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WHEAT DWARF VIRUS

and its impact on the 2020 harvest in some regions of Ukraine

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Goal. *To identify the virus that causes symptoms of dwarfism and no heading of winter wheat, and to investigate its effect on plant yield.* ***Methods.*** *Visual diagnostics, enzyme-linked immunosorbent assay in double sandwich modication (DAS-ELISA), polymerase chain reaction, biometric determining of yield and its structure, statistical data analysis.* ***Results.*** *Investigation of winter wheat varieties from Vinnytsia, Khmelnytsky, Kyiv, Chernihiv and Cherkasy regions with symptoms of dwarfism, yellowing of leaves and no heading was performed. ELISA and PCR have shown that the disease is caused by wheat dwarf virus. The absence of the wheat streak mosaic virus, barley yellow dwarf virus, brome mosaic virus, and wheat spindle streak mosaic virus in the tested wheat samples was shown. It was found that wheat dwarf virus significantly reduces the number of seeds per spike (3.3 times), weight of seeds per spike (3.4 times) and weight of 1000 grains (1.9 to 3.3 times) depending on the degree of plant damage (from moderately to severely affected).* ***Conclusions.*** *The circulation of wheat dwarf virus in agrocenoses of five regions of Ukraine and a significant negative impact of the disease on the yield of winter wheat plants have been established. The obtained data indicate the necessity for constant monitoring and testing of plants for the presence of wheat dwarf virus in Ukraine.*

wheat dwarf virus; winter wheat; yield

Cereals are crucial in ensuring Ukraine's food program, the main of which is winter wheat, which occupies an area of more than 6 million hectares. However, its yield over the past seven years is only 4 t/ha, which is much lower than the genetic potential of domestic varieties. Even under favorable conditions for crop production, wheat crop losses from viral diseases can reach more than 60%. The least studied among the viral diseases of wheat in our country is the wheat dwarf virus.

Wheat dwarf virus (WDV) belongs to the genus *Mastrevirus*, family *Geminiviridae*, is in the form of a twinned quasi-isometric virions, which contains a circular single-stranded DNA genome encoding 4 proteins: movement protein (MP; *V1*), capsid protein (CP, *V2*) and proteins associated with replication (RepA, *C1* and Rep, *C1: C2*) [1]. For the main representative of the genus *Mastervirus* — Maize streak virus, its size is 38 nm in length and 22 nm in diameter.

The virus has two non-coding regions: large and small (LIR and SIR, respectively), which contain regulatory elements for replication and transcription of the virus. LIR contains the origin for transcription. SIR has polyadenylation signals and the region to which a short complementary primer is attached for the synthesis of the second chain [2].

WDV was first described by Vacke [3] in the western part of the former Czechoslovak Socialist Republic. The virus causes wheat dwarfism, which is one of the most serious wheat diseases in Europe, Asia and Africa [4]. The presence of the virus has been confirmed in many countries around the world: in Turkey [5] and in China [6]; in Iran [7] and Syria [8], in North Africa (Tunisia [9], Zambia [10]). The spread of this virus has increased in recent years in Europe. VKP was found in Bulgaria [11, 12]; Hungary [13], France [14], Romania 15], Germany [16], Poland [17], Sweden [18], Finland [19], Spain [20], Ukraine [21, 22], Czech Republic [23], Austria and Great Britain [24]. Only in 2017 WDV has appeared in Estonia [25] and Slovenia [26].

The symptoms caused by WDV in different countries of the world are similar and manifest themselves in the form of varying degrees of dwarfism of plants, yellowing of leaves, reduced heading and/or no heading of plants. Initially, WDV isolates were divided into two groups: wheat and barley forms of the virus (WDV-W and WDV-B strains) [27]. Subsequent studies have shown that WDV-B is serologically similar to WDV-W, but the strains differ in nucleotide sequence (84% identity) and genome size: 2749—2750 nucleotides in WDV-W and 2734 nucleotides in WDV-B [5]. Later, based on examination of cereal field samples and DNA sequences of different virus isolates, Schubert et al. [4] proposed to divide WDV into three types: WDV, barley dwarf virus (BDV), and oat dwarf virus (ODV). Muhire et al. [28] suggested that ODV should be considered as a separate species and WDV should be divided into five strains (referred to as A — E) based on many phylogenetic analyzes of its genome, which were approved by the International Committee on Taxonomy of Viruses (ICTV). In 2015, ICTV proposed to divide the WDV isolates into strains A — F, and A and F isolates of the virus were mainly isolated from barley, while B, C, D, E originated from wheat plants [29]. WDV is transmitted persistently, nonpropagatively by the vector *Psammotettix alienus* Dahlb. (order Hemiptera, family *Cicadellidae*) on plants of barley, wheat, oats, rye, triticale and wild grasses of the family *Poaceae*. The virus does not reproduce in insects and is not transmitted by eggs [30]. Adults of the second generation begin to appear in late summer and are present until late autumn. The main spread of WDV is in winter wheat fields, infection occurs in autumn, when adult cicadas migrate to freshly sown fields. The incidence can range from a few percent to about 75% [31]. Even at low insect densities; the virus can cause significant crop losses. In the spring and early summer, secondary spread occurs in the form of newly hatched cicada nymphs that have acquired the virus from plants previously infected in the autumn. In addition, tillage methods have changed and many fields of winter wheat are sown immediately after harvesting winter wheat without plowing. This means that early seedlings of winter wheat plants can become infected in the autumn and be a source of infection next spring.

It has been shown that WDV can lead to significant yield losses of up to 80% [32]. In Finland, the virus caused crop losses of 20 to 40%, in some cases up to 100% [19]. In Sweden, it has been shown that losses from WDV are close to 35% to 90%, and that early crops suffered more from dwarf wheat than fields sown at the optimal time. Some other agronomic factors have also been associated with higher yield losses, such as crop failure and irregular germination [33]. In the Czech Republic, different varieties of wheat were studied by mechanically inoculating them with WDV, and it was shown that there is a decrease in grain yield from 87.3 to 100%, reduction of plant heading (in some cases by 100%) and ear sterility in certain varieties [34]. In addition, it was found that even resistant varieties of wheat with the defeat of the WDV are characterized by a decrease in grain yield: by 72% in very susceptible varieties, 35% and 21% in susceptible and moderately susceptible varieties, respectively [35].

In 2020, we received many appeals from farms and research institutions from different regions of Ukraine about the presence of winter wheat plants with symptoms of severe dwarfism and no heading. Therefore, the aim of the study was to identify the virus that causes these symptoms and to investigate its effect on crop yields.

Materials and methods.

Agroecological monitoring of winter wheat crop disease by viral diseases was carried out in Vinnytsia, Khmelnytsky, Kyiv, Chernihiv and Cherkasy regions by visual diagnostics [36, 37, 38]. Identification of viruses was carried out using enzyme-linked immunosorbent assay (DAS-ELISA) using commercial test systems for viruses: wheat streak mosaic virus (WSMV), wheat dwarf virus (WDV), barley yellow dwarf virus (BYDV), brome mosaic virus (BrMV), and wheat spindle streak mosaic virus (WSSMV) (Loewe, Germany).

Leaf samples from healthy plants were also included as a negative control. Positive controls were commercial (Loewe, Germany). The analysis was performed in three replicates. Plant samples (leaves) were ground with the addition of 0.1 M PBS in a ratio of 1: 2 (m/V). To remove plant residues in the homogenate, samples were centrifuged at 3 000 rpm. for 20 minutes followed by selection of the supernatant, which was used for analysis [39]. The reaction results were recorded on a Termo Labsystems Opsis MR reader (USA) with the Dynex Revelation Quicklink software at wavelengths of 405/630 nm [37, 38, 39] Values that exceeded the negative control at least three times were considered positive. DNA isolation was performed using the GeneJet PlantRNA Puntification Kit (cat. number K 0801, Thermo Scientific, USA) according to the manufacturer's recommendations. Amplification was performed using DreamTaq ™ Green PCR Master Mix (Thermo Fisher Scientific, USA) in a thermocycler (Genetic Research Instrumentation LTD, UK) with oligonucleotide primers to the capsid protein (CP) gene of WDV which code for the sequence of length 783 bp: WDV-F 5'-ATGGTGACCAACAAGGACTCC-3' and WDV-R 5'-TTACTGAATGCCGATGGCTTTG-3' [40]. Amplification regime: denaturation for 3 min at 95 °C, then 30 cycles (95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min), 72 °C for 5 min. The PCR products were separated in a 1.5% agarose gel, ethidium bromide solution at a concentration of 0.5 μg/ml, with DNA markers CSL-MDNA-100bp (Cleaver Scientific, UK) and visualized under UV light. Biometric measurements, yield and its structure were performed by conventional methods according to Dospekhov [41]. Mathematical data processing was performed using the Microsoft Office Excel computer software package. To analyze the crop and its structure, mean values of traits and their standard error (10 plants of each variant) were determined. Differences between mean values of the traits were evaluated by t-test.

Results.

In 2020, wheat plants with severe symptoms of dwarfism and no heading were found in five regions of Ukraine (Vinnytsia, Khmelnytsky, Kyiv, Chernihiv, and Cherkasy). In addition to dwarfism, the plants were characterized by yellowing of various degrees and streaked stripes (Fig. 1—12).

In Fig. 3 (photo from the drone) there are clearly visible differences between dwarf wheat plants. Greener plants were observed on the northern slope. Due to the well-heated soil, the plants of the southern slope were more populated by vectors (*Psammotettix alienus* cicadas) and therefore the plants looked much yellower (affected).

Using ELISA we have found that wheat plants of 13 samples with these symptoms were affected by wheat dwarf virus (Fig. 7, 8).

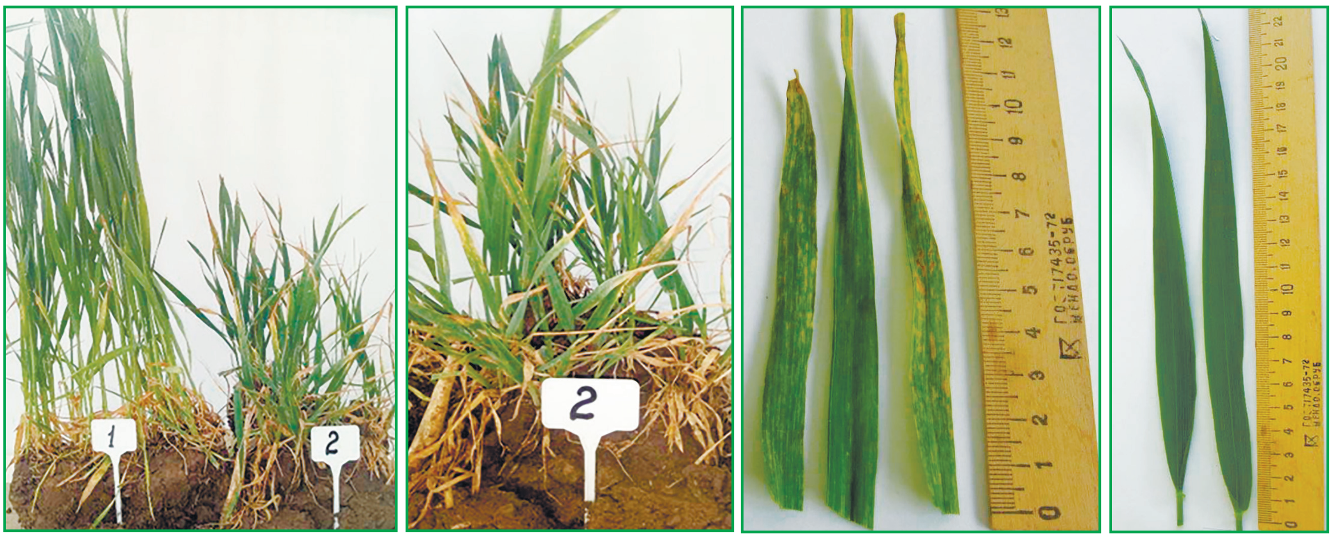
Wheat samples with symptoms of dwarfism were also tested for the presence of other viruses circulating in Ukraine [42] that can cause similar symptoms of the disease, namely: WSMV, BYDV, BrMV and WSSMV. ELISA results showed the absence of all these viruses in the studied wheat varieties with symptoms of dwarfism, streaked stripes, yellowing and no heading. Thus, these symptoms are caused only by WDV.

The ELISA results were confirmed by PCR, which showed the presence of 783 bp amplicons corresponding to the wheat dwarf virus capsid protein gene (Fig. 9).

Studies have shown that the wheat dwarf virus has a significant negative impact on plant productivity and wheat yield, because in many varieties it leads to the reduction of the heading, or, in general, to no heading (Fig. 10, 11). In fig. 11 well-marked habitats of harvested field sheaves of healthy and affected by WDV winter wheat plants Producent from Khmelnytsky region. Plants affected by the wheat dwarf virus have a dark (blackened) appearance due to the population of secondary fungi, which is characteristic of thin and weakened plants. It is on such plants that fungi colonize due to adverse environmental factors, in particular weather conditions. Individual spikes of healthy and affected plants are shown in Fig. 12, 13. Well-marked blackened WDV-affected spikes (Fig. 13).

Observations of healthy and affected by WDV plants showed a large unevenness of plant damage by this virus in 2020, and hence the impact on plant productivity. Depending on the variety, growing conditions, the degree of damage to plants by the dwarf wheat virus, we observed large fluctuations in the percentage of no heading of plants — from more than 50% to 100% in some areas. In the mown-off affected WDV plants, we recorded a different degree of damage to the ear (Fig. 13). The spikes looked too weak, the grains of them are shown in Fig. 14.

Grain size of the spike and weight of 1000 grains play the most important role in the formation of spike productivity. Many years of research have shown that the weight of 1000 grains depends on the varieties and growing conditions, plant diseases, etc. [37]. In recent years, the newest varieties have mass of 1000 grains over 50 g. For Myronivka

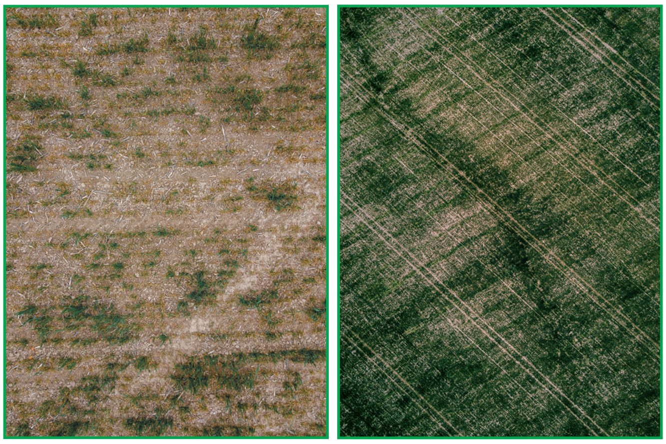


a *b* *c* *d* *e*

Figure. 1. Monoliths of wheat plants Akter with symptoms of infection caused by WDV: a — healthy plants; b, c — WDV-infected plants; d — individual leaves of WDV-infected plants; e — leaves of healthy wheat plants (19 May 2020), Vinnytsia region

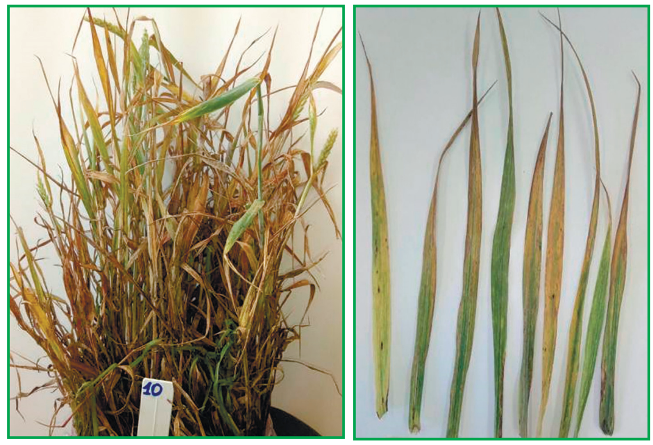
a *b* *c* *d*

Figure 2. Symptoms of WDV infection in Matrix wheat plants: a — infected plants in the foreground, healthy — behind; b, c — WDV-infected plants and leaves from them; d — leaves of healthy wheat plants (19 May 2020, Vinnytsia region)



a b

Figure 3. Field with dwarf wheat plants, (Vinnytsia region, 19 May 2020): a — southern slopes; b — northern slopes (photo from the the drone)



a b

Figure 4. Symptoms of WDV on winter wheat plants D-13 (Kyiv region, June 16, 2020): a — infected plants; b — individual leaves of diseased plants

Figure 5. Symptoms of WDV on spring wheat plants D-35, (Kyiv region, June 22, 2020)

a b

Figure 6. Symptoms of WDV on winter wheat plants (Chernihiv region): a — moderately affected plants; b — severely affected, diseased (no heading)

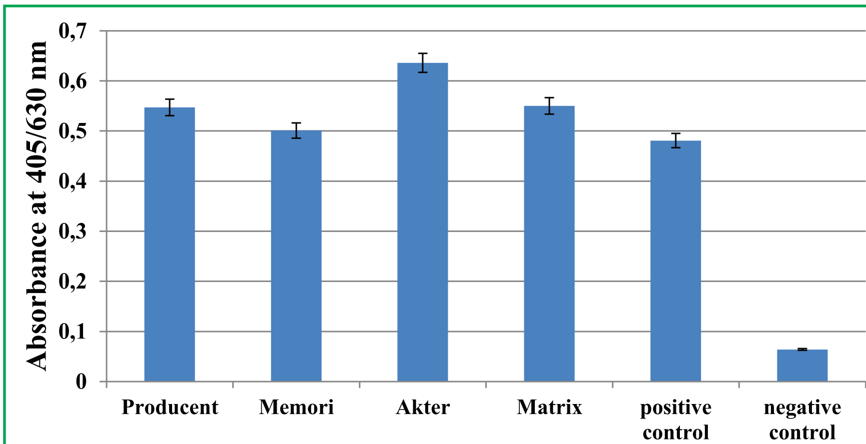


Figure 7. The content of WDV antigens in wheat plants from Vinnytsia and Khmelnytsky regions (May — July 2020)

varieties, the standard is the variety Podolyanka, where this parameter is 43.1 — 45.5 g.

An integral indicator of spike productivity is the grain weight of one spike [34, 37].

The height of WDV-infected plants of wheat variety Akter (Vinnytsia region, 2020) is less than that of healthy plants, but the most significant negative impact ($P < 0.001$) of the virus was found on such indicators as: the number of grains in the spike (3.3 times less), mass of grains per spike (3.4 times), and mass of 1000 grains (1.9 — 3.3 times), depending on the degree of damage of plants — from moderately to severely affected (Table).

Similar results on a significant reduction in wheat yield under the WDV infection have been previously obtained by scientists in Western Europe [32—35].

Some farms of Vinnytsia region received a significant shortage of wheat grain, namely: in the wheat fields of the Akter variety, where the plants affected by WDV were first detected in mid-May 2020, only 2.5 t/ha of wheat grain were harvested. From other varieties, from 4 to 5 t/ha were obtained, depending on the spread of the virus and the degree of damage to plants in the fields by this virus. In the absence of damage to the WDV, the yield of the variety Kolonia was 6.0 t/ha. For comparison, in the Cherkasy region. 8 t/ha of grain were harvested in some unaffected fields of the Patras wheat. Such a harvest of the Patras variety was obtained in the fields of Chernihiv region. Yield of the Berehynia variety in Chernihiv region was

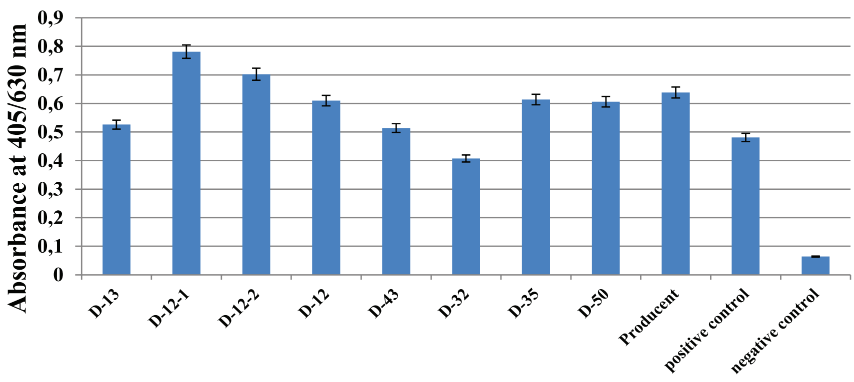


Figure 8. The content of WDV antigens in wheat plants from Kyiv and Chernihiv regions (July — 2020)

Figure 12. Healthy spikes of winter wheat Akter (Vinnytsia region, 2020)

10.1 t/ha. From the given data it is clearly visible to what considerable losses WDV infection leads.

We did not find data on the impact of WDV on the wheat harvest in our country. In 2009, the wheat dwarf virus was isolated from winter wheat plants in Kyiv and Odesa regions and a phylogenetic analysis of three its isolates belonging to different groups was performed [21]. In 2011, WDV was detected on winter barley and wheat plants in Odesa region and on wild cereals in Odesa and Kherson regions [22]. However, the symptoms of wheat and barley plants affected by wheat dwarf virus were not presented in detail and the actual results of virological studies were not shown. However, later, for the last almost ten years, there was no information about the detection of the WDV on the territory of Ukraine. Of importance is the appearance of dwarf wheat virus in 2020 in as many as five regions of Ukraine, although previously it was found mainly in

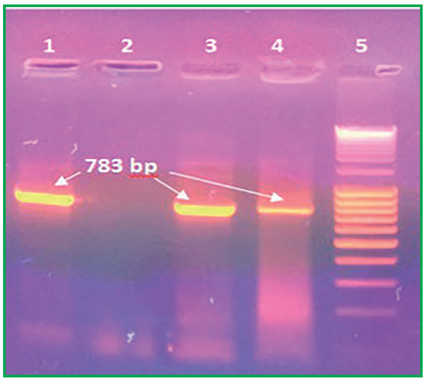


Figure 9. Electrophoregram of PCR products with the size of 783 bp of three Ukrainian WDV isolates with application of primers to the CP gene:
1 — wheat of the Acter variety (Vinnytsia region);
2 — negative control;
3 — wheat D-1 (Kyiv region);
4 — wheat of the Producent variety (Khmelnytsky region);
5 — DNA marker CSL-MDNA-100bp (Cleaver Scientific)

Figure 13. Spikes of winter wheat Akter, infected with WDV (Vinnytsia region, 2020): a — severely affect-ed; b — moderately affected

a b

Figure 10. Influence of WDV on winter wheat of the Matrix variety (Vinnytsia region, 2020): a — WDV-infected; b — moderately affected and healthy plants

Figure 11. Influence of WDV on winter wheat plants of Producent variety (Khmelnytsky region): 5 — VWDV-infected; 6 — healthy in the phase of full maturity

Figure 14. Grains of winter wheat variety Akter (Vinnytsia region): a — from healthy plants; b, c — from WDV-infected plants

Table. Influence of WDV on wheat yield of Akter variety (Vinnytsia region, 2020)

Productivity parameter	Variants	
	Healthy plants	WDV infected plants
Plant height, cm	63.5 ± 2.5	51.4 ± 3.0*
Spike length, cm	9.8 ± 0.6	9.1 ± 0.5
Number of spikes, pcs	24.1 ± 2.1	21.2 ± 1.2
Number of grains per spike, pcs	46.5 ± 2.8	14.2 ± 1.5**
Weight of grains per spike, g	2.4 ± 0.4	0.7 ± 0.1**
Weight of 1000 grains, g	48.9 ± 2.1	moderately affected 25.4 ± 1.9** severely affected 15.0 ± 1.1**

Note: the differences are significant at * $P < 0.01$; ** $P < 0.001$

Western Europe (France, Czech Republic, Slovenia, Sweden, Finland, Bulgaria, Hungary, Romania, Poland, Spain, Austria, the United Kingdom and more recently in Estonia), Asia, and Africa. The cause for the spread of this virus in Ukraine is probably the intensive reproduction of cicada vectors, which may be associated with climate change.

Thus, the periodic variability of viruses (WSMV or BYDV) described by us earlier [38], as well as the appearance of the little-described WDV in our country, is obviously closely related to climate change, both regional and global.

CONCLUSIONS

The results of the agroecological monitoring of wheat crops of different varieties in five regions of Ukraine in the 2020 season revealed plants with symptoms of significant growth stunting, yellowing, streaked stripes. Wheat dwarf virus antigens were detected in these plants. The results of virological studies showed that 13 analyzed varieties and lines of winter and spring wheat do not contain antigens of wheat streak mosaic virus, barley yellow dwarf virus, brome mosaic virus and wheat spindle striped mosaic virus. There was a significant decrease (more than twice) in the yield of winter wheat variety Akter (Vinnytsia region) when infected with the virus. Analysis of the elements of productivity showed that the plants infected with WDV significantly reduce the number and weight of grains from a spike. Wheat dwarf virus can destroy crops by 100% in some areas of the fields. Yield reduction depends on the number of infected plants (virus prevalence) and the degree of damage to wheat plants. The obtained results indicate the need for constant monitoring and testing of plants for the presence of wheat dwarf virus in Ukraine.

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Вірус карликовості пшениці (*Wheat dwarf* virus) та його вплив на урожай 2020 р. у деяких областях України

Мета. *Ідентифікувати вірус, який спричинює симптоми карликовості та невиколошування пшениці озимої (зниження або відсутності колосоутворення), та дослідити його вплив на урожайність рослин.* **Методи.** *Візуальна діагностика, імуноферментний аналіз в модифікації подвійний сендвіч (DAS-ELISA), полімеразна ланцюгова реакція, біометричні, визначення урожаю та його структури, статистична обробка даних.* **Результати**. *Проведено дослідження сортів пшениці озимої із Вінницької, Хмельницької, Київської, Чернігівської та Черкаської областей із симптомами карликовості, пожовтіння листків та невиколошування. Методами ІФА та ПЛР доведено, що захворювання спричинене вірусом карликовості пшениці (Wheat dwarf virus). Показано відсутність у досліджуваних зразках вірусу смугастої мозаїки пшениці (Wheat streak mosaic virus), вірусу жовтої карликовості ячменю (Barley yellow dwarf virus), вірусу мозаїки бромусу (Brome mosaic virus) та вірусу веретеноподібної смугастої мозаїки пшениці (Wheat spindle streak mosaic virus). Встановлено, що вірус карликовості пшениці суттєво знижує кількість зерен у колосі (у 3,3 раза), масу зерен із колосу (у 3,4 раза) та масу 1000 зерен (у 1,9—3,3 раза) залежно від ступеня ураженості рослин (від помірно до сильно уражених).* **Висновки.** *Встановлено циркуляцію вірусу карликовості пшениці в агроценозах п'яти областей України та суттєвий негативний вплив захворювання на урожайність рослин пшениці озимої. Одержані результати свідчать про необхідність постійного моніторингу та тестування рослин на наявність вірусу карликовості пшениці в Україні.*

вірус карликовості пшениці; *Wheat dwarf* virus; пшениця озима; урожайність

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Вирус карликовости пшеницы (*Wheat dwarf virus*) и его влияние на урожай 2020 г. в некоторых областях Украины

Цель. *Идентифицировать вирус, который вызывает симптомы карликовости и отстутствие выколашивания (снижение или отсутствие образования колосьев), исследовать его влияние на урожайность растений.* **Методы.** *Визуальная диагностика, иммуноферментный анализ в модификации двойной сэндвич (DAS-ELISA), полимеразная цепная реакция, биометрические, определения урожая и его структуры, статистическая обработка данных.* **Результаты.** *Проведены исследование сортов пшеницы озимой из Винницкой, Хмельницкой, Киевской, Черниговской и Черкасской областей с симптомами карликовости, пожелтения листьев и отсутствия выколашивания. Методами ИФА и ПЦР доказано, что заболевание вызвал вирус карликовости пшеницы (Wheat dwarf virus). Показано отсутствие в исследуемых образцах вируса полосатой мозаики пшеницы (Wheat streak mosaic virus), вируса желтой карликовости ячменя (Barley yellow dwarf virus), вируса мозаики бромуса (Brome mosaic virus) и вируса веретеновидной полосатой мозаики пшеницы (Wheat spindle streak mosaic virus). Установлено, что вирус карликовости пшеницы существенно снижает количество зерен в колосе (в 3,3 раза), массу зерен с колоса (в 3,4 раза) и массу 1000 зерен (в 1,9—3,3 раза) в зависимости от степени пораженности растений, от умеренно до сильно пораженных.* **Выводы.** *Установлена циркуляция вируса карликовости пшеницы в агроценозах пяти областей Украины и существенное негативное влияние заболевания на урожайность растений пшеницы озимой. Полученные результаты свидетельствуют о необходимости постоянного мониторинга и тестирования растений на наличие вируса карликовости пшеницы в Украине.*

вирус карликовости пшеницы; *Wheat dwarf virus*; пшеница озимая; урожайность

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