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WHEAT VIRUSES DON'T KNOW BORDERS: IDENTIFICATION IN UKRAINE, MOLDOVA AND THEIR IMPACT ON YIELD UNDER GLOBAL WARMING

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Summary. The early spring of 2024 contributed to emergence of viruses both in Ukraine and in neighboring Moldova. Already in April, wheat plants with signs of virus damage were selected in Vinnytsia and Dnipropetrovsk regions, and almost at the same time in Moldova. In May, the *Wheat stripe mosaic virus* (WSMV) was also discovered in Poltava. WMSV was detected in both countries, which has a significant epidemic potential, leading to a decrease in yield. In addition, in Ukraine, we continued to observe the manifestation of cold stress due to a daily temperature drop of more than 20ºC, which led to the appearance of symptoms such as purple leaves and the development of chlorotic and yellowish-brown stripes, which are very similar to viral damage. The absence of viruses in such stressed wheat plants has been conclusively proven. A reliable decrease in wheat yield was established not only under the influence of a viral infection, but also in variants with a daily temperature difference in May-June, that is, the phase of emergence into the tube and the beginning of earing.

Keywords: Wheat, *Wheat streak mosaic virus*, symptoms, crop, Moldova, Ukraine.

Introduction

Wheat (*Triticum aestivum* L.), as the main food crop in Ukraine, is of great economic importance, while serving as a critical contributor to global food security. However, climate change affects water availability, temperature, and atmospheric CO_2 concentration, which, in turn, directly affects plant growth processes and, finally, the ability of plants to efficiently produce protein, starch, and other plant products that are valuable for human nutrition [1]. The issue of climate change is also relevant for our country, because in recent years, abnormal climatic phenomena have been observed, which create a number of significant problems for grain production. Thus, late spring frosts in 1999, 2001 and 2004, a long-term ice crust in 2003, waterlogging in June 2001, 2006, and moisture deficit in September-October 2005, 2007 [2] and 2011, 2023 years led to a catastrophic crop condition winter wheat and significant financial losses due to grain shortage. Modern warming causes a significant change in the agroclimatic conditions for the growth,

development and productivity of agricultural crops. It is accompanied by a significant increase in air temperature in the winter months, an increase in the number of prolonged thaws, a temporal shift in the development of natural processes, changes in the duration of the seasons, an extension of the frost-free period and the duration of the growing season of agricultural crops, and an increase in the heat supply of the growing season. More than 30% of the territory of Ukraine with the most fertile lands, where the production of about 65% of commercial grain is concentrated, is in the zone of insufficient hydration. The main feature of the warming period was the unevenness of precipitation within the year and in individual years, which led to an increase in the number of drought events. It was shown that the climate of Ukraine is highly sensitive to global climate change. It was found that annual temperature changes towards warming over a century period are 0.7–0.9 °C in Polissia and Forest Steppe, and 0.2–0.3 °C in the Steppe. Therefore, the problem of assessing the impact of expected climate changes on agroclimatic conditions of cultivation, productivity and gross harvest of agricultural crops is extremely relevant [3].

It is known that such climate changes can significantly affect crop yield. Thus, the productivity of grain crops is closely related to the presence of precipitation and temperatures during the growing season. It has been established that precipitation during the vegetation period is a positive factor for grain yield, while the influence of high temperatures leads to its decrease [4, 5]. An increase of temperature above the optimum for every 1 °C negatively affects wheat yield with a predicted 5.3% [6] and 6% [7] reduction in yield. In wheat, exposure to frost or high temperatures during pollination significantly affects yield [8, 9].

Climate change affects the level of losses from plant diseases, because the environment significantly affects not only plants, but also pathogens and their vectors. The appearance, prevalence, harmfulness of phytoviral diseases, as well as the subsequent co-evolution of plants and their pathogens, which leads to changes in the species composition of viruses in a certain region, the appearance of differences in the properties of virus isolates, the occurrence of epiphytotia directly depend on changes associated with global warming (increasing temperature, changes in the amount of atmospheric precipitation, increasing the level of CO_2 and ozone, drought, etc.) [10, 11, 12]. However, only a few of them are devoted to viruses of cereals [13, 14, 15, 16]. It should be noted that these pathogens are transmitted by vectors*: Barley yellow dwarf virus* (BYDV) – aphids, *Wheat streak mosaic virus* (WSMV) by microscopic mites, and *Wheat dwarf virus* (WDV) by leafhoppers. In this regard, changes in climate impacts may indirectly affect the intensity and prevalence of WSMV, VYDV and WDV diseases through effects on the reproduction/survival of their vectors. Thus, in the conditions of a mild winter, a high intensity of aphid migration in spring and a high frequency of cases of plant damage are noted. In this regard, climate changes impacts may indirectly affect the intensity and prevalence of WSMV, VYDV and WDV through effects on the reproduction/survival of their vectors. Thus, in the conditions of a mild winter, a high intensity of aphid migration in spring and a high frequency of cases of plant damage are noted. The harmfulness of viral diseases is largely determined by the amount of inoculum and the duration of infection, which are affected by the winter survival of its hosts. For some viruses, increased temperatures also cause more severe symptoms. The survival rate of aphids increases with a milder winter, and higher spring and summer temperatures increase their development and reproductive level and lead to a greater intensity of the disease. Mild winters increase the survival of weeds, which are virus reservoirs. A raise of the frequency and intensity of summer storms with high winds, rain and hail will increase plant damage, leading to an enhancing in mechanical and livestock transmission of viruses.

In recent years, in Ukraine, and in particular, in the Poltava region, it has become very noticeable that winter wheat practically does not freeze, but suffers from significant temperature changes in the phases of emergence into the tube and the beginning of earing [17, 18, 19], which leads to significant economic losses. New WSMV diagnostic approaches are also shown (Mishchenko et al, 2021) [20]. Innovation may be the biggest challenge in developing crop varieties to win the race between climate change and food security [21].

In connection with climate changes, it is often difficult to distinguish the symptoms of viral infections from other abiotic factors. Therefore, the diagnosis of wheat viral infections is an old and at the same time a new topic

The goal of our research was the identification of viruses on wheat crops in Ukraine and Moldova in 2024 and the influence of the *Wheat streak mosaic virus* and the biotic factor (daily temperature difference in May-June) on the yield of the crop (wheat).

Material and methods

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) were employed to identify viruses in wheat leaf sap, using specific antibodies to wheat streak mosaic virus (WSMV), wheat dwarf virus (WDV), barley yellow dwarf virus (BYDV-PAV), brome mosaic virus (BMV), wheat spindle streak mosaic virus (WSSMV), barley stripe mosaic virus (BSMV) (all Loewe, Germany) and High Plains wheat mosaic virus (HPWMoV) (Agdia, USA). Wheat leaf tissue was ground in PBS buffer (pH 7.4) in a ratio of 1:2 (w/v) to prepare antigen samples. Leaf samples from healthy wheat plants were used as negative controls, and commercial samples from Loewe, Germany, as positive controls. The results were recorded with an Opsis MR reader (Termo Labsystems, USA) and Dynex Revelation Quicklink software, at a wavelength of 405 nm. Samples were considered positive when their absorbance values at 405 nm were at least three times higher than those of the negative controls [22].

RNA extraction, RT-PCR.

Total RNA was extracted from fresh leaves using the RNA kit from Thermo Scientific (https://www.thermofisher.com/se/en/home/life-science/dna-rna-purification-analysis/rna-extraction/rna-types/total-rna-extraction.html). Reverse transcription polymerase chain reaction (RT-PCR) was conducted in two steps. First, reverse transcription was performed with RevertAid Reverse Transcriptase (Thermo Scientific, USA), which incorporates genetically modified Moloney Murine leukaemia virus reverse transcriptase (MMuLV RT), following the manufacturer's protocol. Amplification was then carried out using a thermocycler (Genetic Research Instrumentation LTD, Great Britain) and specific oligonucleotide primers targeting a portion of the WSMV CP gene (WS-8166F (5′ GAGAGCAATACTGCGTGTACG 3′) and WS-8909R (5′ GCATAATGGCTCGAAGTGATG 3′) [23]. This resulted in amplification of a 750 bp DNA product. The amplification reaction contained 12.5 μL Dream Taq PCR Master Mix (2x) buffer (comprising Dream Taq DNA polymerase, 2x Dream Taq buffer, 0.4 mmol/L of each dNTP, 4 mmol/L MgCl2), 7.5 μL nuclease-free water, 1 μL of each primer (10 mol/L) and 3 μL cDNA. The temperature profile of the amplification reaction was as follows: initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. The final extension step was performed at 72°C for 10 min. The PCR products were separated on a 1.5% agarose gel alongside DNA markers (MassRuler DNA Ladder Mix, ready-to-use, SM 0311, Thermo Scientific, USA), stained with ethidium bromide and visualised under UV light. Following gel electrophoresis, the PCR products were purified from the agarose gel using the QIAquick Gel Extraction Kit (Qiagen, UK).

Five biological replicates, each containing leaves pooled from five individual plants, were used in each ELISA measurement. Three technical replications were conducted for each biological replicate and, when appropriate, the mean value was calculated for each biological replicate. DAS-ELISA was performed in three replicate runs. Yield structure was measured on 10 replicates, while all biochemical measurements were performed on six replicates. Data processing was performed using Microsoft Office Excel v.14.7.3 (Microsoft, Redmond, WA, USA) or Prism Graph 8 (GraphPad Software, San Diego, CA, USA). Differences between mean values of traits were evaluated by the Student's t-test at probability level $p \leq 0.05$.

Results and discussion

Phytopathological monitoring of wheat crops in Ukraine and Moldova in April - May 2024 revealed symptoms of viral diseases in both countries (Fig. 1, A-D). Infected plants had symptoms of light green and yellow stripes of various lengths extending parallel to the venation and characteristic chlorotic. Especially classical symptoms of WSMV were observed in Moldova and Poltava region. WSMV was identified by DAS-ELISA. In May in the Poltava region there were clearly visible symptoms on different genotypes and lines of wheat, yellow-brown stripes and spots that resembled viral or fungal diseases (Fig. 1, E-H). The conducted studies (light microscopy for fungi) and immunological (DAS-ELISA) did not reveal fungi and none of the 7 viruses listed in the methods. PCR analysis confirmed this (Fig. 2). WSMV was detected in Moldovan and Vinnytsia isolates of of winter wheat.



A **B** **C** **D**

E **F** **G** **H**

Figure 1. Symptoms of WSMV on wheat: A–C – Moldova, 24.04.2024; D – Vinnytsia region, 17.04.2024; E – G – lines and variety Patras (H) in the Poltava region, 15.05.2024

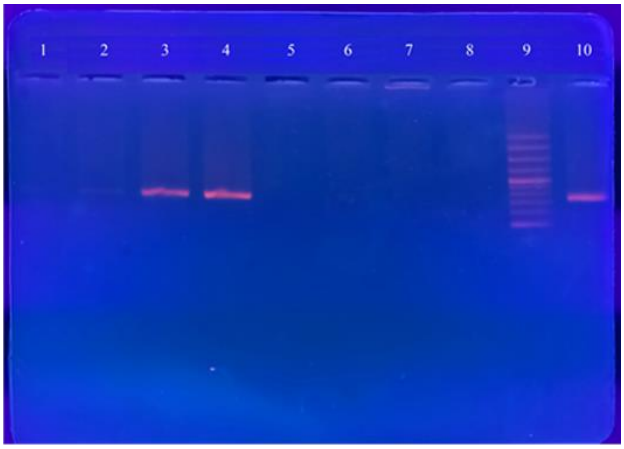


Figure 2. Detection of WSMV in infected wheat leaves by RT-PCR. Electrophorogram showing PCR DNA fragments of 750 bp in size, amplified using Dream Taq PCR Master Mix (2X) (Thermo Scientific); Tracks 1–4 – isolates from Moldova; 5 – lines No. 22, 6 – 32, 7 – 41; 8 – variety Patras; 9 – DNA markers (O'GeneRuler 100 bp Plus DNA Ladder, Thermo Scientific), 10 – Vinnytsia region.

The conducted harvest studies showed that the mass of 1,000 grains in the WSMV-infected wheat plants of the Bohdana variety (Poltava region) decreased by 25% compared to the control (healthy plants) and by 32% under the action of cold stress. It is 54.6 g for healthy plants, 40.9 g for virus-infected plants, and 36.8 g under conditions of cold stress. For the Odesa line in the field experiment of the Poltava region the mass of 1,000 grains in healthy plants is 46.2 g, and with the effect of a daily temperature drop of more than 20º C – only 38.1 g.

Conclusions

Conducted researches of wheat plants of various genotypes, affected by the WSMV in Ukraine and Moldova, confirmed that viruses really do not have borders. The PCR analysis revealed amplicons of 750 bp size, corresponding to the expected WSMV product for wheat from neighboring Moldova and Vinnytsia region. The next step will be to study the phylogeny of the detected viruses. A reliable decrease in wheat yield was established not only under the influence of a viral infection, but also in variants with a daily temperature difference in May-June, that is in the phase of emergence into the tube and the beginning of earing. The symptoms of the these plants are very similar to viral and fungal infections of wheat. Therefore, diagnosis is extremely necessary for decision-making.

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