

Prevalence and natural impact of major wheat viruses in Azerbaijan

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Abstract: Cereal viruses such as *Wheat streak mosaic virus* (WSMV), *Barley yellow dwarf viruses* (BYDV), and *Wheat dwarf virus* (WDV) often occur alongside other wheat pathogens, making it difficult to diagnose and manage these diseases effectively. To better understand the current situation with these important DNA and RNA viruses, we collected 157 wheat samples showing virus-like symptoms during the 2022–2024 growing seasons. The samples were taken from wheat-growing areas of Azerbaijan, including Absheron, Jalilabad, and Shamakhi, and were analysed using ELISA and RT-PCR. During our field surveys, we observed potential signs of viral infections, such as stunted growth, yellowing or streaking of leaves, and reduced grain yields. After extracting total RNA and DNA from the samples, we used specific primers to amplify regions of the viruses' genomes. DNA fragments of 404 bp, 178 bp, and 550 bp were successfully amplified in 25, 39, and 44 samples infected with BYDV, WSMV, and WDV, respectively. These results provide new insights into the prevalence of these viruses in wheat fields across Azerbaijan and provide essential information for improving management strategies to protect wheat productivity.

Key words: cereal viruses, survey, detection, ELISA, RT-PCR, WSMV, BYDV, WDV

Razširjenost in naravni vplivi pomembnejših virusov pšenice v Azerbajdžanu

Izvleček: Virusna obolenja žit, kot so virus mozaika pšenice (*Wheat streak mosaic virus*, WSMV), virus rumene pritlikavosti ječmena/žit (*Barley yellow dwarf viruses*, BYDV) in virus pritlikavosti pšenice (*Wheat dwarf virus*, WDV), se pogosto pojavljajo skupaj z drugimi patogeni pšenice, kar otežuje natančno diagnostiko in učinkovito obvladovanje teh bolezni. Za boljše razumevanje trenutnega stanja teh pomembnih DNK in RNK virusov smo med rastnima sezonomi 2022–2024 zbrali 157 vzorcev pšenice, ki so kazali simptome, podobne virusnim. Vzorce smo odvzeli iz območij pridelave pšenice v Azerbajdžanu, vključno z Absheronom, Jalilabadom in Šamakhi, ter jih analizirali z uporabo ELISA in RT-PCR. Med terenskimi pregledi smo opazili morebitne znake virusnih okužb, kot so zavrti rast, rumenjenje ali progasto obarvanje listov ter zmanjšani pridelek zrn. Po ekstrakciji celotne RNK in DNK smo iz vzorcev s specifičnimi primernimi sekvencami namnožili dele genomov virusov. DNK fragmenti dolžine 404 bp, 178 bp in 550 bp so bili uspešno amplificirani v 25, 39 in 44 vzorcih, okuženih z BYDV, WSMV oziroma WDV. Ti rezultati nudijo nove vpoglede v razširjenost teh virusov na poljih pšenice po celotnem Azerbajdžanu in zagotavljajo ključne informacije za izboljšanje strategij upravljanja in zaščite pridelave pšenice.

Ključne besede: virusi žit, pregled, odkrivanje, ELISA, RT-PCR, WSMV, BYDV, WDV

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1 INTRODUCTION

Cereals are a cornerstone of global food production, providing essential staples for billions of people worldwide. Wheat, rice, maize, and barley are among the most important of these crops, grown in many parts of the world. Growing cereals is no easy task—it requires careful planning and management, including planting, irrigation, fertilization, and pest control. These practices are vital not only for ensuring food security but also for maintaining economic stability, particularly in regions where cereal farming is a key industry. In Azerbaijan, bread wheat (*Triticum aestivum* L.) has been a staple crop for centuries, and it remains the most widely grown grain in the country. In 2020, Azerbaijan harvested nearly 1.87 million tonnes of wheat, with production continuing to rise. By 2021, wheat output had reached 2.16 million metric tons, with a yield of 32,101 kilograms per hectare. However, the country's wheat demand is slightly higher—around 3.2 million tons annually (Figure 1). To meet this growing need, research and technological advancements are helping to sustain wheat yields, ensuring that supply can keep pace with demand. Despite the successes, wheat farming faces several challenges, with viral diseases being one of the most significant threats. Viral dwarfness (Morca *et al.*, 2024), in particular causes serious damage to wheat crops, leading to reduced yields and lower grain quality. These diseases can result in considerable economic losses for farmers, making it essential to find effective ways to manage them. Approaches such as planting resistant varieties and implementing strong biosecurity measures are key to controlling the spread of these viruses. A growing body of research is also focused on the links between crop diseases and climate change. As global temperatures rise and weather patterns shift, the spread and severity of viral plant diseases are also changing. Factors like higher CO₂ level, changes in ozone, and more frequent droughts can alter the relationship between plants and their pathogens, making some diseases more widespread and more damaging (Mishchenko *et al.*, 2013; 2014). In Europe alone, more than 30 different viruses are known to affect cereals. These include *Wheat streak mosaic virus* (WSMV), *Barley yellow dwarf viruses* (BYDV), and *Wheat dwarf virus* (WDV). WDV, transmitted by the leafhopper *Psammotettix alienus* (Dahlbom, 1850), poses a major threat to cereals, with the virus spreading faster due to increasing temperatures, which extend the activity of the vectors and broaden their range. This results in a higher risk of infection across large areas of Europe. In some regions, like Sweden, yield losses can reach between 35-90 % in winter wheat fields, and in southern Finland, losses have been recorded as high as 100 % (Lindblad *et al.*, 2022).

As temperatures rise, the infection window is expected to get longer, which could lead to more outbreaks and higher infection rates (Habekub *et al.*, 2009). The growing presence of WDV, alongside *Wheat streak mosaic virus* (WSMV), is prompting increased research into ways to develop virus-resistant wheat varieties (Pfrieme *et al.*, 2023). WSMV, an RNA virus from the Potyviridae family, is a major concern for wheat production worldwide. The virus is spread by wheat curl mites (*Aceria tosicella* Keifer, 1969), which pick up the virus while feeding on infected plants and spread it to healthy ones. Similarly, BYDVs are also RNA-based viruses transmitted by aphids, such as *Rhopalosiphum padi* (L., 1758) and *Sitobion avenae* (Fabricius, 1775), which feed on infected plants and spread the virus to others. These viruses cause symptoms like yellowing and stunting of plants, ultimately reducing yields and impacting a wide range of cereal crops, including wheat, barley, oats, and rye. To reduce the impact of these viruses, understanding their genome structure, transmission methods, and the conditions that drive their spread is crucial. This research is necessary for developing better control strategies. This study aims to assess the prevalence of wheat viruses in Azerbaijan's primary cereal-growing regions and evaluate the seed transmission potential of WSMV isolates. While progress has been made, there is still much to learn about how these viruses spread and how they evolve. Only a few wheat varieties with genetic resistance to these diseases have been identified, so there is a strong need for further research. This study examines ongoing efforts to develop reliable diagnostic tools, assess epidemiological risks, and implement effective preventive strategies, which are of critical importance for the timely detection of these viruses, accurate risk assessment, and the adoption of appropriate management measures.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

This study explored the presence and distribution of three major wheat viruses—*Wheat streak mosaic virus* (WSMV), *Barley yellow dwarf viruses* (BYDV), and *Wheat dwarf virus* (WDV)—in Azerbaijan's main cereal-growing regions: Absheron, Jalilabad, and Shamakhi. Between April and May, and during the early days of June, a total of 157 wheat samples were collected from eight different fields, representing various wheat cultivars. The samples were analysed using a combination of serological (DAS-ELISA) and molecular (RT-PCR) methods to detect the viruses. The samples were taken from plants

showing clear signs of virus infection, such as mosaic patterns on leaves, rolling, yellowing, stunting, and deformation. Infected plants also exhibited symptoms like mottling, fewer spikes, and in severe cases, necrosis that could result in the complete death of the plant. Once collected, the leaf samples were transported to the laboratory and stored at 4 °C until further testing could be carried out. The research was carried out in the Bioadaptation Laboratory at the Institute of Molecular Biology and Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan.

2.2 DOUBLE-ANTIBODY SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY (DAS-ELISA)

To identify the possible presence of WSMV, BYDV, and WDV, DAS-ELISA tests were carried out using antisera developed by DSMZ (Germany), following the manufacturer's guidelines. For the ELISA procedure, all buffers were prepared according to the provided instructions. Wheat leaf samples were homogenized at a ratio of 1:5 (w/v) in Tris extraction buffer using a sterile mortar and pestle. Next, 100 µl of the extracts were added to microtiter plate wells pre-coated with 100 µl of antibodies diluted at 1:1000 in carbonate coating buffer. The plates were incubated overnight at 4 °C. After incubation, the wells were rinsed with washing buffer and then treated with 100 µl of alkaline phosphatase-conjugated IgG diluted 1:1000 in conjugate buffer. This step was followed by a 3-hour incubation at 37 °C. The wells were then washed again and incubated with 100 µl of substrate for 1 hour at room temperature. Absorbance was measured at 405 nm using a Stat Fax Microplate Reader (Awareness Technology, USA). Each sample was tested in duplicate, and the results were considered positive if the mean absorbance was at least three times higher than the average reading of negative (healthy) controls. In addition to ELISA, the presence of these viruses was also confirmed using RT-PCR.

Table 1: Primers used for viral DNA amplification.

Name	Sense	sequence [5'-3']	Tm (°C)	PCR Fragment size
WSMV1	C	TGCGGAACCTTATCGACAACA	61.4	178
WSMV2	V	AATCACACGCTGCCACAATA	56.2	
BYDV1	C	CCGGCGCTATCTTTATTGAA	62.8	404
BYDV2	V	CCATTGGCCTTGTAGAGCAT	57.4	
WDV3	C	TTTRTCTTGCTCGTAGCCGAGC	53.3	550
WDV5	V	AATAATCGGCATACAAATCAGACC	58.8	

2.3 RNA EXTRACTION

Total RNA was extracted using a modified CTAB method based on Sambrook et al. (1989). For this, 200 mg of scraped bark tissue from basal nodes, petioles, or midribs, as well as 100 mg of leaf tissue, were processed. Leaf tissue was added to 1 ml of pre-warmed extraction buffer containing β-mercaptoethanol inside sterile extraction bags. The samples were incubated at 60 °C for 20 minutes with occasional vortex. An equal volume of chloroform : isoamyl alcohol (24:1) was then added, and the mixture was vortexed for 10 minutes. After centrifugation at 10,000 rpm for 15 minutes at 4 °C, the upper aqueous phase was carefully collected. To precipitate RNA, 1/3 volume of lithium chloride (LiCl) was added, and the samples were incubated on ice at 4 °C, followed by centrifugation at 10,000 rpm for 20 minutes at 4 °C. The resulting pellet was resuspended in DEPC-treated water, along with 0.2 volumes of sodium acetate (pH 5.2) and 2 volumes of 100 % ethanol. The mixture was incubated at -20 °C for 2 hours and then centrifuged at 10,000 rpm for 15 minutes. After discarding the supernatant, the pellet was washed with 70 % ethanol, air-dried, and stored at -20 °C or -80 °C for future use. The concentration and purity of the extracted RNA were assessed using a Nano-Drop 2000C Spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 230, 260, and 280 nm, and the A260/A280 and A260/A230 ratios were calculated. Additionally, RNA integrity was confirmed via 1 % agarose gel electrophoresis, with the RNA bands visualized under UV light after ethidium bromide staining.

2.4 REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR)

RT-PCR was performed using virus-specific primers targeting a region of the coat protein (CP) gene (see Table 1 for details).

RT-PCR was carried out in two steps using an "Applied Biosystems 2720 Thermal Cycler" (USA). To begin, 2 µl of extracted RNA was reverse transcribed into

complementary DNA (cDNA) in a final reaction volume of 20 μ l. The reaction mix included 2 μ l of 10 x RT buffer (containing 0.5 M Tris-HCl, 0.7 M KCl, 0.1 M MgCl₂, pH 8), 1 μ l of DTT (100 mol μ l⁻¹), 1 μ l of dNTPs (10 mmol μ l⁻¹), 0.5 μ l of RNase inhibitor (10 mmol μ l⁻¹), and 2 μ l of reverse primer (100 mmol μ l⁻¹). This mixture was incubated for one hour at 47 °C, along with 0.5 μ l of MMuLV reverse transcriptase (200 U μ l⁻¹).

For the PCR step, 5 μ l of the reverse transcription product was used. The PCR reaction was carried out in a final volume of 20 μ l, containing 20 ng of cDNA, 10 mM of each dNTP (Solis BioDyne, Estonia), 1.6 mM MgCl₂, 1U of Taq DNA polymerase (Solis BioDyne, Estonia), 0.5 μ l of each primer (10 pmol μ l⁻¹), and 1X PCR buffer. PCR products were stored at 4 °C or -20 °C and analyzed via electrophoresis on a 1.5 % agarose gel, using 1X Tris-Borate-EDTA (TBE) buffer and staining with ethidium bromide (EB). The DNA bands were visualized using a UV-Gel Doc system (UK), and the size of the PCR products was estimated with a 2-log DNA Ladder (NEB, UK).

2.5 TESTING METHOD FOR SEED-TRANSMITTED VIRUSES

The percentage of virus seed transmission (ST) was calculated using the following formula:

$$ST = (n * 100) / N$$

Where n represents the number of virus-infected plants, confirmed through symptom observation, ELISA,

and RT-PCR, and N is the total number of plants grown from virus-infected seeds under controlled, protected soil conditions (Albrechtsen, 2006). Statistical analysis of the experimental data was performed using parametric tests for normal distribution. To ensure more reliable results, advanced statistical software such as R or SPSS was employed for data analysis. These tools provide robust methods for calculating standard deviations, analysing variance, and testing hypotheses, offering greater accuracy and reproducibility in experimental outcomes.

3 RESULTS AND DISCUSSION

Wheat is the second most widely grown cereal grain in the world, after maize, and its global trade exceeds that of any other crop. In 2020, total wheat production worldwide reached 760 million tons. China, India, and Russia are the top three producers, together accounting for about 41 % of global wheat output. In Azerbaijan, wheat, particularly winter wheat, is vital to the country's food security. Despite its importance to the national economy, the average wheat yield over the past decade has been around 32,101 kilograms per hectare (Figure 1).

To study the presence of viruses in wheat, 157 samples showing virus-like symptoms such as mild chlorosis, downward leaf rolling, leaf mosaic, streaking, distortion, and plant stunting were collected from eight fields in Azerbaijan during the 2022-2024 growing seasons (Figure 2). Yellowing leaves and stunted plants

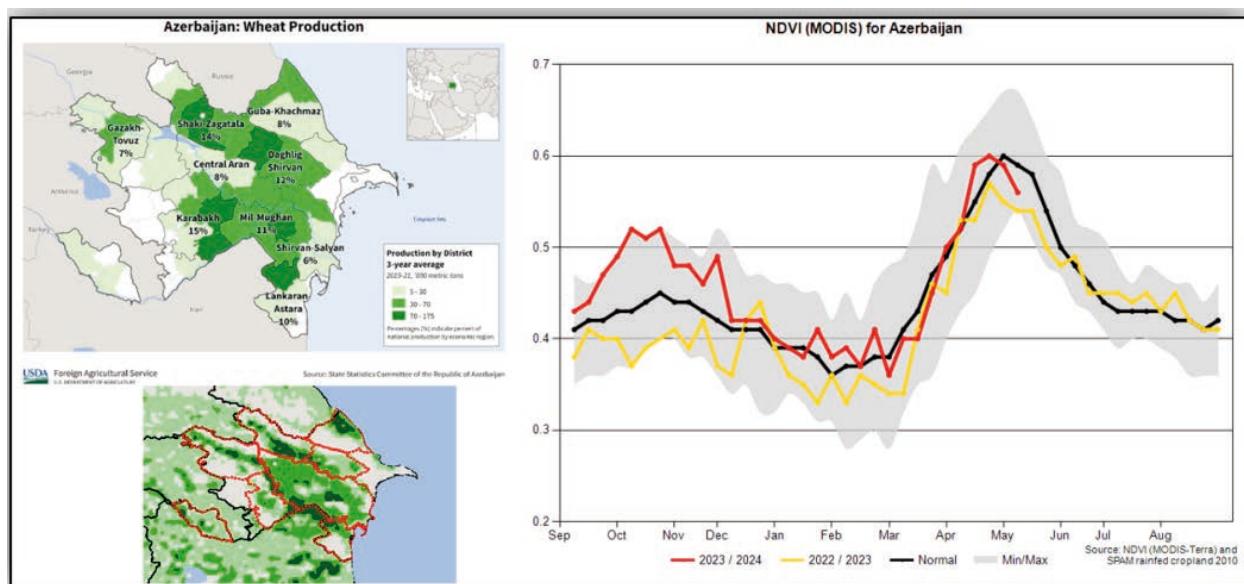


Figure 1: Wheat area, yield, and production in Azerbaijan (Source: State Statistical Committee of the Republic of Azerbaijan, <https://ipad.fas.usda.gov/>).



Figure 2: Virus-induced symptoms observed on wheat plants during the survey in Jalilabad, including mild chlorosis, downward leaf rolling, leaf mosaic, streaking, distortion, and plant stunting.

were common signs of viral infections in cereals, with a noticeable reduction in the root system. No significant differences were observed in the occurrence of virus symptoms across regions and fields based on visual assessments.

Our research, based on serological tests, showed that out of all the samples exhibiting virus-like symptoms, 24.84 % (39 out of 157) tested positive for WSMV, 28.03 % (44 out of 157) for WDV, and 15.92 % (25 out of 157) for BYDV (Figure 3).

Notably, incidence of BYDV increased steadily over the years, with 16.29 % of samples testing positive in 2022, 21.22 % in 2023, and 27.17 % in 2024 (Figure 4). Long-term monitoring of wheat fields from 2022 to 2024, coupled with virus detection through serological and molecular methods, revealed an alternating dominance of WSMV, BYDV, and WDV across the study period.

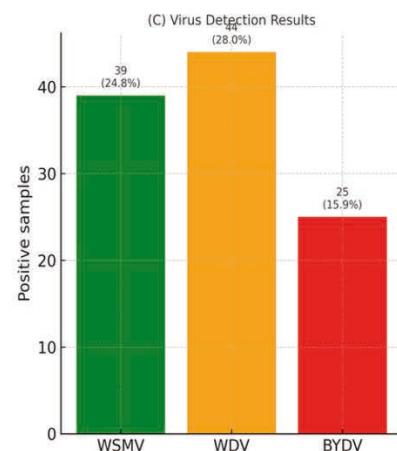
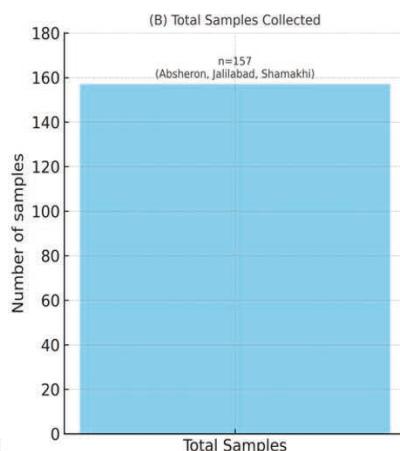
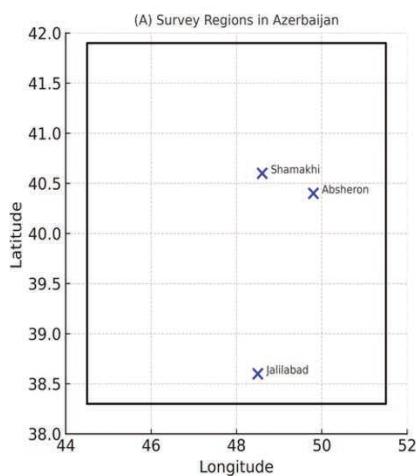


Figure 3: Frequency of wheat virus occurrence in Azerbaijan during the 2022-2024 growing seasons. (A) Map of Azerbaijan with highlighted study regions (Absheron, Jalilabad, Shamakhi), (B) Sample distribution (total n = 157 across 3 regions), (C) Virus detection results (WSMV, WDV, BYDV) with counts and percentages.



Figure 4: ELISA test results for wheat viruses WSMV, WDV, and BYDV during the 2022-2024 growing seasons.

No instances of co-infection with the studied viruses were detected. To further confirm the results from the serological tests, molecular analyses (RT-PCR and PCR) were performed. Phloem scrapings from infected leaves were examined using RT-PCR. Specific primers, BYDV1/BYDV2, WSMV1/WSMV2, and WDV3/WDV5, were used to amplify a segment of the coat protein gene (CP) for each virus. cDNA was synthesized from the total RNA extracted from the samples. The RT-PCR results showed amplification products of 550 bp, 178 bp, and 404 bp, confirming the presence of WDV, WSMV, and BYDV, respectively (Figure 5).

Total RNA was extracted from juvenile leaves of 12 different wheat samples. The expected amplification products were 550 bp for WDV, 178 bp for WSMV, and 404 bp for BYDV, confirming the presence of these viruses. Lane M represents the DNA marker (2-Log DNA Ladder, NEB, UK), and lanes 1-13 show the results from

the tested wheat samples. The RNA and RT-PCR products were separated in a 1.5 % agarose gel.

A thorough review of global literature on wheat viruses reveals a range of viral pathogens affecting wheat crops, posing significant risks to agricultural productivity and food security. The most extensively studied wheat viruses include *Wheat streak mosaic virus* (WSMV), *Barley yellow dwarf virus* (BYDV), and *Wheat dwarf virus* (WDV), all of which exert widespread impacts on wheat production. BYDVs, in particular, are regarded as the most significant cereal viruses due to their global distribution and their capacity to cause considerable yield losses, estimated at 15–25 % in wheat, barley, and oats (Karaozan & Usta, 2020). Furthermore, these viruses have a remarkably broad host range, infecting not only cereals but also numerous weed species and more than 150 grass varieties occurring in meadows and pastures. Research by Spaar (2008) and Schubert *et al.* (2015) emphasizes the economic importance of WSMV, which is transmitted by the wheat curl mite and can lead to significant yield losses. Studies on BYDV, such as those by Karaozan (2020), highlight its complex transmission mechanisms, involving various aphid species and contributing to yellow dwarf disease complexes that affect wheat worldwide (Jones, 2003). Additionally, WDV, first identified in Europe, has been spreading globally, with Lindsten and Vacke (1991) documenting its expansion and its detrimental effects on wheat and barley crops. The spread of WDV has also been linked to the increased movement of infected plant material and environmental changes (Przybył *et al.*, 2020). These studies collectively underscore the need for continuous surveillance, advanced diagnostic techniques, and integrated manage-

ment approaches to combat wheat viruses and protect global wheat production (Sahragard *et al.* 2010; Wosula *et al.*, 2014).

Seed-transmitted wheat viruses are of particular concern for wheat production, as they can be passed through infected seeds and establish infections in future generations. WSMV and BYDV are among the key viruses transmitted via seeds, and their impact on wheat yield and quality can result in considerable economic losses (Kashiwabara *et al.*, 2007; Li *et al.*, 2011). Detecting and managing these seed-transmitted viruses rely on advanced diagnostic methods, such as serological assays and molecular techniques, which can identify viral pathogens in seeds (Xie *et al.*, 2017). However, these methods can sometimes overestimate transmission rates, as they may detect inactivated viruses within seed parts. Typically, only viruses capable of infecting the seed embryo can be transmitted to the next generation. However, certain viruses, such as those from the genus *Tobamovirus*, can survive in or on seeds without entering the embryo and can still infect subsequent plants (Chen *et al.*, 2016). Furthermore, embryo-transmissible viruses can remain in an inactivated state in seed parts like the endosperm or testa, even if the embryo itself is not infected (Elena & Lenske, 2008). Understanding the specific mechanisms and conditions under which these viruses are transmitted via seeds is crucial for developing effective control strategies (Baranwal *et al.*, 2019).

To investigate seed transmission of WSMV, twelve seeds from WSMV-infected Azamatli cultivar plants were treated with 3 % hydrogen peroxide for 20 minutes, followed by rinsing with water. The seeds were then sprouted in Petri dishes under moist conditions

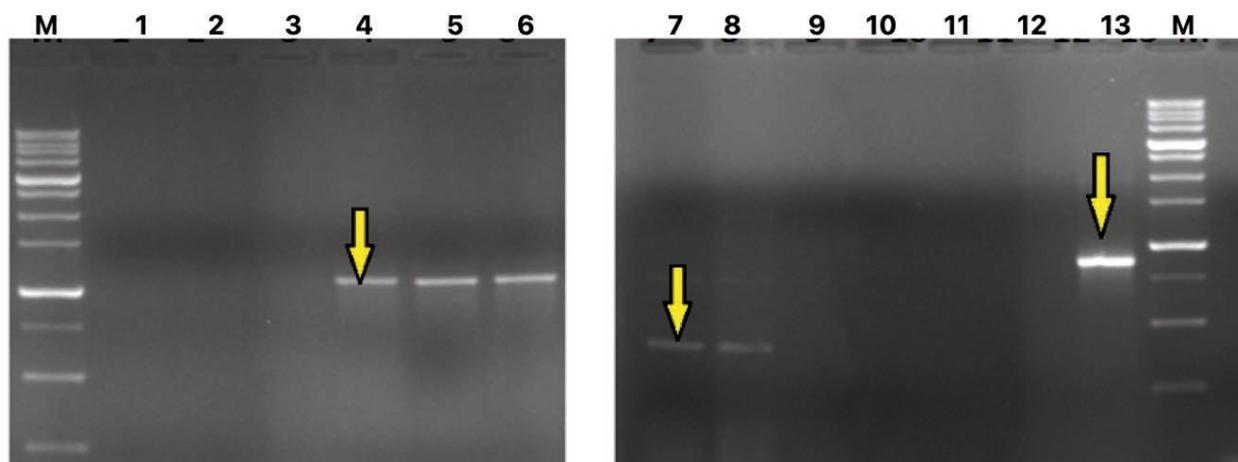


Figure 5: Agarose gel electrophoresis results showing the RT-PCR detection of WDV (4-6) WSMV (7-8), and BYDV (13) using the primer pairs WDV3/WDV5, WSMV1/WSMV2, and BYDV1/BYDV2 respectively. 1- water; 2,3-negative control, WDV; 9,10-negative control, WSMV; 11,12-negative control, BYDV. M-DNA marker-2-log DNA Ladder (NEB, UK).

and planted into pots with sterile soil in a vector-free greenhouse, with a temperature range of 23-24 °C and illumination of 105–135 $\mu\text{mol m}^{-2} \text{s}^{-1}$ lux. When the plants reached the 2-4 leaf stage, the number of successfully grown plants was recorded. The wheat plants were inspected for viral symptoms and tested for WSMV using ELISA. Virus seed transmission (ST) was calculated based on the formula from Albrechtsen (2006). The ELISA results were confirmed by the absence of WSMV symptoms in the wheat plants. It was found that both the grains and leaves of new-generation wheat plants did not contain WSMV antigens, indicating that the virus was not transmitted via seeds. Similar findings were reported in earlier studies (Mishchenko, 2009; Mishchenko et al., 2018; Knudson et al., 2010). Robust seed health testing and certification processes are essential for reducing the spread of seed-transmitted wheat viruses, ensuring the health and productivity of wheat crops (Ellis et al., 2014). The findings of this study highlight critical gaps in existing knowledge, particularly the absence of comparative assessments of agroclimatic factors influencing virus dissemination across the contrasting regions of Azerbaijan. In addition, the dynamics of co-infections remain under-explored, and there is limited evidence regarding the performance of molecular diagnostic tools under local field conditions. By addressing these issues, the present research provides an important step toward characterizing the regional determinants of viral disease prevalence in cereal crops under diverse agroecosystems of Azerbaijan.

4 CONCLUSIONS

This study highlights the significant impact of viral infections, particularly WSMV, BYDV, and WDV, on wheat crops in Azerbaijan, with a notable increase in the prevalence of BYDV over the three-year study period. Long-term monitoring and the use of advanced diagnostic techniques, such as ELISA and RT-PCR, confirmed the presence and dynamics of these viruses, emphasizing the importance of continuous surveillance and diagnostic precision for effective management. The absence of seed transmission of WSMV, as demonstrated through controlled experiments, underscores the necessity for robust seed health testing and certification processes to prevent the spread of viral infections through infected seeds. The findings underline the need for integrated virus management strategies, combining field surveillance, molecular diagnostics, and vector control, to mitigate the risks posed by wheat viruses and ensure sustainable agricultural productivity in Azerbaijan and beyond.

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