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MOLECULAR-GENETIC ANALYSIS OF CEREAL YELLOW DWARF VIRUS-RPV (CYDV-RPV) AFFECTING WHEAT (*TRITICUM AESTIVUM* L.) IN UZBEKISTAN

T.KH. MAKHMUDOV[1,4]*, Z.N. KADIROVA[1], Z.M. ZIYAEV[1,4], B.SH. ADILOV[1], A.G. SHERIMBETOV[1], N.E. CHORSHANBIEV[1], O.R. ERGASHEV[1], Z.A. UMAROV[2], S.U.NOROVA[2], M.R. TOKHIROVA[2], K. ASTANAKULOV[3], R. EGAMBERDIEV[3], U.X. YULDASHOV[4], and V.B. FAYZIEV[5]

[1]Institute of Genetics and Plants Experimental Biology, Academy of Sciences, Tashkent, Uzbekistan
[2]Scientific-Research Institute of Horticulture, Viticulture and Winemaking named after Academician M. Mirzaev, Tashkent, Uzbekistan and
National University of Uzbekistan named after Mirzo Ulugbek, Tashkent City, Uzbekistan
[3]Tashkent Institute of Irrigation and Agricultural Mechanization Engineers, National Research University, Tashkent City, Uzbekistan
[4]Research Institute of Plant Genetic Resources, Tashkent, Uzbekistan
[5]Chirchik State Pedagogical University, Department of Natural Sciences, Chirchik, Uzbekistan
*Corresponding author's email: tohir_m@inbox.ru
Email addresses of co-authors: zarifakodirova@yahoo.com, zafaruzripi@gmail.com, bakhti.genetics@gmail.com, sher.anvar1981@gmail.com, nurik_1980@mail.ru, zafarumarov1982@gmail.com, 1985.sarvinoz@gmail.com, tohirovamalohathon@gmail.com, komiljon.astanaqulov@gmail.com, ramish_1976@mail.ru, utkirbekyul@gmail.com, fvaxid@mail.ru

SUMMARY

Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal worldwide, a staple food for 40% of the world's population that contributes 20% of total dietary requirements. Yellow dwarf viruses are viruses affecting the grain yield and its quality and damaging the cereal crops economically. Yellow dwarf viruses are the most economically important plant viruses impacting cereal production worldwide, which include viruses from the genus *Luteovirus* BYDV-PAV, MAV, and *Polerovirus* CYDV-RPV. The Cereal Yellow Dwarf Virus (CYDV-RPV) is one of the most dangerous YDV viral species. Barley/cereal yellow dwarf viruses cause yellow dwarf disease, which is a continuous risk to cereal crop production globally. These viruses cause leaf yellowing and stunting, resulting in yield reduction of up to 80%. They are the most critical viral diseases of cereals worldwide. They have a wide host

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range, which includes wheat, barley, oats, and over 150 grass species. These viral infections have the potential to spread epidemics in Uzbekistan's wheat fields during specific seasons and play a crucial role in the epidemiological process. Using the KibrayT1 isolate of the CYDV-RPV strain, the following study aimed to determine the prevalence of CYDV-RPV in wheat fields in the District of Kibray, Tashkent Region, Uzbekistan. The use of reverse transcription-polymerase chain reaction succeeded in making the diagnosis.

Keywords: Wheat (*T. aestivum* L.), cereal yellow dwarf virus (CYDV), RT-PCR, CP gene, phylogenetic analysis, KibrayT1

Key findings: The CYDV-RPV isolate detection was successful by RT-PCR in wheat (*T. aestivum* L.). The determination of the nucleotide sequence of the coat protein gene prevailed in this isolate. The phylogenetic analysis revealed its close relatedness to the Jordan CYDV-RPV isolate 'Jordon' (HQ206716.1).

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal crop in the world, grown on more land than any other commercial crop. In 2021, wheat production reached 771 million tons worldwide, making it the second most-produced cereal after maize (FAO, 2022). As a member of the Poaceae family, wheat is considerably essential to livelihoods, economics, and food security. In wheat production, EU nations lead the globe (up to 23%), followed by China (19%) and India (15%) (FAO, 2018). For the world's population, wheat is the primary source of carbohydrates and protein, supplying about 55% and 21% of the world's carbohydrates and food calories, respectively. Wheat is also the leading source of vegetable protein in human food, with a protein content of about 13%. Wheat growing can survive in a wide range of climates, from the Arctic Circle to higher elevations near the equator. The optimum required growing temperature is about 25 °C. World trade in wheat is greater than for all other crops combined.

Fungal, bacterial, viral, nematodal, physiological, and other diseases caused by a lack of mineral nutrition and other abiotic factors occur on wheat (Sherimbetov *et al.,* 2020, 2024). The most crucial thing a nation can do to prevent the spread of potentially fatal viruses, bacteria, and other pathogens causing life-threatening foodborne illnesses (salmonellosis and *E. coli* infections) is ensuring food safety and good health worldwide. In the present circumstances, food safety is crucial to lessen the impact of financial losses caused by various pathogens and especially the viruses. Numerous viral diseases and other environmental stresses frequently target the wheat crop, potentially lowering its yield and quality. Nevertheless, there is no known efficient chemical control to address the viral diseases in cereals. Among the top four causes of the decline in wheat yield, barley yellow dwarf group (BYDV) viruses cause significant losses annually globally. The BYDV infection in wheat can cause symptoms like spotting, yellowing, crimson leaves, resetting, loss of green color, dwarfing, and necrosis, which are frequently misattributed to abiotic causes (Erkan and Yılmaz, 2009).

Several diseases may be the cause of severe symptoms in wheat. *Luteovirus*, *polerovirus*, and *enamovirus* are the three genera of wheat viruses belonging to the family Luteoviridae. Typically, BYDV viruses are prevalent in the cytoplasm, parenchyma cells, and nucleus. Based on genetic characteristics, dividing the viruses of this category (RPV, SGV, MAV, RMV, and PAV) into five species depended on their vectors (Aradottir and Crespo-Herrera*,* 2021; Lister and Ranier*,* 1995). Following 5612 nucleotide sequence

analysis, the virus formerly known as Barley yellow dwarf virus (RMV) bore renaming as the maize yellow dwarf virus (RMV), being classified in the genus *Polerovirus* (Miller *et al.,* 2002).

Transmission of these viruses transpires by more than 25 species of aphids worldwide; however, in temperate climates, the most relevant aphid vectors responsible for BYD viruses are *Rhopalosiphum padi, Rhopalosiphum maidis, Sitobion avenae,* and *Schizaphis graminum*. The aphids feed upon and transmit the viruses to various grain crops, such as wheat, barley, oats, rye, and corn, as well as over 150 non-crop grass species in the family Poaceae (Miller and Rasochová, 1997).

Presently, different viral diseases occur in wheat plants that weaken the wheat crop's immunity. Viral diseases have a greater impact on wheat yield, and their infection in other grain crops has also been evident. As an important cause of plant diseases, dividing plant viruses can have the following types according to their genome characteristics: Solemoviridae is a typical family of viruses with a positive single-stranded RNA (+ssRNA) genome and currently includes four genera (*Enamovirus*, *Polemovirus*, *Polerovirus*, and *Sobemovirus*) (Walker *et al.,* 2021).

Barley yellow dwarf viruses (PAV and MAV) belong to the genus *Luteovirus* and family Tombusviridae, with the cereal yellow dwarf virus (RPV) also being detected in many wheat-growing countries (Nancarrow *et al.,* 2023). The CYDV is the most common virus in crop plants belonging to the Poaceae family. In wheat, the CYDV symptoms are similar to those of BYDV and include dwarfing, yellowing, and reddish and pink coloration of wheat leaves and result in stunted plant growth (Erkan and Yılmaz*,* 2009).

Similarly, Miller and Rasochova and Pike determined the average yield losses caused by BYDVs or the CYDV to be between 11% and 33% and sometimes up to 80% in the wheat fields. YDVs are the most destructive virus diseases on cereal crops, comprising a complex virus group (Miller and Rasochová, 1997).

The CYDV transmission is by aphids from diseased plants into healthy ones, spreading through the phloem layer, infecting all the organs of the plant, and resulting in yield losses of 5% to 10%. The CYDV is not spread mechanically, by pollen grains, or by seeds. The CYDV main aphid vectors are the *Rhopalosiphum padi* and *Schizaphis graminum* (Yang *et al.,* 2008). The CYDV is a widely studied threat in various countries and is one of the most common viruses in plants found in the family Poaceae. These viruses have reports of infestations in Brazil, Australia, Turkey, and also in Uzbekistan (Makkouk *et al.,* 2001; Parizoto *et al.,* 2013; Nancarrow *et al.,* 2014; Karaozan and Usta*,* 2020).

In Uzbekistan, this virus is prevalent; therefore, the conduct of research progressed on the isolation and molecular characterization of the viruses that infect grain plants, including corn, millet, wheat, and barley; however, the viral infection severity relied on the plant genotype (Sobirova *et al.,* 2020, 2023*;* Makhmudov *et al.,* 2023, 2024). Determination of the molecular genetic characteristics of viruses infecting other crop plants emerged, including plums and potatoes, and their effect on some strain-specific physiological characteristics of crop plants (Sattorov *et al.,* 2020; Fayziev *et al.,* 2020; Yusubakhmedov *et al.,* 2024). Research has been progressive on the preparation of specific serum necessary for diagnosing an endemic strain of the virus and its use in diagnosing the virus (Akhmadaliev *et al.,* 2024; Jovlieva *et al.,* 2024).

The scientific research has been ongoing on various wheat viruses, including the barley yellow dwarf virus, wheat streak mosaic virus, and soil-borne wheat mosaic virus in Uzbekistan. The genetic diversity of the CP gene of BYDV-PAV isolates T-UZB1 and T-UZB2 has been in distribution to wheat fields of the Tashkent and Navoiy regions (Makhmudov *et al.,* 2023, 2024). In the presented study, the genetic diversity of the CP gene of CYDV-RPV KibrayT1 isolate underwent investigation, with its spread probed in wheat fields using RT-PCR (Figure 1).

Figure 1. Electropherogram of RT-PCR performed with RPV-L/RPV-R primers in wheat samples from Tashkent Region; M - DNA 100 bp DNA Ladder; 3,5 Wheat samples.

Table 1. Name and sequence of forward and reverse PCR primers designed for detecting CYDV-RPV.

Target region	Name of the primers	Sequence (5′-3′)	Product size (bp)	Source
CP	RPV-L	ATGTTGTACCGCTTGATCCAC	400	Deb and Anderson (2008)
CP	RPV-R	GCGAACCATTGCCATTG		

MATERIALS AND METHODS

Sample collection

In April–May 2023, an extensive survey succeeded in the cereal-growing areas of the Tashkent Region, Uzbekistan. Virus-like symptoms of CYDV disease gained visual studies in wheat fields. A collection totaling 60 cereal leaf samples exhibiting yellowing, reddening, stripe mosaic, irregular necrotic patches, and dwarfing symptoms came from cereal fields in the Tashkent Region. The samples' transport to the laboratory continued for additional procedures after being kept at -20 °C in an Alpicool (Russia) refrigerator. Plant leaves, cut and placed in labeled plastic bags, remained stored at -80 °C to prevent the viral RNA's degradation.

Extraction and purification of total RNA

Leaf samples of 0.1 g received homogenization in liquid nitrogen before extracting the total RNA according to the manufacturer's protocol, using the Invitrogen™ PureLink™ RNA Mini Kit (Thermo Fisher Scientific, USA). Measurements based on quantity and quality of total RNA also ensued using a spectrophotometer NanoDrop Eight (Thermo Fisher Scientific, USA), and then, storing the RNA samples at -80 °C until used for RT-PCR.

Synthesis of the cDNA

The cDNA's synthesis used the SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, USA) and buffers in a final volume of 20 µl. From the total extracted RNA solution, 5 ml incurred mixing with 1 µl of 10 µM reverse primer Lu4 (Table 1), 4 µl of 2.5 mM dNTP, and 3 µl of nuclease-free water. Afterward, the samples' incubation commenced at 65 °C for 5 min before quenching on ice for 3 min. The other reaction components' addition was in the following order: 4 µl of 5X PCR buffer, 1 µl of 0.1 M of dithiothreitol, 0.5 µl (20 U) of SuperScript IV reverse transcriptase, and nuclease-free water to give a reaction volume of 20 µl. The tubes underwent incubation for 1 h 55 min at 41 °C on a T960 PCR Thermal Cycler, followed by 10 min of heating at 70 °C to denature the enzyme.

Amplification by PCR

Reverse (RPV-R) and forward primer (RPV-L) tend to amplify a 400 bp PCR product within the viral coat protein gene of CYDV. The conduct of PCR reactions used the Platinum Hot Start PCR 2x master mix (Thermo Fisher Scientific, USA, https://www.thermofisher.com) in a 25 µl volume, containing 4 µl of 2X Master Mix, 0.5 µl of each primer (10 µM), 0.9 µl of 25 mM MgCl, and 4 µl of the cDNA. The following thermocycling program operated for PCR: initial denaturation at 94 °C for 2 min, then 45 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, elongation at 72 °C for 1 min, and 1 cycle of final elongation at 72 °C for 5 min. The names and sequences of the forward and reverse PCR primers used to detect CYDV appear in Table 1.

Electrophoresis in agarose gel

Analysis of PCR products proceeded by electrophoresis on 2% agarose gel prepared in 1x Tris-borate-EDTA buffer (TBE, Thermo Scientific) stained with ethidium bromide. The adding of 2 µl of DNA Gel Loading Dye (6X) and 1.3 µL of 100 bp DNA Ladder to the first well of agarose gel ensued. Following this was the addition of 10 µl of PCR products with 3 µl of DNA Gel Loading Dye (6X) to the prepared agarose wells. Electrophoresis took place using the horizontal electrophoresis system SE-1 (Helicon, Russia) at 100 V for 100 min. The PCR products reached visualization by the UV light, as photographed by using a gel document imaging system BK-AG100 (Biobase, China).

Sequencing of PCR products

For sequence analysis, PCR products' purification from agarose gels used the PureLink™ Quick Gel Extraction Kit (Thermo Fisher Scientific, USA). The cycle sequencing reaction progressed using the BigDye® Terminator v 3.1 kit (Thermo Fisher Scientific, USA). The cycle sequencing reaction consists of ddH^2O-3.5 µl, BigDye-1 µL, 5x Seg. buffer-2 µl, sequencing primer-0.5 µl, and purified PCR product-2 µl. RPV-L1 and RPV-R primers succeeded in usage for sequencing. The following thermocycling program served for the cycle sequencing reaction: initial denaturation stage at 96 °C for 1 min; denaturation at 96 °C for 10 s, annealing at 53 °C for 10 s, and elongation at 60 °C for 3 min, repeated for 40 consecutive cycles. The product of the sequence reaction remained stored at 4 °C. The sequencing reaction products reached purification from fluorescently labeled terminator nucleotides using the Dynabeads Sequencing Clean-Up Kit (Thermo Fisher Scientific, USA). The separation and analysis of DNA sequence reaction products by capillary gel electrophoresis with the laser-induced fluorescence detection ran on the Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific) sequence alignments and phylogenetic analyses. Using the SnapGene 5.3.1 program edited the raw data obtained from the DNA sequence analysis system. Nucleotide sequences' scrutiny employed the BLASTN program (basic local alignment search tool nucleotide-nucleotide, http://www.ncbi.nlm.nih.gov/BLAST/) (Camacho *et al.*, 2009). Multiple sequence alignments engaged the ClustalW. Phylogenetic tree construction by the neighbor-joining (NJ) method used the Kimura 2-parameter model, with the bootstrap method of interior-branch test for phylogenetic trees providing statistical support (Kimura, 1980). Bootstrap test of phylogeny and visualization of the final phylogenetic tree transpired by the Molecular Evolutionary Genetics Analysis version 11 (MEGA11 software package) (Tamura *et al*., 2021) (Figure 2).

RESULTS

The monitoring and identification of CYDV symptoms commenced in April–May 2023 in the wheat fields of the Tashkent Region, Uzbekistan. During the field survey, wheat plants showed typical symptoms of virus infection, like yellowing, reddening, stripe mosaic, irregular necrotic patches, and dwarfing symptoms (Figure 3). Moreover, during the field survey, the large populations of

Figure 2. Neighbor-Joining (NJ) tree for the *CP* gene of BYDV-PAV isolates. Numbers above branches are bootstrap support values based on 1000 replicates.

Figure 3. CYDV disease symptoms in wheat (*Triticum aestivum* L.) in fields of Tashkent Region, Uzbekistan.

aphid vectors *Rhopalosiphum padi* and *Schizaphis graminum* were visibly colonizing wheat. In addition to the aphid vectors *Rhopalosiphum padi* and *Schizaphis graminum*, the prevalence of *Sitobion avenae* and *Rhopalosiphum maidis* prevailed in wheat fields in the Tashkent Region. The collected samples of the wheat-infected plants came from the surveyed fields (41°24′52.0″N 69°28′21.0″E, 41°04′51.3″N 69°19′38.1″E, 41°11′08.0″N 69°19′48.0″E, and 41°06′28.5″N 68°58′33.2″E).

In the typically symptomatic wheat samples, the viral RNAs' amplification occurred by an approximately 400 bp fragment, and the occurrence of CYDV gained confirmation by sequencing and BLAST analysis. The obtained sequence succeeded in being deposited into the GenBank NCBI database under accession number PP083321. A phylogenetic tree, which was also generated from the alignment of the nucleotide sequence of the Kibray-T1 isolate, confirmed the isolates were from Jordan, Germany, Turkey, and the USA. The

evolutionary tree demonstrated that all CYDV strains' grouping can comprise three main clades. The Clade-I virus strains were mainly from Jordan and Turkey, the Clade-II virus strains were primarily from Estonia and the USA, while the Clade-III virus strains mainly came from Germany.

The BLAST analysis of Uzbekistan enunciated that the isolate Cereal yellow dwarf virus RPV isolate Kibray-T1 (PP083321.1) emerged against a nucleotide sequence database, authenticating that said isolate was also similar to other isolates ranging from 93.20% to 96.46%. The said isolate showed the highest genetic similarity (96.46%) with the Jordan Cereal Yellow Dwarf Virus-RPV isolate (HQ206716.1). However, it showed the lowest similarity rate (93.20%) with the Germany Cereal yellow dwarf virus RPV isolate 102A (KU975373.1).

For phylogenetic analysis, the partial nucleotide sequence of replicase and coat protein genes of the Uzbekistan CYDV-RPV Kibray-T1 (PP083321.1) isolate was compared with 13 strains of CYDV-RPV available in the GenBank. According to the constructed phylogenetic tree, the CYDV Kibray-T1 isolate achieved clustering with the CYDV-RPV 'Jordon' isolate (HQ206716.1) and with two Turkey CYDV-RPV isolates (KT933457 and KT923556). Phylogenic analysis and multiple sequence alignment (using ClustalW) considerably suggested that 16 mutation events, i.e., 438C>T, 450C>T, 459C>T, 524C>T, 541A>T, 556A>T, 581A>T, 602A>G, 618A>T, 643T>C, 652G>A, 655C>T, 661A>T, 685C>T, 688G>A, and 742T>C, involving the CYDV-RPV isolate Jordon and TR-6, have considerably contributed to the evolutionary history of CYDV-RPV Kibray-T1 Uzbekistan haplotype (Figure 4). However, the one mutation, 556A>T, was a missense mutation that resulted in amino acid substitutions N186Y in the RNA-dependent RNA polymerase (Figures 5, 6, and 7). The other 15 (438C>T, 450C>T, 459C>T, 524C>T, 541A>T, 581A>T, 602A>G, 618A>T, 643T>C, 652G>A, 655C>T, 661A>T, 685C>T, 688G>A, and 742T>C) were the silent (synonymous) mutations that have no effect on the amino acid sequence coded for by the *RDRP* gene (Figure 8).

DISCUSSION

Climate change has been an alarming issue since the beginning of the 21st century. Besides, deteriorating earth and abrupt climate change have direct and indirect effects on biotic and abiotic stresses faced by crop plants. Considering the increasing rate of the world's population and climate change-induced increase in temperature, expect wheat production to reduce by 2050 (Ray *et al.,* 2013). Barley yellow dwarf virus symptoms were first notable in 1951 in California (Guy *et al.,* 1987), and shortly thereafter, these proliferated elsewhere in North America, Europe, and Australasia.

Currently, the barley yellow dwarf virus is active within the world's cereal-growing regions and grasslands and significantly suppresses the wheat grain yield. One of the biotic factors damaging the wheat is aphids. Aphids not only damage the crop by sucking the leaf sap but, as vectors, also transfer the BYDV/CYDVs into plant cells. In mitigating the aphid problems, farmers mostly used some insecticides at the adult stage, which worked effectively; however, for controlling viruses, some host resistance is essential. The *Bdv2* genes were well reported to have potential against BYDV/CYDVs. Gene expression profiling of wheat and barley genotypes has progressive studies to identify novel genes associated with resistance to BYDV-PAV. Although some resistance genes are known to confer resistance, such as the *BYd2* gene, their incorporation into wheat or barley varieties remains limited (Jarošová *et al.,* 2016).

The CYDV is widespread in the world and infects the cultivated and wild plant species belonging to the family Poaceae, causing 15%–25% yield losses in wheat, barley, and oats (Lister and Ranier, 1995). Wheat viruses have a wide range of hosts and infect both cereals and weeds and more than 150 species of meadow, pasture, and grass crops (Gould and Shaw, 1983). The potential study evaluated the prevalence of cereal yellow dwarf viruses in wheat plants in the Tashkent Region, Uzbekistan. Typical viral infection symptoms, such as redness of flag leaves, dwarfing, and the leaf chlorotic stripe patterns,

Figure 4. Alignment of replicase and coat protein genes sequences CYDV-RPV isolate TR-6 Turkey, Jordon (Jordan), and CYDV-RPV Kibray-T1 (Uzbekistan).

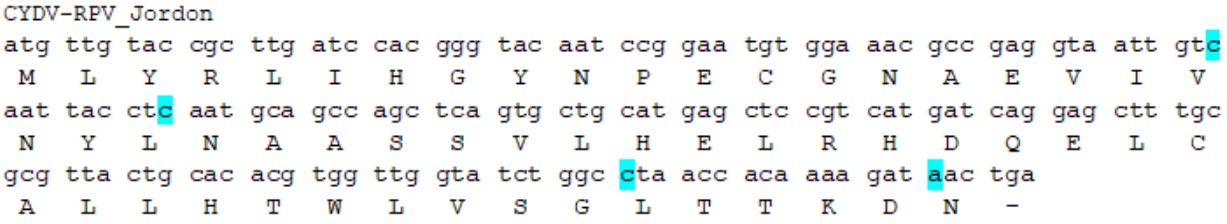

Figure 5. Deduced RNA-dependent RNA polymerase gene of the Jordon isolate (Jordan).

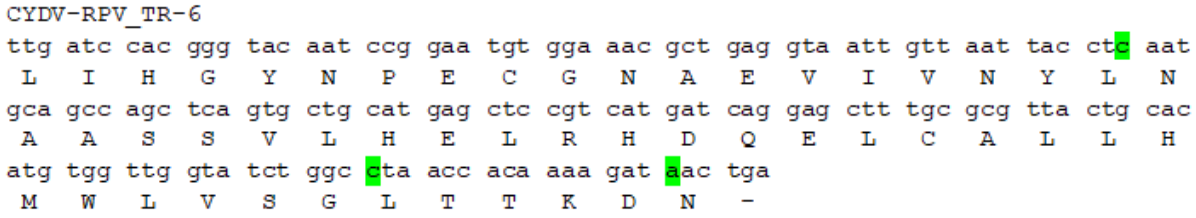

Figure 6. Deduced RNA-dependent RNA polymerase gene of the TR-6 isolate (Turkey).

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CYDV-RPV_Kibray T1
atg ttg tac cgc ttg atc cac ggg tac aat ccg gaa tgt gga aac gcc gag gta att gtt
 M   L   Y   R   L   I   H   G   Y   N   P   E   C   G   N   A   E   V   I   V
aat tac ctt aat gca gcc agc tca gtg ctg cat gag ctc cgt cat gat cag gag ctt tgc
 N   Y   L   N   A   A   S   S   V   L   H   E   L   R   H   D   Q   E   L   C
gcg tta ctg cac acg tgg ttg gta tct ggc tta acc aca aaa gat tac tga
 A   L   L   H   T   W   L   V   S   G   L   T   T   K   D   Y   -
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Figure 7. Deduced RNA-dependent RNA polymerase gene of the Kibray T1 isolate (Uzbekistan).

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                          10        20        30        40        50        60        70        80        90       100
                           |         |         |         |         |         |         |         |         |         |
CYDV_RPV_KibrayT1  ----------------------------------------------------------------------------------------------------
CYDV_RPV_Jordan    ----------------------------------------------------------------------------------------------------
CYDV-RPV_TR-6      ----------------------------------------------------------------------------------------------------
CYDV_RPV_87_2      MLPDDMEVRNRLTIDCNELTRHLRAVWLQGISNSILCLSDGTMLSQVKPGVQKSGSYNTSSTNSRIRVMAAYHCGASWAIAMGDDALEAPDTDLSKYKDL

                         110       120       130       140       150       160       170       180
                           |         |         |         |         |         |         |         |
CYDV_RPV_KibrayT1  ----------------------------MLYRLIHGYNPECGNAEVIVNYLNAASSVLHELRHDQELCALLHTWLVSGLTTKDY
CYDV_RPV_Jordan    ----------------------------MLYRLIHGYNPECGNAEVIVNYLNAASSVLHELRHDQELCALLHTWLVSGLTTKDN
CYDV-RPV_TR-6      ---------------------------------LIHGYNPECGNAEVIVNYLNAASSVLHELRHDQELCALLHMWLVSGLTTKDN
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Figure 8. Alignment of RNA-dependent RNA polymerase gene sequences of CYDV-RPV Kibray T1 isolate (Uzbekistan) with CYDV-RPV TR-6 isolate (Turkey) and CYDV-RPV "Jordon" (Jordan).

were evident in wheat plants of the Tashkent Region, which showed consistency with those symptoms recorded by various researchers.

Sequencing and phylogenetic analysis of the *CP* gene of the CYDV-RPV Kibray-T1 isolate revealed similarities and differences with other strains worldwide. In the phylogenetic tree analysis, one can see that the CYDV-RPV 'Kibray-T1' isolate PP083321.1, isolated from wheat in the Tashkent Region, showed the highest similarity (96.46%) with the CYDV-RPV 'Jordon' isolate (HQ206716.1). The CYDV-RPV "TR-6" (KT923457.1) and "TR-6" (KT923456.1) isolates gave distinction by 96.23%-96.50% similarities.

The RT-PCR assay and molecular identification of the CYDV proved effective in the molecular diagnosis of CYDV infection in wheat in Uzbekistan for early diagnostics and timely conduct of control strategies to prevent the viral disease's spread in crop fields. In the presented study, the RT-PCR assay proved desirable in the molecular diagnosis of CYDV infection in wheat plants in Uzbekistan. Understanding virus diversity will help us to develop the appropriate wheat virus control strategies. Virus resistance is a considered crucial factor in the selection of wheat genotypes and their cultivation.

CONCLUSIONS

Studying viral diseases of wheat crops by visual inspection proceeded in the Tashkent Region, Uzbekistan. Based on the results, the CYDV-RPV strain was prevalent in the Tashkent Region. The nucleotide sequence of CYDV-RPV, as probed before, received the name Kibray-T1 isolate. According to the phylogenetic tree generated with 16 different homologous sequences of the cereal yellow dwarf virus RPV, the cereal yellow dwarf virus RPV Kibray-T1 isolate sequence succeeded in being clustered. The Clade-I virus strains were mainly from Jordan and Turkey, the Clade-II virus strains mainly came from Estonia and the USA, while Clade-III virus strains were primarily from Germany. The RT-PCR assay and molecular identification of CYDV proved

useful for the early detection of CYDV infection and timely control strategies to prevent the spread of viral diseases in wheat fields.

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