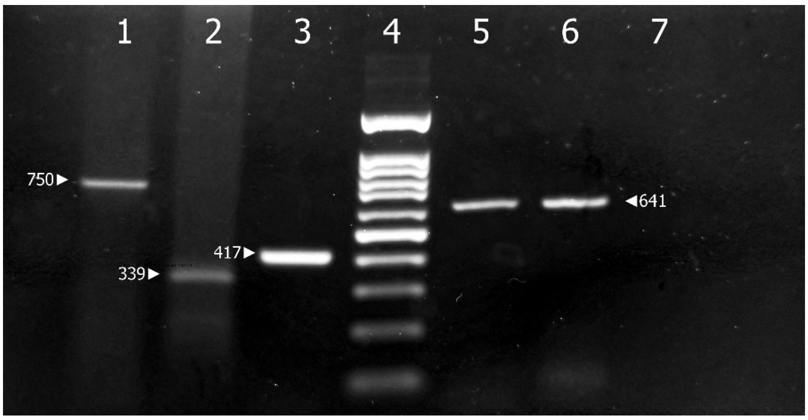

Fig. 4. Visualization of PCR products in the agarose gel under UV light for cereal viruses.
Tracks: 1, 2, 3, 5, 6 – WSMV, HPWMoV, BSMV, BYDV-PAV, and BYDV-MAV amplicons of expected size, respectively; 4 – DNA marker CSL-MDNA-100BP (Cleaver Scientific); 7 – negative control

Conclusions. Summarizing the results of serological screening, direct detection, and molecular detection we can say about the circulation in the agrocenoses of Ukraine at least five cereal viruses, in particular, WSMV, HPWMoV, BSMV, BYDV-PAV, and BYDV-MAV in 2020–2021. Significant spread and the dominant role of WSMV have been shown, with the degree of damage to symptomatic plants reaching 52 %. The spread of HPWMoV in Kyiv and Poltava regions has been established, although previously the virus was detected only in the eastern part of our country [10; 37]. The difference in the symptoms of diseases, associated with the most common pathogens, WSMV and HPWMoV, in mono-infection and in their co-infection, was shown.The EFSA (European Food Safety Authority) Panel on Plant Health considered that HPWMoV fulfills the criteria that are within the remit of EFSA to assess for it to be regarded as a potential Union quarantine pest [38]. WSMV was more likely to induce monoinfection (70 %of virus-positive plants), while HPWMoV was detected in both monoinfection and mixed WSMV infection in the ratioon average ~1:1 (Fig. 1), which may indicate prolonged WSMV circulation in Ukrainian agrocenoses, but probably recent distribution of HPWMoV. The obtained results regarding the significant spread of WSMV and BYDV in agrocenoses of Ukraine coincide with other studies of Ukrainian scientists [5; 7; 9; 12; 16; 23]. However, we managed to determine which types of yellow dwarf viruses affected cereal crops in Ukraine in 2020–2021.

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І. Пожилов[1], асп.,
Г. Снігур[1,2], канд. біол. наук
[1]ННЦ "Інститут біології та медицини", Київський національний університет імені Тараса Шевченка, Київ, Україна,
[2]Лабораторія вірусів рослин, Інститут мікробіології і вірусології імені Д. К. Заболотного НАН України, Київ, Україна

ЗАХВОРЮВАНІСТЬ ТА ПОШИРЕННЯ ВІРУСІВ ЗЛАКОВИХ КУЛЬТУР У 2020–2021 РОКАХ В УКРАЇНІ

Зернові культури займають значну частку в українській економіці та сільському господарстві. Вірусні хвороби можуть бути причиною серйозного зменшення врожаю. Тому метою пропонованої роботи було ідентифікувати та визначити поширення вірусів зернових культур в основних зерносіючих регіонах України в період 2020–2021 років, використовуючи різні методи детекції вірусів. Для ідентифікації вірусів використовували імуноферментний аналіз із застосуванням комерційних тест-систем до вірусів смугастої мозаїки пшениці (WSMV), штрихуватої мозаїки ячменю (BSMV), мозаїки бромусу (BMV), карликовості пшениці (WDV), мозаїки пшениці Високих рівнин (HPWMoV), жовтої карликовості ячменю-PAV (BYDV-PAV), жовтої карликовості ячменю-MAV (BYDV-MAV) і жовтої карликовості злакових RPV (CYDV-RPV). Для прямої детекції вірусів застосовували трансмісійну електронну мікроскопію. Для ідентифікації вірусів молекулярними методами проводили полімеразну ланцюгову реакцію зі зворотною транскрипцією. Узагальнюючи результати різних методів, можна сказати про циркуляцію в агроценозах України WSMV, HPWMoV, BSMV, BYDV-PAV і BYDV-MAV у 2020–2021 роках. Показано значне розповсюдження та домінуюча роль WSMV, ступінь ураження яким симптоматичних рослин сягав 52 %. Установлено поширення HPWMoV у Київській і Полтавській областях, хоча раніше цей вірус був виявлений лише у східній частині нашої країни. WSMV частіше індукував моноінфекцію (70 %). Отже, в агроценозах України у 2020–2021 роках при дослідженні симптоматичних рослин найбільш поширеними були WSMV і HPWMoV (відповідно 52 і 19 %), також виявлено BYDV-PAV на озимій пшениці та BYDV-MAV – на вівсі.

Ключові слова: зернові культури, вірус, виявлення, імуноферментний аналіз, полімеразна ланцюгова реакція.