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Research Article

Concurrent detection of five *Yellow dwarf viruses* (B/CYDVs) in wheat in Mardin (Turkey) and phylogenetic relationship of BYDV-PAV

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Abstract

Wheat (*Triticum aestivum* L.) is a natural host of many viruses. *Yellow dwarf viruses* belonging to the Luteoviridae family are important virus species that cause economic loss by restricting wheat production worldwide. Surveys were conducted in 2017 to determine *Yellow dwarf viruses* (BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV, and CYDV-RPV) and their infection rates in wheat production areas in Mardin province. 400 fresh leaf samples collected were tested by Multiplex reverse transcription-polymerase chain reaction (m-RT-PCR). The overall infection rate was found to be 3%. BYDV-PAV has been identified as the most widespread virus with a 2.5% presence rate. It was found out that BYDV-SGV, CYDV-RPV, and BYDV-RMV infections were lower, with rates of 1.75%, 0.5% and 0.25% respectively. In the current study, double infections were detected in 8 samples. The overall infection rate of the detected viruses (BYDV-PAV, BYDV-SGV, CYDV-RPV, BYDV-RMV) was found to be lower than the records reported in previous similar studies. No BYDV-MAV infection was found in any of the wheat samples tested. The cDNA of the coat protein (CP) gene of a BYDV-PAV isolate randomly selected from virus-positive samples was cloned, bidirectionally sequenced, and the phylogenetic relationship revealed. According to the phylogenetic analysis with 19 different isolates in the NCBI database of BYDV-PAV Mardin isolate, it showed the highest genetic similarity by 95.52% with the Germany isolate (KY634926) while the lowest similarity rate was 89.22% with the Germany and Pakistan isolates (KY634886 and JQ811489). The presence of BYDV-PAV, BYDV-SGV, CYDV-RPV, and BYDV-RMV were reported for the first time with this survey study conducted in Mardin.

Keywords: cloning; mardin; molecular characterization; multiplex RT-PCR; survey; wheat

Introduction

Wheat, grown in a wide geography and climate at the present time, is one of the first plants to be cultivated and its history goes back to 10,000 years ago (Kün, 1996; Ilbağı, 2003; Anonim, 1996). Wheat, a member of the Poaceae family, is the most important cultivated plant after corn. In world's wheat production, EU countries rank first with a 23% share, China comes second with 19% and India ranks third with 15% (FAO, 2018).

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Compared to other crops, wheat production (%38.1) outweighs in Turkey. The Central Anatolia Region ranks first in wheat production (32%) followed by the Marmara Region (18%) and the Southeastern Anatolia Region (15%). Located in the Southeastern Anatolia region, Mardin is the sixth of the most wheat producing provinces in Turkey with a 3.87% rate and 833.009 tonnes of wheat production (TUIK, 2018).

Wheat is often negatively affected by viral diseases as well as abiotic factors, fungal and bacterial pathogens. An effective chemical treatment method for viruses that cause significant economic losses is still unknown (Ilbağı, 2017). Viruses in barley yellow dwarf group (BYDVs), which are among the top 4 viruses that cause the highest yield loss in wheat, are the pathogens that cause serious product losses worldwide every year (Rybicki, 2014). BYDVs infection in wheat -though confused with abiotic factors- can cause spotting, yellowing, reddish leaves, rosetting, loss of green color, dwarfing, and necrosis in the wheat. It is also possible to notice severe symptoms in the wheat due to the synergistic effect of the existing two or multiple infections (Erkan ve Yılmaz, 2009).

The Luteoviridae family, which encompasses wheat viruses, has three genera, Luteovirus, Polerovirus and Enamovirus. BYDVs are a member of Luteovirus while CYDVs are a member of Polerovirus genus (Domier, 2012; Sathees, 2015). BYDVs were first identified in barley in 1950 and then their presence in cereals such as wheat, oats, rice, and corn was reported later (Oswald and Houston, 1951; Chain *et al.*, 2006). BYDV viruses with a genome consisting of a single strand (+) RNA have isometric particles of 25-28 nm in diameter. BYDV viruses are concentrated in the nucleus, cytoplasm, and parenchyma cells in most cases (Domier, 2008; Hogenhout *et al.*, 2008). Viruses in this group (PAV, SGV, RPV, MAV, and RMV) were previously named to be specific to their vector and were then considered five different species, taking into account their genomic characteristics (Gray *et al.*, 1998; Uzunoğulları ve Gümüş, 2017). However, the genomic RNA of the virus identified as *Barley yellow dwarf virus*-RMV was performed with 5612 nucleotide sequence analysis and it was proposed that its name would be changed to *Maize yellof dwarf virus*-RMV and be classified in the genus Polerovirus (Krueger *et al.*, 2013).

Unlike other viruses that are transported by mechanical means, pollen, contact, and seeds, BYDVs are transmitted only by aphids in a persistent manner. Although it is reported to be carried by about 25 aphids, in most cases *Rhopalosiphum padi, Macrosiphum (Sitobion) avenae, Schizaphis graminum, Rhopalosiphum maidis, Acrosternum hilare* vectors are the primary important (Deligöz *et al.*, 2011; Siddiqui *et al.*, 2011).

B/CYDVs are the most common group of viruses in Poaceae family plants studied extensively in many continents and countries on a global scale. The presence of these viruses have been reported in some countries like Brazil (Parizoto *et al.*, 2013), Australia (Nancarrow *et al.*, 2014), Pakistan (Siddiqui *et al.*, 2011), Iraq (El-Muadhidi *et al.*, 2001), Tunisia (Hamdi *et al.*, 2020), İran (Rastgou *et al.*, 2005), Hungary (Áy *et al.*, 2008), England/UK (Kendall *et al.*, 1996), China (Tao *et al.*, 2012), Southern Iran (Pakdel *et al.*, 2010), Latvia (Bisnieks *et al.* 2004), Turkey (Usta *et al.*, 2020; Hassan *et al.*, 2018; Ilbagi *et al.*, 2019), Chech Republic and Sweden (Pokorny, 2006), and Bulgaria (Bakardjeiva *et al.*, 2006).

In Turkey there have been numerous reports about the presence of BYDVs in cereals. However, the studies concerning the South-Eastern Anatolia region is limited. In this study, B/CYDVs causing infections in wheat fields were screened using the m-RT-PCR method and revealed the infection rates in Mardin province. In addition, the genetic diversity of the CP gene of detected BYDV-PAV isolate was investigated.

Materials and Methods

Survey and virus source

In April and May 2017, seventy-four fields from seven different geographical regions of Mardin province were surveyed and 400 wheat leaf samples were collected randomly, with at least 5 fresh leaf samples from each field. The collected samples were stored at -20 °C until total RNA extraction.

Total RNA ısolation, primers, and cDNA synthesis

Total RNA isolation was achieved by making minor modifications in the silica-based method reported by Foissac *et al.* (2001). In cDNA synthesis, Universal Yan Reverse primer (Malmstrom and Shu, 2004) and RevertAid First Strand cDNA kit (Thermo-Fermantas, Vilnius, Lithuania) were used to detect all of BYDVs. Resulted cDNAs were kept in deep-freeze until use.

Detection of B/CYDVs by multiplex RT-PCR and RT-PCR assays

To ensure the separation of Group1 (BYDV-PAV, MAV, SGV) and Group2 (CYDV-RPV, BYDV-RMV), temperature cycles and primers that give 832 bp and 372 bp fragments, respectively, in PCR tests were adjusted as defined by Malmstrom and Shu (2004). The more precise detection was accomplished using group-specific primers described by Malmstrom and Shu (2004), Deb and Anderson (2008) and Usta *et al.* (2020) on group-positive samples.

The 50 µl of PCR-mix contained of 31.6 µl of RNase free water, 5 µl of 10X PCR Buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, 1 µl of 20 µM of each primer sets (Shu-F, Yan R, S2a-F, S2b-F), 0.4 µl of Dream Taq DNA polymerase enzyme (5U/µl) (Thermo Scientific), and 5 µL of cDNA. During PCR tests, Yellow dwarf virus isolates previously determined by Usta *et al.* (2020) were used as positive control and healthy wheat leaves were used as a negative control. PCR products were electrophoresed on the EtBr-added 1% agarose gel and visualized in the gel imaging and analysis system (Syngene™ UV Transilluminator).

Molecular cloning, sequencing, phylogenetic analysis of BYDV- PAV isolate

Among the viruses detected, the complete CP gene of BYDV-PAV, CYDV-RPV, and the partial CP gene of BYDV-SGV and BYDV-RMV were successfully introduced to the appropriate cloning vector and their nucleotide sequences were determined and submitted in the GenBank. The specific primers used to amplify the complete or partial CP genes were adopted as reported by various researchers (Deb and Anderson, 2008; Usta *et al.*, 2020). Temperature cycles and PCR parameters described by Malmstrom and Shu (2004) were used to amplify related genes of viruses. The amplified DNA fragments were purified from agarose gel (Thermo Scientific) and cloned into the prokaryotic cloning vector (pGEM T-Easy vector, Promega) and transformed into competent bacteria *E. coli* (JM109 strain) cells. Recombinant plasmids were purified from transformed bacteria (GeneJET Plasmid Miniprep Kit, Thermo Scientific) and bidirectional sequencing was performed by a commercial firm (Sentebiolab, Bilkent-ANKARA). Sequence information for each viral genome was stored in GenBank (NCBI).

The phylogenetic relationship and nucleotide similarity of BYDV-PAV sequence with other world isolates were analysed using CLC Main Workbench program (version 6.7.1). For better branching of phylogenetic tree, *Zucchini yellow mosaic virus* (ZYMV) (JF792368) isolate was chosen as an out-group.

Results

Field observations and infection rates in 2017

A survey was carried out in Mardin province to identify Yellow dwarf viruses (BYDV-PAV, -SGV,-MAV, -RMV and CYDV-RPV). 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).

As seen in Figure 2 (Panel A), DNA fragments (832 bp and 372 bp) were obtained in the m-RT-PCR test performed with the primers reported by Malmstrom and Shu (2004) indicating that associated samples were infected with one or more viruses. Species-specific primers provided species discrimination of B/CYDVs. The performed tests confirmed that there were BYDV-PAV (≈600 bp), BYDV-SGV (≈254 bp), CYDV-RPV (≈615 bp) and BYDV-RMV (≈365 bp) in the samples collected. (Figure 2, Panel B, C, D, E). PCR tests produced neither healthy wheat plants nor any fragments belonging to the genome of BYDV-MAV.

B/CYDV-positive reactions were detected in 12 (3%) of 400 samples in Mardin province. The highest infection rate was determined in the Nusaybin region and the lowest was in the Artuklu region. The most common virus was BYDV-PAV with a rate of 2.5% (10 samples), followed by BYDV-SGV, CYDV-RPV, and BYDV-RMV with an infection rate of 1.75% (7 samples), 0.5% (2 samples) and 0.25% (1 sample), respectively. Furthermore, double infections were determined that containing PAV+SGV (1.75%) in 7 samples and PAV+RPV (0.25%) in 1 sample. Multiple virus infections containing more than two viruses were not detected. The overall status of virus infections in wheat samples in the province of Mardin on a regional basis is given in Table 1.

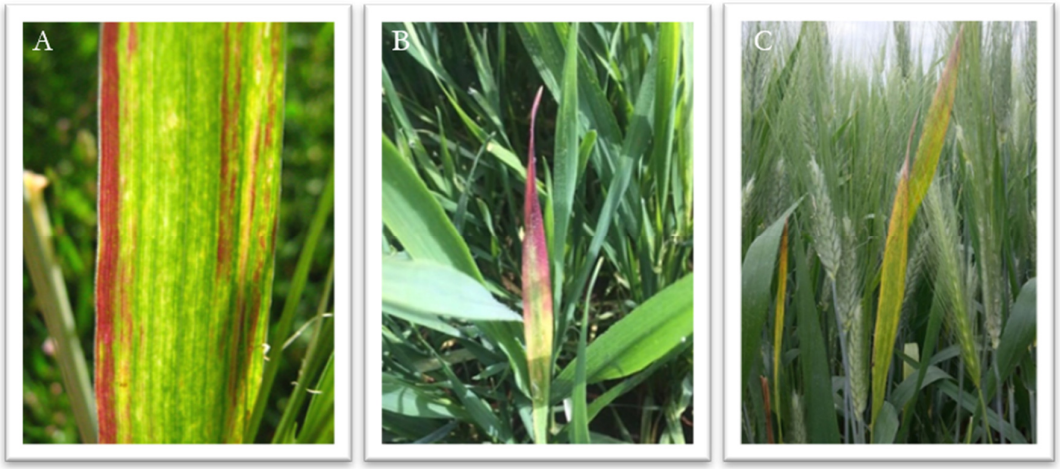

Figure 1. Symptomatic wheat samples in surveys in wheat planting areas of Mardin province
Flag leaf redness (A), Chlorotic striped patterns and dwarfing in the early period (B, C)

Table 1. Surveyed regions in Mardin province, the number of samples tested and the distribution of detected viruses

B/CYDV - Positive samples								
District	Samples	PAV	SGV	RPV	PAV+SGV	PAV+RPV	RMV	%
Kızıltepe	170	4	2	1	2	1	1	2.95
Derik	75	2	2	-	2	-	-	2.5
Artuklu	45	1	1	-	1	-	-	2.22
Nusaybin	40	2	2	1	2	-	-	7.5
Savur	30	1	-	-	1	-	-	3.33
Dargeçit	25	-	-	-	-	-	-	-
Midyat	15	--	-	-	-	-	-	-
Total	400	10	7	2	7	1	1	3.0

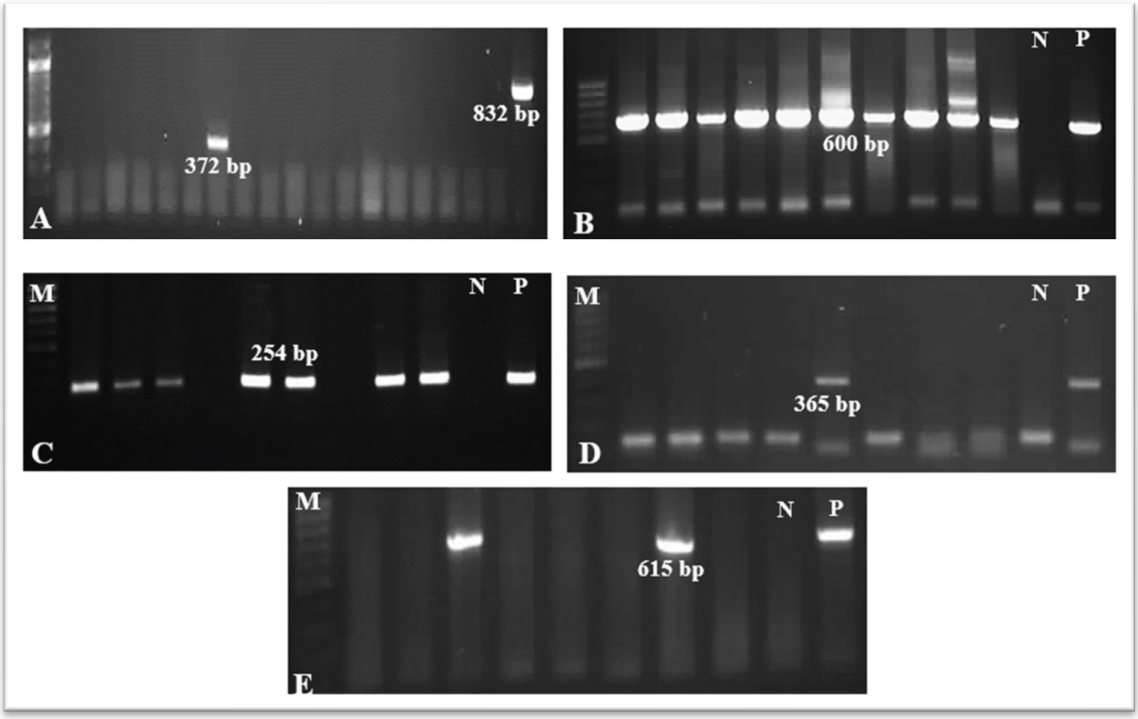

Figure 2. Panel A: Agarose gel image of m-RT-PCR test performed with detection primers in Mardin leaf samples; Positive samples associated with Group2 (372 bp) and Group1 (832 bp); Panel B: Determination of BYDV-PAV with specific primers; Panel C: Determination of BYDV-SGV with the primers of Deb and Anderson (2008); Panel D: Agarose gel image showing the determination of BYDV-RMV with specific primers; Panel E: Agarose gel image showing the determination of CYDV-RPV with specific primers, N: Negative control, P: Positive control, M: 1000 bp DNA ladder

Bioinformatic analysis and phylogenetic relationship of BYDV-PAV

Partial or complete CP genes of a randomly selected isolate from BYDV-PAV, CYDV-RPV, BYDV-SGV and BYDV-RMV were successfully cloned and sequenced. The nucleotide sequence data of BYDV-PAV, CYDV-RPV, BYDV-SGV and BYDV-RMV were deposited in the GenBank with accession numbers MK732034, MK732035, MK940529 and MK955886 respectively. It was revealed that the CP gene of the BYDV-PAV and CYDV-RPV were 603 bp and 615 bp, respectively, while the partial CP gene of the BYDV-SGV and BYDV-RMV were 250 bp and 365 bp, respectively.

It was revealed that the CP gene of the BYDV-PAV was 603 bp, the CP gene of the CYDV-RPV was 615 bp, the partial CP gene of the BYDV-SGV was 250 bp, and the BYDV-RMV partial CP gene was 365 bp.

The results of multiple nucleic acid comparisons of BYDV-PAV Mardin isolate (MK732034) revealed that this isolate was similar to other isolates at rates ranging from 89.22-95.52%. This isolate was found to be similar to Germany (KY634926) with the highest rate of 95.52% and to the Netherlands and Pakistan (KY634886 and JQ811489) with the lowest rate of 89.22%. This isolate was also found to be similar to the regional Turkey-Van isolate (KC900900) by 92.54%. A total of 59 nucleotide changes (corresponding to 9.8%) were detected which are spread across all of the CP gene of the Mardin BYDV-PAV isolate.

Multiple comparison and phylogenetic trees were not created since there were no standard length sequences stored in the GenBank belonging to partial or complete CP nucleotide sequences of CYDV-RPV, BYDV-SGV, and BYDV-RMV. Multiple comparison and phylogenetic tree was created only for BYDV-PAV isolate. According to the phylogenetic tree generated with 19 different homologous sequences of BYDV-PAV, Mardin PAV sequence (MK732034) was clustered with Germany isolate (KY634926) isolated from the corn,

supported by a high bootstrap value (Figure 3). Accordingly, it has been determined that host and geographic affinities are not effective in forming groups of viruses.

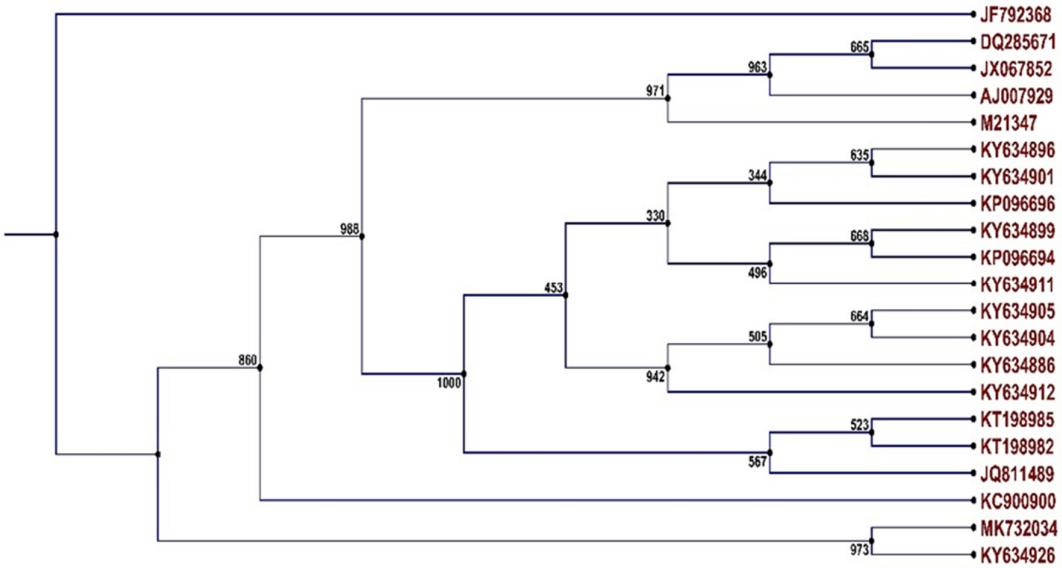

Figure 3. Phylogenetic tree created by Neighbor-joining method with 1000 repeats of the nucleic belonging sequence of the complete coat protein gene of the PAV isolates registered in the NCBI gene bank with the BYDV-PAV (MK732034) isolate. KY634886, (Netherlands) (*Lolium multiflorum*); JX067852, (*Triticum aestivum*) (Brazil); DQ285671 (-) (USA); AJ007929, (-) (France); KY634901 (*Hordeum vulgare*) (France); JQ811489 (*Zea mays*) (Pakistan); KY634899 (*Hordeum vulgare*) (China); KY634904 (*Hordeum vulgare*) (England); KT198985 (*Triticum aestivum*) (Pakistan); KT198982 (*Hordeum vulgare*) (Pakistan); KY634912 (*Triticum aestivum*) (Sweden); KY634926 (Zea mays) (Germany); KY634896 (*Triticum aestivum*) (Germany); KY634905 (Avena sativa) (Germany); KY634911 (*Hordeum vulgare*) (Czech Republic); KP096696 (*Triticum aestivum*) (Hungary); KP096694 (*Triticum aestivum*) (China); M21347 (-) (Australia); MK732034 (*Triticum aestivum*) (This Study, Turkey); KC900900 (*Triticum aestivum*) (Turkey)

Discussion

B/CYDVs are the most important virus diseases seen in all cereal production fields worldwide, causing 15-25% yield losses in wheat, barley, and oats (Lister and Ranieri, 1995). Wheat viruses have a wide range of hosts, and infect not only cereals but also weeds and more than 150 species of meadow, pasture, and grass crops (Gould ve Shaw 1983; İlbağı *et al.*, 2014).

This study evaluated the prevalence of *Barley yellow dwarf viruses* in wheat plants in Mardin province located in the Southeastern Anatolia Region. Molecular tests revealed at varying rates the presence of BYDV-PAV, BYDV-SGV, CYDV-RPV, and BYDV-RMV viruses in five regions (Kızıltepe, Derik, Artuklu, Nusaybin, and Savur) except Dargeçit and Midyat. The current study in Mardin province is the first survey reporting of the mentioned viruses following the study by Hassan *et al.* (2018) conducted in the South Eastern Anatolia region.

Typical viral infection symptoms, such as redness of flag leaves, dwarfing, and chlorotic stripe patterns on leaves, observed in wheat plants in Mardin province showed consistency with those symptoms recorded by various researchers (Chay *et al.*, 1996; İlbağı *et al.*, 2005).

There are 9 species of yellow dwarf viruses (İlbağı, 2017). Numerous studies carried out in Turkey recorded that BYDV-PAV, BYDV-SGV, and CYDV-RPV are common viruses at various hosts (İlbağı *et al.*, 2008; Hamamcı, 2012; Aydın, 2017).

BYDV-PAV is highly prevalent in Turkey and all over the world, as identified in the current study (2.5%). (Kumari *et al.*, 2006; Parizoto *et al.*, 2013; Adhikari *et al.*, 2020). The presence of the second common virus, CYDV-RPV (0.5%), has been previously reported at rates ranging up to 17.73 in Turkey (Aydın, 2017; Usta *et al.*, 2020). Mix infection of B/CYDVs is a common picture worldwide (Conti *et al.*, 1990; El-Yamani ve Hill, 1990; Deligöz *et al.*, 2011). In this study, it was revealed that PAV+RPV (0.25) and PAV+SGV (1.75%) mix infections were at low frequency. Mix infections have also been reported in various regions from Turkey (Hassan *et al.*, 2018; Usta *et al.*, 2020).

According to molecular tests, BYDV-MAV infection was not found in Mardin province, in correlation with those surveyed in the Eastern Anatolia Region (10 provinces) with 900 wheat samples by Usta *et al.* (2020) and in Samsun province with 184 samples Toksöz ve Yılmaz (2016). However, the occurrence of BYDV-MAV has been confirmed at varying proportions (25%, 2.14%, and 2%) in surveys conducted by Köklü (2004), İlbağı (2017), Erkan and Yılmaz (2009) in Turkey.

The lowest virus infection in wheat (0.25%, 1 sample) was recorded for BYDV-RMV. This virus, which is one of the most important pathogens of corn, has been reported to be an important host for barley, oats, triticale, bird meal and various weeds besides wheat (Pocsai *et al.*, 2003; İlbağı and Çıtır, 2014). This viral infection is thought to be due to the fact that corn farming in the region creates a suitable habitat for the vector of this virus (*Rhapalosphum maidis*) (Siddiqui *et al.*, 2011). overall infection incidences obtained in this study, which was conducted for the first time in the province of Mardin in the Southeastern Anatolia region, were similar to the results of the study conducted by Hassan *et al.* (2018).

The CP gene sequences of Mardin isolates were recorded in the NCBI gene bank with access numbers MK732034 (603 nt) for BYDV-PAV, MK940529 (250 nt) for BYDV-SGV, MK955886 (365 nt) for BYDV-RMV and MK732035 (615 nt) for CYDV-RPV. It has been demonstrated that the CP gene of BYDV-PAV Mardin isolate is 603 bp long as in other isolates in the world, but with 59 base changes. It is thought that BYDV-PAV genome located in the genus *luteovirus* in the luteoviridae family does not have an ORF-0 region (which acts as an RNA silencer) in the CP gene, causing a high rate of nucleotide substitution (Mangwende *et al.*, 2009; Peter *et al.*, 2009). Another hypothesis is the possibility of new species developing from B/CYDV species (Robertson and French, 2007; Svanella-Dumas *et al.*, 2013). It has been reported that there is a high degree of genetic diversity in RNA genome plant viruses such as B/CYDV due to high recombination, mutation, rapid replication, and large population size (Chare and Holmes, 2006; Sanjuán *et al.*, 2010). In addition, Wu *et al.* (2011) emphasised that BYDV-PAV is predisposed to divergent strains due to high homologous recombination. They examined the molecular evolution and recombination rates in genomes of BYDV-PAV isolates of different geographic origin and reported at least 22 recombination events involving multiple ORFs in only 58 genomes. These results are in line with the high rate of nucleotide substitution in our study.

The infection rate (3%) of B/CYDVs recognized by this study in Mardin province is not at a level to create an epidemic in wheat agriculture, possibly due to inappropriate agroecosystem conditions. It is uncertain that this advantage will not yield a high yield loss in the future. The main factor affecting the spread of the disease is the presence of at least 25 types of aphids in 15 different genera (Halbert and Voegtlin, 1995). For this reason, it is necessary to pay attention to integrated control strategies by carrying out potential vector scanning in the related region.

Authors' Contributions

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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