

Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology



ISSN: 1019-763X (Print), 2305-0284 (Online) http://www.pakps.com

MOLECULAR IDENTIFICATION BASED ON COAT PROTEIN SEQUENCES OF THE BARLEY YELLOW DWARF VIRUS FROM UZBEKISTAN

^aMakhmudov T. Khalimovich. *, ^aKadirova Nasirovna., ^aAdilov B. Shukhratovich., ^aAbdikarimov B. Qurombayevich ., ^aAbduvaliev B. Abdurashitovich ., ^aZiyaev Z. Mashrabovich., ^aSherimbetov A. Gulmirzayevich, ^bKurganov Sardor

Institute of Genetics and Plants Experimental Biology, Academy of Sciences of Uzbekistan, Tashkent Region, Kibray District, Yuqori-Yuz, 111226 Uzbekistan.
 Institute of Biophysics and biochemistry, 100095, Talabar Street 174.

ABSTRACT

Wheat has long been considered an important plant in the national economy, and several phytopathogenic viruses have been studied to infect this plant, the most common of which is *Barley yellow dwarf virus*. *BYDV* causes great economic damage in agriculture. This article presents the results of research conducted on the *BYDV-PAV* strain isolated from Uzbekistan. As a result of the conducted research, plant samples (leaf, root, stem) collected from wheat fields in Tashkent region were diagnosed by *BYDV-PAV* RT-PCR method using special primers based on nucleotide sequence of ORF3 gene of BYDV-PAV coat protein. As a result of research, the presence of the *BYDV-PAV* strain was found in the collected samples, and the nucleotide sequence of *BYDV-PAV* was studied. BLASTN and phylogenetic analysis of the virus isolate based on the determined nucleotide sequence showed the highest genetic similarity by 99.81% with the Morocco PAV-type isolate MA9501 (AJ007929.1) while the lowest similarity rate was 87.10% with the Tunisian isolate PAV-TN4 (JX402456.1).

Keywords: Wheat, RT-PCR, Cloning, Molecular characterization, Phylogenetic analyses.

INTRODUCTION

Nowadays, ensuring food security is the most important task for every country worldwide. Wheat is one of the first plants cultivated in wide geographical and climatic conditions, and its history goes back 10,000 years. Wheat (*Triticum aestivum* L.) a member of the *Poaceae* family, is the most important cultivated plant after maize. In the world wheat cultivation, EU countries are in first place with 23% share, followed by China with 19% and India in third place with 15% (FAO, 2018). Viral and fungal diseases, various insects, as well as abiotic factors negatively affect the wheat production. Barley yellow

Submitted: April 08, 2023 Revised: May 17, 2023 Accepted for Publication: June 05, 2023 * Corresponding Author: Email: tohir_m@inbox.ru © 2017 Pak. J. Phytopathol. All rights reserved. dwarf virus (BYDV), which infects wheat, is one of the 4 viruses that cause the most yield loss in wheat, with severe effects on yields worldwide each year (Rybicki, 2015). Symptoms of BYDV infection in wheat are similar to those caused by abiotic factors, causing spots, yellowing, reddish, pink color on wheat leaves, plant stunting, and stunting. (Erkan and Yilmaz, 2009). BYDV affects over 100 species in the family Poaceae, including numerous wild grasses and widely grown crops like barley, wheat, oats, sorghum, rye, triticale, corn, and rice.

BYDV is not spread by mechanical means, flower pollen, seeds, only aphids are virus vectors, and about 25 species of aphids are listed in the literature, the main ones are *Rhopalosiphum padi, Macrosiphum (Sitobion) avenae, Schizaphis graminum, Rhopalosiphum maidis, Acrosternum hilare* (Deligöz *et al.,* 2011). BYDV has been widely studied in many countries and is the most common virus in plants of the Poaceae family. BYDV in Brazil (Parizoto et al., 2013), Australia (Nancarrow et al., 2014), Pakistan (Siddiqui et al., 2011), Iraq (El-Muadhidi et al., 2001), Russia and also recorded in Uzbekistan. Today, a number of viruses have been studied in Uzbekistan, including Potato X virus, Plum pox virus, Alfalfa mosaic virus, Sorghum powdery virus, and molecular identification using the PCR method (Fayziev et al., 2020; Sunnatovich et al., 2020; Sobirova et al., 2020). In addition, the spread of viral diseases depends on environmental factors such as the type of plant, soil type (Ramazonov et al., 2020), the discomfort of the environment has a negative effect on the growth and development of the plant, as a result, depending on the level of disease, the amount of pigments that provide important physiological processes such as photosynthesis in it decreases by several leads to a decrease in productivity and a decrease in productivity (Fayziev et al., 2020).

In Uzbekistan, regarding viruses that infect wheat, the wheat streak mosaic virus (WSMV) has been identified (Makkouk *et al.*, 2001).

The degree of spread of viruses found in grain plants was studied in the cross-section of regions. In this study, the *BYDV-PAV* strain spread in the wheat fields of Tashkent region was diagnosed using the RT-PCR method, and the genetic diversity of the CP gene of the identified *BYDV-PAV* strain T-UZ1 isolate was studied.

MATERIALS AND METHODS

Sample collection: Fifty wheat plants with typical symptom of *BYDV* including leaf yellowing with interveinal chlorosis, reddening of the flag leaf, stunting, delayed maturity were collected from wheat growing fields of the Tashkent region in April-May 2022. The samples were transported to the laboratory, the plants' leaves were cut off, placed in plastic bags with labels, and kept at -80°C to prevent viral RNA degradation.

Extraction and purification of total RNA from infected wheat: Total RNA was extracted from the symptomatic leaves using Invitrogen[™] PureLink[™] RNA Mini Kit from Thermo Fisher USA. Leaf samples (0.1 g) were ground in liquid nitrogen, homogenized in the Table 1. Structure of BYDV ORF 3 CP gene primers

lysis buffer with the addition of mercaptoethanol and incubated for 3 min at room temperature. The tubes were centrifuged at 5000 r min for 5 min, and the supernatant was transferred to a new centrifuge tube. The next stages (RNA precipitation using ethanol, placing the sample on a spin-column, washing the columns with RW1 and RPE buffers and elution with elution buffer) were carried out according to the manufacture's protocol. Measurement of quantity and quality of total RNA were performed using a spectrophotometer NanoDrop Eight (Thermo Fisher Scientific, USA), then the RNA samples were stored at – 80°C until used for RT-PCR.

Preparation of cDNA by reverse transcription: For obtaining cDNA based on viral matrix genomic RNA: 2 μ l (20 pmol) of oligonucleotide reverse primer Lu4 (table 1) was used and 5 μ l of total RNA were placed in thin-walled microcentrifuge tube (SnapSeal а Graduated Microtubes, SSIbio, USA). The mixture was placed in a thermostat "HB120-S DLAB" at 65°C for 5 minutes. After incubation, the tubes were placed on ice and the RT mixture was added. The following reagents were used to prepare the RT mixture (1 reaction): 3 µl water (Water, nuclease-free, Thermo Scientific), 1 µl Lu4, 4 µl deoxynucleotide triphosphates (dNTPs), 5 µl RNA, 4 µl 5x RT buffer RT, 1 µl 0.1 M DTT, 1 µl RNA ase out, 1 µl SuperScript II(Invitrogen[™]). Reverse transcription thermocycling program: 41°C - 115 minutes, 70°C - 1 cycle per 10 minutes.

Amplification of BYDV by PCR: Amplification of BYDV was conducted by polymerase chain reaction (PCR). For this, 4 μ l of the cDNA was taken and PCR-mixture (for 1 reaction) 6.6 ddH₂O, 12.5 μ l of 2X Master Mix, 0.9 μ l of MgCl2, 0.5 μ l of Lu1 primer (table 1), 0.5 μ l of Lu4 primer were added. PCR was performed on the T960 PCR Thermal Cycler (China). The following thermocycling program was used for PCR: initial denaturation 94°C, 2 min, denaturation 94°C, 1 min, annealing 41°C, 1 min, elongation 72°C, 20 min – 1 cycle, denaturation 94°C, 1 min, annealing 41°C, 1 min, annealing 41°C, 5 min.

Primer name	Sequence (5´-3´)	Tm value °C	G+C (%) content	Size of amplicon (bp)		
Lu1	CCAGTGGTTRTGGTC	41.0	56.65	521		
Lu4	GTCTACCTATTTGG	41.0	42.9	- 551		

Analysis of PCR products was performed by electrophoresis on 2% agarose gel prepared in 1x tris-borate-EDTA buffer (TBE, Thermo Scientific) stained with ethidium bromide. 2 μ l of DNA Gel Loading Dye (6X) and 1.3 μ L of 100 bp DNA Ladder was added to the first well of agarose gel, then 10 μ l PCR products with 3 μ l of DNA Gel Loading Dye (6X) were added to the following agarose wells. Electrophoresis was performed using horizontal electrophoresis system SE-1 (Helicon, Russia) at 100V for 100 minutes. PCR products were visualized by UV light and photographed by using a gel document imaging system BK-AG100 (Biobase, China).

Sequencing of PCR products: One PCR band was excised using a razor blade, transferred to a 1.5 ml centrifuge tube and purified using the PureLink^M Quick Gel Extraction Kit (Invitrogen, USA) according manufacturer's instructions. Cycle sequencing reaction was performed using the BigDye® Terminator v 3.1 kit (Applied Biosystems, USA). Cycle sequencing reaction consists of ddH2O-3.5 µl, BigDye-1 µL, 5x Seg.buffer-2 µl, sequencing primer - 0.5 µl, purified PCR product-2 µl. Lu1 and Lu4 primers were used for sequencing.

The following thermocycling program was used for cycle sequencing reaction: initial denaturation stage 96° C 1 minute; denaturation at 96° C for 10 secs, annealing at 41° C for 10 secs, and elongation at 60° C for 3 min are repeated for 45 consecutive cycles. The product of the sequence reaction was stored at 4° C.

The sequencing reaction products was purified from fluorescently labeled terminator nucleotides using the Dynabeads Sequencing Clean-Up Kit (Applied Biosystems, USA).

Separation and analysis of DNA sequence reaction products

by capillary gel electrophoresis with laser induced fluorescence detection was performed on Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific)

Sequence alignments and phylogenetic analyses: Snap Gene 5.3.1 program was used to edit the raw data obtained from the DNA sequence analysis system. Nucleotide sequences were examined using BLASTN program (basic local alignment search tool nucleotide-nucleotide, http://www.ncbi.nlm.nih.gov/BLAST/). DNA sequences of the best-matching viral strains were searched using BLASTN and sequences were subsequently downloaded and used for the phylogenetic analysis.

Multiple sequence alignments were performed by ClustalW and T-Coffee software. A phylogenetic tree was constructed by the neighbor-joining (NJ) method using Kimura 2parameter model (Kimura 1980) and statistical support was performed by bootstrap method of interior-branch test for phylogenetic trees. Data sets were bootstrapped (1,000 replicates) to assess the confidence values of the phylogenetic trees, and bootstrap values < 50% were omitted. Bootstrap test of phylogeny and visualization of final phylogenetic tree was conducted by MEGA11 software package: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al.*, 2021).

RESULTS

Field observations in 2022: A survey was carried out in Tashkent region to identify *Barley yellow dwarf viruses*. During the survey, wheat plants showing typical signs of viral infection, such as reddish colour of flag leaves, dwarfing, and chlorotic strip patterns on leaves, were observed (Figure 1).



Figure 1. BYDV disease symptoms in wheat fields of Tashkent region.

a) Infected fields b) Wheat plant infected with -BYDV.

Molecular characterization of BYDV isolate: RT-PCR was performed to amplify the partial sequence of CP gene on 8 samples. PCR products with universal Lu 1/Lu 4 primers were obtained, as predicted 531 bp long (Figure 2). BLASTN of the non-redundant nucleotide sequence database showed that investigated sequence belonged to the BYDV-PAV serotype.



Figure 2. Agarose gel image of RT-PCR performed with universal Lu 1/Lu 4 primers in wheat samples from Tashkent region; M - DNA 100 bp DNA Ladder; 1–8 wheat samples

The result of BLASTN analysis of Uzbekistan isolate T-UZB1 against a nucleotide sequence database revealed that this isolate was similar to other isolates at rates ranging from 87.10%-99.81%. This isolate showed the highest genetic similarity by 99.81% with the Moroccan PAV-type isolate MA9501 (AJ007929.1) while the lowest similarity rate was 87.10% with the Tunisian isolate PAV-TN4 (JX402456.1).

Phylogenetic analysis: The CP gene partial nucleotide sequence of Uzbekistan isolate T-UZB1 0Q087005

https://www.ncbi.nlm.nih.gov/nucleotide/0Q08700 5.1?report=genbank&log\$=nucltop&blast_ra

nk=1&RID=4YR2MNYA01N was compared with 16 strains of BYDV-PAV available in the_GenBank. According to the phylogenetic tree generated with 16 different homologous sequences of BYDV-PAV, BYDV-PAV T-UZB1 isolate sequence was clustered with two Morocco isolates MA9501 (AJ007929.1) and MA9502(AJ007918.1) and one Egypt Egy-Ho isolate KP708704.1





Moreover, the phylogenetic network strongly suggests that one mutation event, involving the PAV-PAV_MA9501_Morocco, have contributed to the evolutionary history of PAV_T-UZB1_Uzbekistan haplotype (Figure 4). The occurrence of haplotype in different countries could be explained by migration of aphids having the same origin.

AV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ATGGATTCAGTAGGTCGTATAGGACCTAAACGCGCAAATCAAAATGGCCCAAGAAGGAGG
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CCAGTGGTTGTGGTCCAACCCAATCGAGCAGGACCCAGACGA CGCCGTAAAACAGTTCGGCCAGTGGTTGTGGTCCAACCCAATCGAGCAGGACCCAGACGA *********************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGAAATGGTCGACGCAAGGGAAGAGGAGGAGGGGCAAATCCTGTATTTAGACCAACAGGCGGG CGAAATGGTCGACGCAAGGGAAGAGGAGGGGGGCAAATCCTGTATTTAGACCAACAGGCGGG ************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA ACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA *******************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACCAT TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACCAT ******************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGTTACAAGATCACAAGTATCCGAGTTGAGTTTAAGTCACACGCGTCCGCCACTACGGCC CGTTACAAGATCACAAGTATCCGAGTTGAGTT
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	GGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCAGCCCTGGGTAGCTACATT GGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCAGCCCTGGGTAGCTACATT ********************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG *********************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AAGGAATTCCAGGAATCAACGATAGACCAATTTTGGATGCTCTACAAGGCCAATGGAACC AAGGAATTCCAGGAATCAACGATAGACCAATTTTGGATGCTCTACAAGGCCAATGGAACC *********************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ACCACTGACACGGCAGGACA <mark>G</mark> TTCATCATCACGATGAGTGTCAGTTTGATGACGGCCAAA ACCACTGACACGGCAGGACA <mark>A</mark> TTCATCATCACGATGAGTGTCAGTTTGATGACGGCCAAA *******************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	TAG TAG
AV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ATGGATTCAGTAGGTCGTATAGGACCTAAACGCGCAAATCAAAATGGCCCAAGAAGGAGG
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CAGTGGTTGTGGTCCAACCCAATCGAGCAGGACCCAGACGA CGCCGTAAAACAGTTCGGCCAGTGGTTGTGGTCCAACCCAATCGAGCAGGAC CCAGACGA *******************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGAAATGGTCGACGCAAGGGAAGAGGAGGGGCAAATCCTGTATTTAGACCAACAGGCGGG CGAAATGGTCGACGCAAGGGAAGAGGAGGGGGCAAATCCTGTATTTAGACCAACAGGCGGG ************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA ACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA *******************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACCAT TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACCAT ******************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGTTACAAGATCACAAGTATCCGAGTTGAGTTTAAGTCACACGCGTCCGCCACTACGGCC CGTTACAAGATCACAAGTATCCGAGTTGAGTT
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	GGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCAGCCCTGGGTAGCTACATT GGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCAGCCCTGGGTAGCTACATT ********************************
	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG
PAV_1-U2B1_U2Dekistan PAV_MA9501_Morocco	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG *********************************
PAV_I-U2B1_UZDEKISTAN PAV_MA9501_Morocco PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG *********************************
PAV_T-UZB1_UZbekistan PAV_MA9501_Morocco PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG *********************************
PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG *********************************

/	AV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ATGGATTCAGTAGGTCGTATAGGACCTAAACGCGCAAATCAAAATGGCCCAAGAAGGAGG
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGCCGTAAAACAGTTCGGCCAGTGGTGGTCCAACCCAATCGAGCAGGACCCAGACGA
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGAAATGGTCGACGCAAGGGAAGAGGAGGGGGCAAATCCTGTATTTAGACCAACAGGCGGG CGAAATGGTCGACGCAAGGGAAGAGGAGGGGGCAAATCCTGTATTTAGACCAACAGGCGGG
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA ACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACCAT TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACCAT
ı	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	CGTTACAAGATCACAAGTATCCGAGTTGAGTTTAAGTCACACGCGTCCGCCACTACGGCC CGTTACAAGATCACAAGTATCCGAGTTGAGTT
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	GGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCAGCCCTGGGTAGCTACATT GGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCAGCCCTGGGTAGCTACATT
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG AATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAACGGG
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	AAGGAATTCCAGGAATCAACGATAGACCAATTTTGGATGCTCTACAAGGCCAATGGAACC AAGGAATTCCAGGAATCAACGATAGACCAATTTTGGATGCTCTACAAGGCCAATGGAACC
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	ACCACTGACACGGCAGGACA <mark>G</mark> TTCATCATCACGATGAGTGTCAGTTTGATGACGGCCAAA ACCACTGACACGGCAGGACA <mark>A</mark> TTCATCATCACGATGAGTGTCAGTTTGATGACGGCCAAA
	PAV_T-UZB1_Uzbekistan PAV_MA9501_Morocco	TAG TAG

Figure 4. Alignment of CP gene sequences of PAV_T-UZB1_Uzbekistan and PAV_MA9501_Morocco

DISCUSSION

Barley yellow dwarf virus (BYDV) is one of the most common and serious viral diseases in the world. The causative agent, BYDV, is widespread and infects cultivated and wild plant species of the Poaceae family. In particular, it causes serious yield losses in barley, wheat and sorghum (Choudhury *et al.*, 2018). Visual observations were made in wheat fields in different ecological regions of our republic. In particular, samples for laboratory PCR analysis were collected from infected wheat plants in Tashkent region. According to the analysis of the results of PCR and molecular genetic methods Comparison of T-UZB1 isolate with other strains of the world from molecular genetic analysis revealed similarities and differences with isolates from other countries of the world. From the phylogenetic tree analysis, it can be seen that T-UZB1 isolate OQ087005 isolated from wheat plant of Tashkent region showed 99.81% similarity with Moroccan *BYDV-PAV* MA9501 (AJ007929.1) isolate. The question arises: what are the causes and potential implications of the observed genetic differences between the Uzbekistani isolate and Moroccan isolate of *BYDV*. The first factor is long-distance migration of aphids. Wheat aphids have been observed to migrate across great distances via air (Sun *et al.*, 2022) and there are some evidences that, in certain circumstances, aphids can migrate by wind thousands of kilometers, for example long-distance migration of grain aphids between Australia and New Zealand (shortest distance = 4163 km) has been observed (Close and Tomlinson, 1975).

In addition, aphids, cicadas, butterflies and moths have reportedly traveled between 1600 and 5700 kilometers across the Atlantic between the Old and New World (Lorenz, 2009). It should be noted that the air travel shortest distance between Morocco and Uzbekistan is 6321 km. Therefore, there is a reasonable possibility that Moroccan isolate could arrived in Uzbekistan some time ago by long-distance migration assisted by jet-stream air currents.

In addition, it should be noted that aphids could also spread by human transportation of infested plant materials (Margaritopoulos et al., 2009). Therefore, there is a second reasonable possibility that Moroccan isolate could have been arrived in Uzbekistan during historical mass human migration, for example during the period of Arab Conquests in the Seventh Century, when Arab armies from Arab Caliphate (consisting of Arabian Peninsula, Middle East and much of North Africa, including present day Morocco) began to travel north into Central Asia invading the countries they passed. But whatever the reason might be, it seems that Moroccan virus invader has been well adapted and survived to date in the present-day Uzbekistan and only underwent slight genetic change (one SNP).

There is also additional interesting implication: previous research conducted by Bencharki *et al.* (1999) showed existence of a correlation between variation in the coat protein sequences of Moroccan barley yellow dwarf virus PAV isolates and the severity of infection (Bencharki *et al.*, 1999). It has been found that Moroccan PAV-type isolates have significant biological variation and classified into two virulence clusters. Cluster 1 grouped the isolates with virulence properties (severity index (ASI) < 4): MA9512, MA9504, MA9502, and MA9501, and the isolates with severe virulence properties (severity index (ASI) > 4) were classified into cluster 2: MA9508, MA9511, MA9415, MA9517, and M A9514 isolates (Bencharki *et al.*, 1999).

As a result of our research T-UZB1 isolate OQ087005 showed high similarity with MA9501 and MA9502 isolates which belong to virulence cluster 1. The results of our studies are consistent with this clusterisation, because our T-UZB1 isolate OQ087005 also affects wheat with moderate damage symptoms.

Joint Moroccan-French research study on screening wheat genotypes from ICARDA (International Center for Agricultural Research in the Dry areas) and CIMMYT (International Maize and Wheat Improvement Center) collections for tolerance to Moroccan PAV isolates belonging to virulence cluster 1, revealed that 80-81-BQCB-10 wheat line, Atlas 68 and Sutter wheat varieties are tolerant to these isolates (Bencharki *et al.*, 1999).

Therefore, it is reasonable to suggest to incorporate these wheat genotypes into Marker-assisted breeding programs in Uzbekistan for developing elite BYDVtolerant wheat cultivars.

The RT-PCR assay used in this study proved to be useful in molecular diagnosis of *BYDV* infection in wheat in Uzbekistan. Understanding virus diversity will help us develop appropriate wheat virus control measures. Virus resistance is considered important in the selection of wheat genotypes and its application to agriculture

CONCLUSION

Viral diseases of wheat crops in Tashkent region were studied by visual inspection. In order to determine whether the wheat fields are infected with BYDV-PAV, samples were taken from wheat plants based on disease symptoms. By reverse transcriptionpolymerase chain reaction (RT-PCR) diagnosis was carried out by RT-PCR method using special primers based on nucleotide sequence of ORF 3 gene of BYDV-PAV protein coat protein. According to the obtained results, the BYDV-PAV strain was detected in the samples as a result of RT-PCR. For the first time in Uzbekistan, the nucleotide sequence of BYDV-PAV was studied and it was named T-UZB1 isolate. T-UZB1 isolate of BYDV-PAV strain isolated from wheat plants was analyzed phylogenetically and was found to be closely related to the Morocco PAV-type isolate MA9501. T-UZB1 isolate of BYDV-PAV strain is stored in the collection of the unique object "Phytopathogenic and other microorganisms" of the Institute of Genetics and Experimental Biology of the Academy of Sciences of the Republic of Uzbekistan.

ACKNOWLEDGMENTS:

The scientific research work was supported by the Ministry of Innovative Development of the Republic of Uzbekistan and was carried out on the basis of the project FZ-2020100685 "Research of the epidemiology of Lute viruses in wheat and leguminous plants in the agro-ecological conditions of Uzbekistan".

REFERENCES

- Bencharki, B., J. Mutterer, M. E. Yamani, V. Ziegler-Graff,
 D. Zaoui and G. Jonard. 1999. Severity of infection of *Moroccan barley yellow dwarf virus* PAV isolates correlates with variability in their coat protein sequences. Annals of Applied Biology, 134: 89-99.
- Choudhury, S., D. Al-Shammari, H. Hu, H. Meinke, G. Westmore, C. Birchall, P. Larkin and M. Zhou.2018. A screening method to detect BYDV-PAV resistance in cereals under glasshouse conditions.Plant Pathology, 67: 1987-1996.
- Close, R. C. and A. I. Tomlinson. 1975. Dispersal of the Grain Aphid *Macrosiphum miscanthi* from Australia to New Zealand. New Zealand Entomologist, 6: 62-65.
- Deligöz, İ., Y. K. Caner and H. Akyol. 2011. Preliminary investigations of *Barley yellow dwarf virus-PAV* and *Barley yellow dwarf virus-MAV* in wheat fields of Samsun and Amasya Provinces in Turkey. Plant Protection Bulletin, 51: 187-193.
- El-Muadhidi, M. A., S. Murad, M. Jerjess, K. M. Makkouk and S. G. Kumari. 2001. Survey for legume and cereal viruses in Iraq. Survey for legume and cereal viruses in Iraq, 10: 1-10.
- Erkan, E. and N. Yılmaz. 2009. Determination of virus diseases on wheat growing areas of Samsun province. Anadolu Tarım Bilimleri Dergisi, 24: 67-75.
- FAO 2018. Preliminary data now available for selected countries and products. Erişim Tarihi. Retrieved 2018 March 03 from http://www.fao.org/statistics/en/
- Fayziev, V., D. Jovlieva, U. Juraeva, J. Shavkiev and F. Eshboev. 2020. Effects of PVXN-UZ 915 necrotic isolate of potato virus X on amount of pigments of *Datura stramonium* leaves. Journal of Critical Reviews, 7: 400-403.

- Lorenz, M. W. 2009. Migration and trans-Atlantic flight of locusts. Quaternary International, 196: 4-12.
- Makkouk, K. M., S. G. Kumari, Z. Kadirova and A. Zueva. 2001. First Record of Barley yellow striate mosaic virus and cereal yellow dwarf virus-RPV Infecting wheat in Uzbekistan. Plant Disease, 85: 1122-1122.
- Margaritopoulos, J. T., L. Kasprowicz, G. L. Malloch and B. Fenton. 2009. Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid. BMC Ecology, 9: 13-13.
- Nancarrow, N., F. E. Constable, K. J. Finlay, A. J. Freeman, B. C. Rodoni, P. Trebicki, S. Vassiliadis, A. L. Yen and J. E. Luck. 2014. The effect of elevated temperature on barley yellow dwarf virus-PAV in wheat. Virus Research, 186: 97-103.
- Parizoto, G., A. Rebonatto, J. Schons and D. Lau. 2013. Barley yellow dwarf virus-PAV in Brazil: seasonal fluctuation and biological characteristics. Tropical Plant Pathology, 38: 11-19.
- Ramazonov, B., K. Mutalov, V. Fayziev and S. Koraev. 2020. Morphogenetic characteristics and biological activity of takyr and meadow soils of the republic of karakalpakstan (on the example of soils of chimbay district) morphogenetic characteristics and biological activity of takyr and meadow soils of the republic of karakalpakstan (on the example of soils of chimbay district). Journal Of Critical Reviews, 7: 243-249.
- Rybicki, E. P. 2015. A Top Ten list for economically important plant viruses. Archives of Virology, 160: 17-20.
- Siddiqui, N. N., M. Ilyas, S. Mansoor, A. Azhar and M. Saeed. 2011. Cloning and phylogenetic analysis of coat protein of barley yellow dwarf virus Isolates from different regions of Pakistan. Journal of Phytopathology, 160: 13-18.
- Sobirova, Z., V. Fayziev and K. Abduraimova. 2020. Effect of the virus of the yellow dwarf corn mosaic growth and development of varieties of corn in various phases. Journal of Advanced Research in Dynamical and Control Systems, 12: 602-606.
- Sun, J. X., Q. Li, X. I. Tan, J. Fan, Y. Zhang, Y. G. Qin, F. Francis and J. I. Chen. 2022. Population genetic structure of *Sitobion miscanthi* in China. Journal of Integrative Agriculture, 21: 178-187.
- Sunnatovich, K. T., K. Z. Nasirovna, D. K. Sotvoldievich and A. Z. Rakhmatovna. 2020. Analysis of

lysimetric parameters of the effect of chemical and biological drugs against Alfalfa Mosaic Virus. The American Journal of Agriculture and Biomedical Engineering, 02: 25-29. Tamura, K., G. Stecher and S. Kumar. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38: 3022-3027.

Contribution of Authors:				
Makhmudov T. Khalimovich	:	Collected samples from infected wheat fields		
Kadirova Z. Nasirovna	:	Help in collecting scientific literature		
Adilov B. Shukhratovich	:	Help in PCR process and analysis		
Abdikarimov B. Qurombayevich	:	Help in collecting samples from infected wheat field.		
Abduvaliev B. Abdurashitovich	:	Extraction of viral RNA from wheat plants with disease symptoms		
Ziyaev Z. Mashrabovich	:	Help in stylistic and orthographic corrections in the article		
Sherimbetov A. Gulmirzayevich	:	Help in analysis of wheat samples		
Kurganov Sardor	:	Help in sequence processes based on CP gene fragment		