sciendo

*Agriculture (Poľnohospodárstvo), 66, 2020 (4): 161 − 170*

Original paper

DOI: 10.2478/agri-2020-0015

# MOLECULAR CHARACTERIZATION OF CEREAL YELLOW DWARF VIRUS-RPV IN GRASSES IN EUROPEAN PART OF TURKEY

HAVVA ILBAĞI[1*], AHMET ÇITIR[1], ADNAN KARA[1], MERYEM UYSAL[2]

[1]Namık Kemal University, Tekirdağ, Turkey
[2]Selçuk University, Konya,Turkey

ILBAĞI, H. – ÇITIR, A. − KARA, A. − UYSAL, M.: Molecular characterization of cereal yellow dwarf virus-RPV in grasses in european part of Turkey. Agriculture (Poľnohospodárstvo), vol. *66,* no. 4, pp. 161 – 170.

Yellow dwarf viruses (YDVs) are economically destructive viral diseases of cereal crops, which cause the reduction of yield and quality of grains. *Cereal yellow dwarf virus-RPV* (CYDV-RPV) is one of the most serious virus species of YDVs. These virus diseases cause epidemics in cereal fields in some periods of the year in Turkey depending on potential reservoir natural hosts that play a significant role in epidemiology. This study was conducted to investigate the presence and prevalence of CYDV-RPV in grasses and volunteer cereal host plants including 33 species from Poaceae, Asteraceae, Juncaceae, Geraniaceae, Cyperaceae, and Rubiaceae families in the Trakya region of Turkey. A total of 584 symptomatic grass and volunteer cereal leaf samples exhibiting yellowing, reddening, irregular necrotic patches and dwarfing symptoms were collected from Trakya and tested by ELISA and RT-PCR methods. The screening tests showed that 55 out of 584 grass samples were infected with CYDV-RPV in grasses from the Poaceae family, while none of the other families had no infection. The incidence of CYDV-RPV was detected at a rate of 9.42%. Transmission experiments using the aphid species *Rhopalosiphum padi* L. showed that CYDV-RPV was transmitted persistently from symptomatic intact grasses such as *Avena sterilis*, *Lolium perenne* and *Phleum exratum* to barley cv. Barbaros seedlings. PCR products of five Turkish RPV grass isolates were sequenced and compared with eleven known CYDV-RPV isolates in the GenBank⁄ EMBL databases. Compared nucleotide and amino acid sequences of CYDV-RPV isolates showed that the identities ranged from 40.38 − 95.86 % to 14.04 − 93.38%, respectively. In this study, 19 grass species from the Poaceae family and two volunteer cereal host plants were determined as natural reservoir hosts of CYDV-RPV in the cereal growing areas of Turkey.

Key words: CYDV-RPV*, polerovirus*, grasses, molecular diversity

The Trakya region is one of the most important cereal growing areas in Turkey. Almost one million ha of arable land, which covers 65% of the region, has been allocated to field crops and cereal production. With the effects of global climate change in recent years, increasing temperatures have gradually caused prevailing pest and diseases. Besides the very damaging fungal pathogens, Yellow dwarf viruses (YDVs) considerably reduced the yield and quality of cereal crops in Trakya. High incidence rates of YDVs causing severe infections and epidemics on cereals have been reported periodically in Turkey. Early sown susceptible winter wheat cultivars were especially affected by the highest incidences of YDVs (Ilbağı *et al.* 2005; Ilbağı *et al.* 2013). Moreover, BYDVs or CYDV infections

Havva Ilbağı (*Corresponding author), Ahmet Çıtır, Adnan Kara, Department of Plant Protection, Faculty of Agriculture, Namık Kemal University, Tekirdağ, Turkey. E-mail: hilbagi@nku.edu.tr
Meryem Uysal, Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya,Turkey

© 2020 Authors. This is an open access article licensed under the Creative Commons Attribution-NonComercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

caused up to 80% yield losses on cereal crops by reducing the number of tillers per plant, number of seeds per tiller, and seed weights as reported by McKirdy *et al.* (2002) and Perry *et al.* (2000). Similarly, Miller & Rasochova (1997) and Pike (1990) determined average yield losses 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. There are currently 10 recognized YDV species within the family *Luteoviridae* (Adams *et al.* 2014), consisting of *Barley yellow dwarf viruses* (BYDVs), *Cereal yellow dwarf viruses* (CYDVs), and *Maize yellow dwarf virus-RMV* (MYDV-RMV). Five species have been assigned to the genus *Luteovirus* (BYDV-Kerll, BYDV-KerIII, BYDV-MAV, BYDV-PAS and BYDV-PAV), three species have been assigned to the genus *Poleovirus* (CYDV-RPV, CYDV-RPS, and MYDV-RMV), and two have not yet been assigned any genus (BYDV-GPV and BYDV-SGV). YDVs have isometric particles of 25−30 nm in diameter and ss(+) RNA genome of approximately 5,600 nucleotides (Rochow & Duffus 1981). There are six open reading frames (ORFs) in the genome, which are numbered 1−6 in the members of *Luteovirus* and 0−5 in the members of *Polerovirus* (Miller *et al.* 2002). These viruses are phloem-limited and are transmitted in a persistent circulative manner by over 25 aphid vectors. Among them, *Rhopalosiphum padi* L. and *Rhopalosiphum maidis* Fitch are the most common and efficient ones (Smith & Plumb 1981). The bird cherry-oat aphid, *Rhopalosiphum padi* L., is a frequent vector of BYDV species (Halbert & Voegtlin 1995). Çalı & Yurdakul (1996) identified four more aphid species as the vector of BYDVs in four provinces of Central Anatolia in Turkey. Seven aphid species in the wheat fields of Tekirdağ province in Trakya were identified by Özder & Toros (1999). The mechanisms associated with YDV infections in the field conditions are complex and influenced by many factors. The wild grasses, perennial pasture grasses, and volunteer cereals play a significant role as inoculum sources of virus and vector reservoirs during summer and throughout the growing seasons (McKirdy & Jones 1997). Because of the direct interactions between the viruses, aphid vectors, and cereal host plants, it is also important to investigate the grass hosts presence in these agroecosystems (Power & Gray 1995). A list of 96 annual, 2 biannual and 111 perennial Poaceae weed hosts worldwide were compiled by D'Arcy (1995). In the subsequent years, the grass hosts of YDVs were reported in different countries by Garrett *et al.* (2004) in the USA, Pokorny (2006) in the Czech Republic, Bisnieks *et al.* (2004) in Latvia and Sweden, Bakardjeiva *et al.* (2006) in Bulgaria. Previously, sporadic infections of BYDV in wheat fields were investigated in the western part of Turkey by Bremer & Raatikainen (1975). Since 1999 the year in the Trakya region, YDVs have become epidemics in the cereal fields, as reported by Ilbağı *et al.* (2013). In addition to the Trakya region, YDV infections were determined in 15 other cereal growing provinces of Turkey (Pocsai *et al.* 2003). Later, Ilbağı (2006) identified common reed (*Phragmites communis* Trin) as a perennial natural weed host of BYDV-PAV; however, CYDV-RPV was not detected in the reed. Following this study, birdseed (*Phalaris canariensis* L.) was also determined as the most susceptible host of both BYDV-PAV and CYDV-RPV in Tekirdağ (Ilbağı *et al.* 2008). Lately, dicotyledonous weed hosts of BYDV-PAV and MAV such as *Juncus compressus* Jacq. and *Geranium dissectum* L. were reported in Trakya, Turkey (Ilbağı *et al.* 2019). Güncan & Karaca (2018) suggested effective weed control for potential reservoir sources of YDVs, as well as competition with cultivated cereals for plant nutrients and water. Del Blanco *et al.* (2014) reported in barley two major QTLs for CYDV tolerance from cultivar Madre Selva on chromosomes 2H and 7H, four minor QTLs from line Butta 12 on chromosomes 3H, 4H, and 2H with potential value to improve barley tolerance to CYDV-RPV. Moreover, Balaji *et al.* (2003) suggested determining BYDV and CYDV by using RT-qPCR for rapid and sensitive diagnosis. Vincent *et al.* (1991) identified nucleotide sequence and genome organization of RPV serotypes. Also, Zammurrad *et al.* (2014) indicated the high similarity of CP sequences of RPV isolates from different geographical regions. Recently, Singh *et al.* (2019) identified CYDV-RPVs in wheat samples by deep sequencing and determined they should be grouped separately from BYDVs in phylogenetic analysis.

The aim of this study was to investigate the presence and prevalence of CYDV-RPV in natural grass

hosts in the Trakya region. In order to determine the phylogenetic relationship of the Turkish five grass RPV isolates, the PCR products were sequenced, and partial nucleotide and amino acid sequences of RPV were compared with published sequences of other RPV isolates available in the GenBank® (Benson *et al*. 2013) and EMBL (Stoesser *et al*. 2000) databases.

## MATERIAL AND METHODS

*Survey studies and sampling*

Extensive survey studies were conducted in the cereal growing areas of the Trakya region, Turkey. A total of 584 grasses and volunteer cereal leaf samples exhibiting yellowing, reddening, stripe mosaic, irregular necrotic patches and dwarfing symptoms were collected from the border of cereal fields in Edirne, Kırklareli, and Tekirdag provinces of Trakya. Moreover, 30 intact grass plants representing each species were collected to determine aphid transmissions and herbariums.

*Aphid transmission*

Thirty symptomatic intact grass plants with colonized aphids were transplanted into 5 L pots filled with a mixture of sterile soil, sand and compost (1:1:1) and were kept alive in greenhouse conditions (21 ± 5°C, L16: D8). Apterous aphid colonies free from their parasites were collected and examined for diagnosis under a Stereomicroscope (Olympus SZ51). They were cultured on potted healthy wheat (cv. Pehlivan, and Atilla 12) and barley (cv. Barbaros) plants grown under greenhouse conditions. Barley (cv. Barbaros) was selected as the indicator plant for RPV. Five seeds were sown into 500 ml pots filled with a sterilized mixture of soil, sand, and compost (1:1:1). Aphid transmissions were performed as suggested by Du *et al.* (2007). Using a camel hairbrush, apteral individuals were collected in petri dishes and placed on each transplanted grass and left to feed for 72 h for the acquisition of RPV viral particles. For inoculation, 1 pot containing 5 barley plants at the 2-leaf stage was allocated and 5 viruliferous aphids were placed on per each plant, saving 1 healthy barley plant in each pot as control. This procedure was repeated for all transplanted grasses. Five days of post-inoculation, the aphids were killed by spraying insecticide and the plants were maintained in insect-proof greenhouse conditions till the plants exhibited viral symptoms.

*Serological test*

A total of 584 grass and volunteer cereal leaf samples, 30 intact grass samples and 50 indicator plant leaves obtained from aphid transmission, were tested by ELISA Reagent Set for Cereal yellow dwarf virus-RPV (BioReba AG, Reinach, Switzerland) for the presence of CYDV-RPV by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assays (DAS-ELISA) as described by Clark and Adams (1977).

*Nucleic acid isolation and cDNA synthesis*

All the samples of this work to investigate CYDV-RPV were subjected to the isolation of the viral nucleic acids by employing the total nucleic acid extraction method based on Trizol as described by Portillo *et al.* (2006). First-strand cDNA was synthesized from total isolated RNA by using a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). In each reaction, 0.5 µg RNA sample and 20 pmol of Reverse complementary primer of CYDV-RPV designed by Deb & Anderson (2007) were used and processed according to the manufacturer's instructions.

*RT-PCR amplifications*

The primer pairs RPV-L (5'-ATGTTGTACCGCTTGATCCAC-3'), RPV-R (5'-GCGAACCATTGCCATTG-3') as designed by Deb & Anderson (2007) were used for the amplification of coat protein region of CYDV-RPV. The amplified fragments were 400 bp long. The PCR reaction for RPV consisted of 3 µl 10x reaction buffer, 2 µl $MgCl_2$ (25 mM), 1 µl dNTP (10 mM), 0.5 µl for each primer, 0.3 µl Tag DNA polymerase enzyme (MBI Fermentas), 2 µl cDNA and 15.7 µl RNAse free water. The amplification protocol for RPV was as follows: initial denaturation step at 94°C for 2 min, followed by 40 cycles at 94°C for 30 sec, 60°C for 45 sec, 72°C for 1 min and the final extension step at 72°C for 10 min in a thermal cycler. PCR products were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and viewed under UV illumination in a gel documentation system (Vilber Lourmet, Marne La Vallee Cedex 1, France).

*Sequencing of PCR products*

For sequence analysis, PCR products were purified from agarose gels using QIAquick Gel Extraction Kit (Qiagen N.V., Venlo, Netherlands) in accordance with the manufacturer's protocol and sequenced by RefGen Biotechnology (Middle East Technical University (ODTU), Ankara, Turkey). Obtained nucleotide and deduced amino acid sequences were aligned with Bioedit Program (version 7.2.5; https://bioedit.software.informer.com/7.2, Hall 1999). The alignments were used as input data to construct phylogenetic trees with the neighbor-joining distance method implemented in the Mega X program (Kumar *et al.* 2018). Pairwise sequence comparisons were calculated with the BioEdit Program. The distance matrix for the neighbor-joining the analysis was calculated using the Kimura two-parameter model (Kimura 1980). Bootstrap analysis with 1,000 replicates was performed to assess the robustness of the branches.

## RESULTS AND DISCUSSION

The present survey study included three provinces of Trakya and resulted in the collection of 584 symptomatic grass and volunteer cereal leaf samples from 33 species belonging to the Poaceae, Asteraceae, Juncaceae, Geraniaceae, Cyperaceae, and Rubiaceae families. Such wild grasses exhibiting yellowing, reddening, stripe mosaic, irregular necrotic patches and dwarfing symptoms were examined for the presence of CYDV-RPV by ELISA and RT-PCR methods, and the transmission experiments were performed using aphid vector *R. padi* L. to confirm the RPV infections. During survey studies, the most common grasses in Trakya were detected to be *Avena sterilis*, *Phragmites australis*, *Hordeum* spp*., Bromus* spp., *Lolium* spp. in the family of Poaceae, which showed typical YDV symptoms infected naturally. Moreover, except for Poaceae grasses, the other weeds such as *Gastridium ventricosum*, *Galium aparine* L., *Cynodon dactylon, Dactylis glomerate, Sonchus asper, Juncus compressus, Geranium dissectum, Dasypyrum villosum, Lactuca serriola, Carex divisa* and *Taeniatherum caput-medusa* showing systemic symptoms were common in the border of the cereal fields and in grassland, which also was examined for the presence of RPV. However, one grass, *A. sterilis* was a common grass in the cereal fields and grassland in Trakya. Similarly, the most of those species were reported as competitive grasses in Turkey's cereal fields by Güncan & Karaca (2018). Among the aphid species known to transmit CYDV-RPV (Halbert & Voegtlin 1995), only *R. padi* has been recorded in the Trakya region was used for the aphid transmission tests in this study. These findings confirmed the observations obtained by Çalı and Yurdakul (1996) that determined aphid vectors in Central Anatolia, and *R. padi* were identified as common aphid vectors in cereal fields in Tekirdag province of Trakya by Ozder and Toros (1999). During the survey studies, *R. padi* were observed to colonize particularly on *A. sterilis* and be locally abundant and also on other grass species such as *L. perenne, P. exaratum, P. austrialis* and *A. fatua* grasses. Such 30 symptomatic intact grasses with aphid colonies were used for the aphid transmission tests of RPV as shown in Table 1. Aphid transmission test results showed that apterous *Rhopalosiphum padi* L. transmitted CYDV-RPV from *A. steriles*. *L. perenne* and *P. exaratum* symptomatic intact grasses to barley (cv. Barbaros) seedlings. According to ELISA and RT-PCR test results, 28 out of 50 indicator plants were found positive for RPV. Thus, the intact grass species found to be infected by RPV is among those observed to be the preferred hosts of *R. padi*. However, *A. sterilis* could have an otherwise greater attractivity for the aphid vectors than other grass species. This could be explained that *R. padi* prefers more to feed and maintain on this grass than in other grass species. The screening test results revealed that 55 (9.42%) out of 584 samples were infected with CYDV-RPV, as shown in Table 2. According to this, 19 grass species and two volunteer cereal samples were found infected with RPV. The results of this study showed that over-summering and over-wintering grasses were potential natural wild grass hosts of CYDV-RPV in the cereal fields of Trakya. Moreover, the infection rates of *Avena sterilis* and *Lolium rigidum* were more significant among the tested grass species than RPV's alternative potential reservoir hosts. Thus, our results revealed that the presence of significant reservoir grasses of RPV in Trakya. Similarly, McKirdy and Jones (1997) and Power and Gray

(1995) reported the wild grasses, perennial pasture grasses, and volunteer cereals are the crucial reservoir plant hosts of YDVs, which are important to know the direct interactions among the viruses, aphid vectors and host plants in the ecological system. Also, the previous findings in the Trakya region reported by Ilbağı *et al.* (2013) revealed *Bromus sterilis, Bromus arvensis, Poa trivialis* and *Sorghum halepense* were also the significant grass hosts of YDVs in the cereal growing areas of Trakya. Nevertheless, the results concerning twelve virus-free weed species including *Gastridium ventricosum*, *Galium aparine* L., *Cynodon dactylon, Dactylis glomerate, Sonchus asper, Juncus compressus, Elymus repens, Geranium dissectum, Dasypyrum villosum, Lactuca serriola, Carex divisa* and *Taeniatherum caput-medusa* were not found as alternative reservoir hosts of RPV as indicated in Table 2. Additionally, *C. dactylon* and *D. glomerate* weed species were identified as reservoir hosts of BYDV-PAV by D'Arcy (1995). Afterward, such grasses like *Echinochloa crus-galli, Seteria pumila,* and *Phalaris canariensis* were identified as the sources of YDVs in the Czech Republic (Pokorny 2006). Also, *Elymus repens*, *Avena fatua,* and *Sorghum halepense* as grass hosts of YDVs were reported in Bulgaria (Bakardjieva *et al.* 2006). Thus, our results confirm their findings related to *S. halepense, A. fatua* as grass hosts of RPV. The present results also showed that *P. australis* was found as an over-summering and over-wintering host of RPV. Similarly, Ilbağı *et al.* (2013) cited that *P. australis* was a widespread perennial grass as a natural reservoir host of YDVs. However, Ilbağı (2006) reported that *P. communis* Trin was not infected with RPV but was found BYDV-PAV in Tekirdağ province of Trakya. Virus detection tests were confirmed by direct sequencing of the PCR products in order to complete the molecular characterization of CYDV-RPV. The partial sequences belonged to five grass isolates originated from Tekirdağ (isolate TR-2 RPV, GenBank code KR005847; isolate TR-6 RPV, KT923457), Edirne (isolate TR-3 RPV, KT923454; isolate TR-4 RPV, KT923455), and Kirklareli (isolate TR-5 RPV, KT923456). The obtained nucleotide sequences were aligned and compared with 11 accessions available in GenBank⁄EMBL database. Multiple sequence alignments and pairwise sequence comparisons were performed using Bioedit software, as shown in Table 3. Sequence analysis among the studied five Turkish isolates of grasses indicated that the intragroup percentage of nucleotide identities were 93.93−98.62%. The lowest level of identity was 93.93% between isolate TR-2 RPV and TR-3 RPV, TR-6 RPV isolates, and also between TR-3 RPV and TR-6 RPV isolates, while the highest level of identity was 98.62% between TR-4 RPV and TR-5 RPV isolate. The comparison of the known RPV isolates with five Turkish isolates revealed that lowest identity was 40.38% between TR-3 RPV and isolate NY RPV and the highest was 95.86% between TR-2 RPV and 44P4b04-RPV from the USA. Amino acid multiple sequence alignments revealed that the lowest identity of five Turk-

T a b l e 1

Intact grass plants used in aphid transmission experiments

| Province | Intact grass species | No. of intact grasses |
|---|---|---|
| Edirne | *Avena sterilis* L. | 5 |
| Edirne | *Phragmites australis* (Cav.) Trin. ex Steudel | 4 |
| Kirklareli | *Phleum exaratum* Hochst. ex Griseb. | 3 |
| Kirklareli | *Avena fatua* L. | 3 |
| Kirklareli | *Avena sterilis* L. | 3 |
| Tekirdağ | *Avena sterilis* L. | 5 |
| Tekirdağ | *Lolium perenne* L. | 4 |
| Tekirdağ | *Avena fatua* L. | 3 |

T a b l e 2

The incidence rate of the naturally infected grass species in the Trakya region of Turkey

| Name of grass species | Family name | Number of samples | Number of RPV infected samples |
|---|---|---|---|
| *Avena fatua* L. | Poaceae | 20 | 2 |
| *Avena sterilis* L. | Poaceae | 60 | 11 |
| *Aegilops triuncialis* L. | Poaceae | 2 | 1 |
| *Alopecurus myosuroides* Huds. | Poaceae | 7 | 2 |
| *Apera spica venti* (L.) P. Beauv. | Poaceae | 4 | 1 |
| *Avena barbata* L. | Poaceae | 20 | 1 |
| *Avena sativa* L. (voluntary) | Poaceae | 31 | 2 |
| *Bromus sterilis* L. | Poaceae | 32 | 2 |
| *Bromus arvensis* L. | Poaceae | 17 | 3 |
| *Bromus hordeceaus* L. | Poaceae | 29 | 3 |
| *Bromus tomentellus* L. | Poaceae | 20 | 1 |
| *Hordeum bulbosum* L. | Poaceae | 10 | 1 |
| *Hordeum murinum* L. | Poaceae | 34 | 3 |
| *Lolium rigidum* Gaudin | Poaceae | 35 | 9 |
| *Lolium perenne* L. | Poaceae | 15 | 1 |
| *Phleum exaratum* (Sali) Aschers and Graebn. | Poaceae | 25 | 3 |
| *Phragmites australis* (Cav.) Trin. ex Steudel | Poaceae | 57 | 2 |
| *Poa trivialis* L. | Poaceae | 23 | 1 |
| *Sorghum halepense* (L.) Pers. | Poaceae | 24 | 3 |
| *Echinocloa cruss-galli* (L.) P.B | Poaceae | 23 | 1 |
| *Triticum aestivum* L. (voluntary) | Poaceae | 2 | 2 |
| *Elymus repens* (L.) Gould | Poaceae | 15 | – |
| *Juncus compressus* Jacq. | Juncaceae | 3 | – |
| *Gastridium ventricosum* (Gouan) Schinz & Thell | Poaceae | 3 | – |
| *Galium aparine* L. | Rubiaceae | 13 | – |
| *Cynodon dactylon* (L.) Pers. | Poaceae | 16 | – |
| *Dactylis glomerata* L. | Poaceae | 9 | – |
| *Sonchus asper* (L.) Hill | Asteraceae | 4 | – |
| *Dasypyrum villosum* (L.) *Cand.* | Poaceae | 9 | – |
| *Carex divisa* Huds. | Cyperaceae | 1 | – |
| *Geranium dissectum* L. | Geraniaceae | 17 | – |
| *Taeniatherum caput-medusa* (L.) Nevski | Poaceae | 2 | – |
| *Lactuca serriola* L. | Asteraceae | 2 | – |
| 33 | 6 | 584 | 55 |
| Rate of virus infections [%] | | | 9.42 |

Table 3

Percentage of nucleotide (above the diagonal) and amino acid (below the diagonal) sequence identity of partial sequencing region among studied RPV isolates

| RPV isolate names | 44P4b04 | 05P1b02 | OH1H | NY | PK | 05YC3 | TR-Van | Mardin117 | 005RPV | MI-RPV | NY-RPV | TR-2 | TR-3 | TR-4 | TR-5 | TR-6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 44P4b04 | | 80.16 | 41.32 | 41.32 | 42.14 | 40.77 | 42.42 | 42.69 | 41.59 | 41.87 | 41.59 | 95.86 | 93.66 | 92.28 | 93.11 | 95.04 |
| 05P1b02 | 61.15 | | 41.32 | 41.04 | 41.87 | 40.77 | 41.04 | 41.04 | 41.32 | 41.87 | 41.32 | 80.44 | 80.99 | 82.09 | 82.92 | 82.92 |
| OH1H | 16.62 | 13.22 | | 98.86 | 94.60 | 88.35 | 96.31 | 95.17 | 98.59 | 98.59 | 98.30 | 41.87 | 41.87 | 42.42 | 41.87 | 42.14 |
| NY | 15.70 | 13.22 | 96.65 | | 94.60 | 87.78 | 96.31 | 95.17 | 98.02 | 97.46 | 98.87 | 41.87 | 40.38 | 42.42 | 41.87 | 41.59 |
| PK | 15.70 | 13.22 | 90.90 | 90.90 | | 86.93 | 94.33 | 95.17 | 93.80 | 93.80 | 94.08 | 42.42 | 41.67 | 42.42 | 42.42 | 42.14 |
| 05YC3 | 15.70 | 14.87 | 76.03 | 74.38 | 73.55 | | 86.96 | 86.93 | 87.60 | 87.04 | 87.32 | 41.04 | 41.59 | 41.87 | 41.87 | 41.32 |
| TR-Van | 17.35 | 12 39 | 91.73 | 91.73 | 89.25 | 73.55 | | 97.73 | 96.61 | 95.49 | 96.33 | 42.42 | 42.42 | 42.69 | 42.14 | 42.42 |
| Mardin117 | 17.35 | 12 39 | 90.90 | 90.90 | 90.90 | 75.20 | 95.86 | | 94.92 | 94.36 | 94.64 | 42.42 | 42.42 | 42.97 | 42.42 | 42.42 |
| 005RPV | 16.52 | 13.22 | 96.69 | 95.04 | 89.25 | 74.38 | 93.38 | 90.90 | | 98.87 | 99.15 | 42.14 | 42.14 | 42.69 | 42.14 | 41.87 |
| MI-RPV | 16.52 | 13.22 | 97.52 | 94.21 | 88.42 | 73.55 | 90.90 | 88.42 | 97.52 | | 98.59 | 42.42 | 42.42 | 42.97 | 42.42 | 42.69 |
| NY-RPV | 16.52 | 13.22 | 95.86 | 97.52 | 90.08 | 73.55 | 92.56 | 90.08 | 97.52 | 96.69 | | 42.14 | 42.14 | 42.69 | 42.14 | 41.87 |
| TR-2 | 93.38 | 63.63 | 15.70 | 14.87 | 14.87 | 14.87 | 16.52 | 16.52 | 15.70 | 15.70 | 15.70 | | 93.93 | 94.21 | 94.49 | 93.93 |
| TR-3 | 86.77 | 63.63 | 15.70 | 14.87 | 15.70 | 17.35 | 16.52 | 16.52 | 15.70 | 15.70 | 15.70 | 85.95 | | 95.04 | 95.86 | 93.93 |
| TR-4 | 83.47 | 66.11 | 14.87 | 14 04 | 14.87 | 16.52 | 15.72 | 15.70 | 14.87 | 14.87 | 14.87 | 87.60 | 90.82 | | 98.62 | 94.76 |
| TR-5 | 82.29 | 66.11 | 14.87 | 14 04 | 14.87 | 16.52 | 15.70 | 15.70 | 14.87 | 14.87 | 14.87 | 86.77 | 92.56 | 97.52 | | 95.59 |
| TR-6 | 85.12 | 68.59 | 15.70 | 14.87 | 15.70 | 16.52 | 16.52 | 16.52 | 15.70 | 15.70 | 15.70 | 86.77 | 87.60 | 90.90 | 91.73 | |

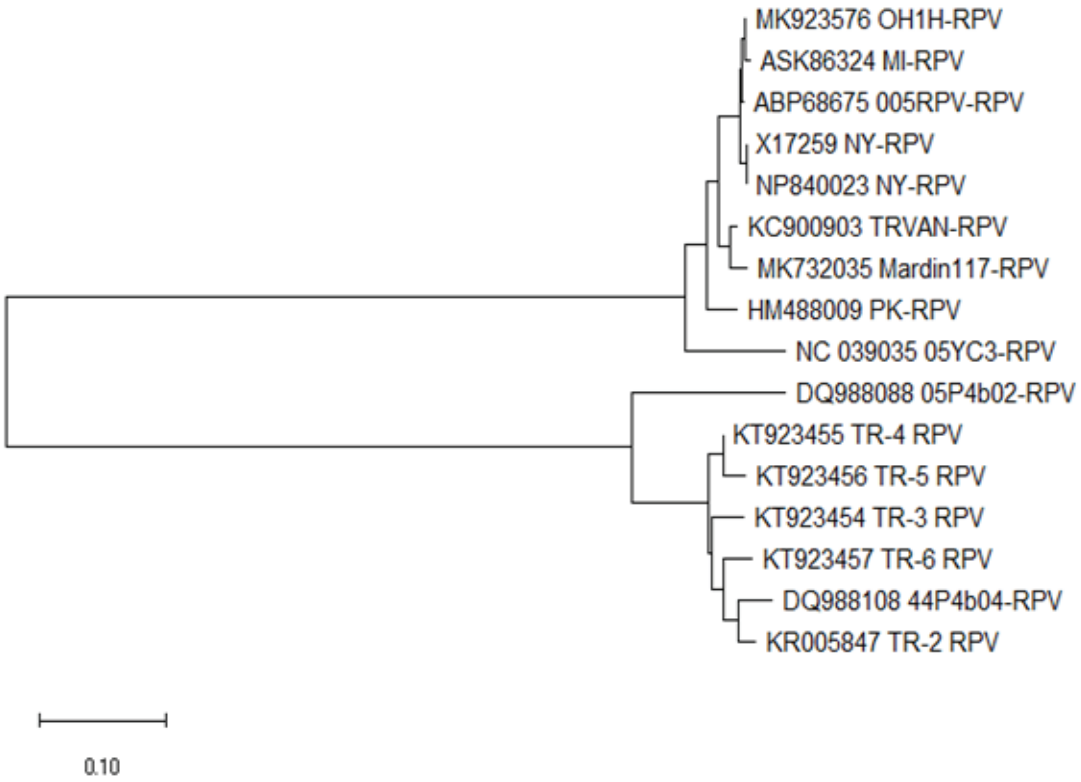


Figure 1. Constructed phylogenetic tree based on partial nucleotide sequencing results of PCR products from 5 Turkish RPV isolates and nucleotide sequences of 11 RPV isolates from the GenBank database.

ish isolates was 14.04% between TR-4 RPV, TR-5 RPV and isolate NY-RPV while the highest was 93.38% between TR-2 RPV and isolate 44P4b04-RPV from the USA. The intragroup percentage of amino acid identities of five Turkish grass RPV isolates was 85.95% between TR-2 RPV and TR-3 RPV while the highest was 97.52% between TR4-RPV and TR-5 RPV isolates. Similar high identity (99.95%) had the Pakistan RPV isolate with two RPV isolates from the USA belonging to different geographical regions as determined by Zamurrad *et al.* (2014). Phylogenetic tree of nucleotide sequences of five Turkish RPV isolates revealed two major groups as shown in Figure 1. Thus, the phylogenetic analysis indicated five Turkish RPV grass isolates clustered into the same group with two RPV isolates from the USA. These two RPV isolates included two ancestral recombination events for some isolates, as described by Robertson and French (2007). Also, Sigh *et al.* (2019) indicated that CYDV-RPV had several recombination events, which are distinct from the commonly detected BYDVs. Similarly, Vincent *et al.* (1991) determined that the sequence similarity and genome relationship of RPV serotype was more closely related to BWYV and PLRV, which typically infect dicotyledonous hosts, than to PAV serotypes of BYDV. This study constitutes the first study revealing the molecular characterization of RPV Turkish isolates on grass species. The results will increase the genetic composition and taxonomical relationship within different host isolates of YDVs in Turkey.

## CONCLUSIONS

YDVs cause economically important virus diseases of cereal crops worldwide. CYDV-RPV is one of the most serious virus species within them. This study was conducted to investigate the presence and prevalence of CYDV-RPV in reservoir grasses and volunteer cereal host plants exhibiting yellowing, reddening, and dwarfing symptoms in Turkey's Trakya region. The screening tests showed

that grasses in the family Poaceae were infected with CYDV-RPV as natural reservoir hosts in the cereal growing areas in Trakya. The incidence of RPV was at a rate of 9.42% in tested 584 symptomatic grass samples. Transmission experiments using the aphid species *Rhopalosiphum padi* L. showed that CYDV-RPV was transmitted persistently from symptomatic intact grasses such as *Avena sterilis*, *Lolium perenne* and *Phleum exratum* to barley cv. Barbaros seedlings. PCR products of five Turkish grass RPV isolates were sequenced and compared with eleven known RPV isolates in the GenBank. A phylogenetic tree of nucleotide sequences of five Turkish RPV isolates revealed two major groups of CYDV-RPV isolates. The conclusion of this study showed that 19 grass species and two volunteer cereal plants from the Poaceae family act as a reservoir of CYDV-RPV in the cereal growing areas in the Trakya region of Turkey.

***Acknowledgements.*** We would like to thank the Turkish Scientific and Technological Research Council (TÜBİTAK) for the valuable support to Project 109O522, this article obtained from part of its result.

## REFERENCES

ADAMS, M.I. – LEFKOWITZ, E.I. –KING, A.M.Q. – CARSTENS, E.B. 2014. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses. In A*rchives of Virology,* vol. *159,* pp. 2831−2841. DOI: 10.1007/s00705-014-2114-3.

BAKARDJIEVA, N. – KRASTEVA, C. – HABEKUSS, A. – RABENSTEIN, F. 2006. Detection of cereal viruses and study of aphid population in Bulgaria. In *Bulgarian Journal of Agricultural Science,* vol. *43,* pp. 499−501.

BALAJI, B. – BUCHOLTZ, B.D. – ANDERSON, J.M. 2003. Barley yellow dwarf virus and Cereal yellow dwarf virus quantification by real-time polymerase chain reaction in resistant and susceptible plants. In *Phytopathology,* vol. *93,* no. 11, pp. 1386−92. DOI: 10.1094/PHYTO.2003.93.11.1386.

BENSON, D.A. – CAVANAUGH, L.M. – CLARK, K. – KARSCH-MIZRACHI, I. – LIPMAN, D.J. – OSTELL,J. – SAYERS, E.W. 2013. GenBank. In *Nucleic Acids Research*, vol. *41*, pp. D36−42. DOI: 10.1093/nar/gks1195.

BREMER, K. – RAATIKAINEN, M. 1975. Cereal disease transmitted or caused by aphids and leafhopper in Turkey. In *Annales Academiae Scientarum. Fennicae,* A. *IV. Biologica,* vol. *203,* pp. 1−14.

BISNIEKS, M. – VARNHEDEN, A. – SIGVALD, R. – VALKONEN, J.P.T. 2004. Molecular diversity of the coat protein-encoding region of *Barley yellow dwarf virus*-PAV and *Barley yellow dwarf virus*-MAV from Latvia and Sweden. In *Archives of Virology,* vol. *149,* no. 4, pp. 843−853. DOI: 10.1007/s00705-003-0242-2.

CLARK, M.F. − ADAMS, A.N. 1977. Characteristics of the Microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. In *Journal of General Virology*, vol. *34,* pp.475−483. DOI: 10.1099/0022-1317-34-3-475.

ÇALI, S. – YURDAKUL, S. 1996. Investigation on virus diseases of wheat in Central Anatolia. In *Book of Abstracts, 5th International Wheat Conference.* June10−14, Ankara, Turkey, p. 120. ISBN:07923-4727-7.

D'ARCY, C.J. 1995. Symptomatology and host range of Barley yellow dwarf. In D'ARCY, C.J − BURNET, P.A. (Eds.), *Barley yellow dwarf 40 years of progress.* St Paul, MN-USA: APS Press, pp. 9−28. ISBN:0890541671.

DEB, M. – ANDERSON, J.M. 2007. Development of a multiplexed PCR detection method for barley and cereal yellow dwarf viruses, wheat spindle streak virus, wheat streak mosaic virus and soil-borne wheat mosaic virus. In *Journal of Virological Methods*, vol. *148*, pp. 17−24. DOI: 10.1016/j.jviromet.2007.10.015.

DEL BLANCO, I.A. – HEGARTY, J. – GALLAGHER, W. – FALK, B.W. – BROWN-GUEDIRA, G. – PELLERIN, E. – DUBCOVSKY, J. 2014. Mapping of QTL for tolerance to cereal yellow dwarf virus in two-rowed spring barley. In *Crop Sci*ence vol. *54*, pp.1468−1475. DOI: 10.2135/cropsci2013.11.0781.

DU, Z-Q. – LI, L. – WANG, X.F. – ZHOU, G. 2007. Evaluation of aphid transmission abilities and vector transmission phenotypes of barley yellow dwarf viruses in China. In *Journal of Plant Pathology*, vol. *89,* no. 2, pp. 251−259. Available at: www.jstor.org/stable/41998385.

GARRETT, K.A. – DENDY, S.P. – POWER, A.G. – BLAISDELL, G.K. – ALEXANDER, H.M. – McCARRON, J.K. 2004. Barley yellow dwarf disease in natural populations of dominant tallgrass prairie species in Kansas. In *Plant Diseases*, vol. *88*, pp. 574. DOI: 10.1094/PDIS.2004.88.5.574B.

GÜNCAN, A. − KARACA, M. 2018. *Weed control.* Konya: Selçuk Press, 334 p. ISBN: 975-448-178-4.

HALBERT, S. – VOEGTLIN, D. 1995. Biology and taxonomy of vectors of barley yellow dwarf viruses. In DARCY, C.J.D – BURNET, P.A. (Eds.), *Barley yellow dwarf 40 years of progress.* MN, USA: APS Press, pp. 217−258. ISBN:0890541671.

HALL, TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic Acids Symposium Series,* vol. *41*, pp. 95−98.

ILBAĞI, H. – CITIR, A. – YORGANCI, U. 2005. Occurrence of virus infections on cereal crops and their identifications in the Trakya region of Turkey. In *Journal of Plant Diseases and Protection,* vol. *112,* no. 4, pp. 313−320. Available at: www.jstor.org/stable/43215632.

ILBAĞI, H. 2006. Common reed (*Phragmites communis*) is a natural host of important cereal viruses in the Trakya region of Turkey. In *Phytoparasitica*, vol. *34,* no. 5, pp. 441−448. DOI: 10.1007/BF02981198.

ILBAĞI, H. – RABENSTEIN, F. – HABEKUSS, A. – ORDON, F. – ÇITIR, A. – CEBECI, O. – BUDAK, H. 2008. Molecular, Serological and Aphid Transmission Studies of *Barley yellow dwarf virus-PAV* and *Cereal yellow dwarf virus-RPV* in Canary Seed (*Phalaris canariensis* L.). In *Cereal Research Communications,* vol. *36,* pp. 225−234. DOI: 10.1556/CRC.36.2008.2.3.

ILBAĞI, H. – KARA, A. – UYSAL, M. – ÇITIR, A. 2013. *Poaceae weed host range of luteoviridae viruses in the Trakya region of Turkey.* In 16th Symposium European Weed Research Society. Samsun-Turkey. June, 24−27, p. 98. ISBN: 978-90-809789-12.

ILBAĞI, H. – ÇITIR, A. – KARA, A. – UYSAL, M. – AZZOUZ OLDEN, F. 2019. First report of barley yellow dwarf viruses (BYDVs) on dicotyledonous weed hosts in Turkey. In *Cereal Research Communications*, vol. *47,* no. 2, pp. 292−303. DOI:10.1556/0806.47.2019.015.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. In *Journal of Molecular Evolution*, vol. *16*, pp. 111−120. DOI: 10.1007/BF01731581.

KUMAR, S. – STECHER, G. – LI, M. – KNYAZ, C. – TAMURA, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. In *Molecular Biology and Evolution,* vol. *35,* no. 6, pp. 1547−549. DOI: 10.1093/molbev/msy096.

MCKIRDY, SJ. – JONES, R.A.C. 1997. Effect of sowing time on barley yellow dwarf virus infection in wheat: Virus incidence and grain yield losses. In *Australian Journal of Agricultural Res*earch, vol. *48*, pp. 199−206. DOI: 10.1071/A96073.

MCKIRDY, S.J. – JONES, R.A.C. – NUTTER, F.W. 2002. Quantification of yield losses caused by *Barley yellow dwarf virus* in wheat and oats. In *Plant Disease*, vol. *86,* pp. 769−773. DOI: 10.1094/PDIS.2002.86.7.769.

MILLER, W.A. – RASOCHOVA, L. 1997. Barley yellow dwarf viruses. In *Annual Review of Phytopathology*, vol. *35,* pp. 167−190. DOI: 10.1146/annurey.phyto.35.1.167.

MILLER, W.A. – LIU, S. – BECKETT, R. 2002. Barley yellow dwarf virus: Luteoviridae or Tombusviridae? In *Molecular Plant Pathology,* vol. *3,* pp.177−183. DOI: 10.1046/j.1364 3703.2002.00112.x.

ÖZDER, N. – TOROS, S. 1999. Investigations on the wheat aphids (Homoptera:Aphidoidea) in Tekirdağ province. In *Turkish Journal of Entomology,* vol. *23*, pp. 101−110.

PERRY, KL. – KOLB, F.L. – SAMMONS, B. – LAWSON, C. – CISAR, G. – OHM, H. 2000. Yield effects of Barley yellow dwarf virus in soft red winter wheat. In *Phytopathology,* vol. *90,* pp. 1043−1048. DOI: 10.1094/PHYTO.2000.90.9.1043.

PIKE, K.S. 1990. A review of barley yellow dwarf virus grain yield losses. In BURNETT, P.A. (Ed.), *World perspectives on barley yellow dwarf.* CIMMYT, Mexico, D.F., Mexico. pp. 356−361. ISBN 968-6127-39-9.

POCSAI, E. – CITIR, A. – ILBAGI, H. – KÖKLÜ, G. – KORKUT, K. – MURÁNYI, I. – VIDA G. 2003. Incidence of barley yellow dwarf viruses, cereal yellow dwarf virus and wheat dwarf virus in cereal growing areas of Turkey. In *Agriculture (Poľnohospodárstvo),* vol. *49,* no. 11, pp. 583−591.

POKORNY, R. 2006. Occurrence of viruses of the family luteoviridae on maize and some annual weed grasses in the Czech Republic. *In Cereal Research Communications*, vol. *34,* no. 2−3, pp. 1087−1092. DOI: 10.1556/CRC.34.2006.2-3.242.

PORTILLO, M. – FENOLL, C. – ESCOBAR, C. 2006. Evaluation of different RNA extraction methods for small quantities of plant tissue: Combined effects of reagent type and homogenization procedure on RNA quality-integrity and yield. In *Physiologia Plantarum*, vol. *128,* pp. 1−7. DOI: 10.1111/j.1399-3054.2006.00716.x.

POWER, AG. – GRAY, SM. 1995. Aphid transmission of barley yellow dwarf viruses: Interactions between viruses, vectors and host plants. In D'ARCY, C.J. – BURNETT, P.A. (Eds.), *Barley yellow dwarf 40 years of progress*. MN, USA: APS Press, pp. 259−292. ISBN:0890541671.

ROBERTSON, N.L. – FRENCH, R. 2007. Genetic structure in natural populations of barleycereal yellow dwarf virus isolates from Alaska. In *Archives of Virology*, vol. *152*, pp. 891−902. DOI: 10.1007/s00705-006-0913-x.

ROCHOW, W.F. – DUFFUS, J.E. 1981. Luteoviruses and yellow diseases. In KURSTAK, E. (Ed.), *Handbook of Plant Virus Infections: Comparative Diagnosis,* Elsevier/North-Holland: Biomedical Press, USA, pp. 147−170. ISBN: 9780444803092.

SMITH, P.R. – PLUMB, R.T. 1981. Barley yellow dwarf virus infectivity of cereal aphids trapped at 2 sites in Victoria. In *Australian Journal of Agricultural Research,* vol. *32,* no. 2, pp. 249−255. DOI: 10.1071/AR9810249.

SINGH, K. – JAROSOVA, J. – FOUSEK, J. – HUAN, C. – KUNDU, J.K. 2019. Virome identification in wheat in the Czech Republic using small RNA deep sequencing. In *Journal of Integrative Agriculture,* vol. *18,* pp. 2−10. DOI: 10.1016/s2095-3119(19)62805-4.

STOESSER, G. – BAKER, W. – VAN DEN BROEK, A. – CAMON, E. – HINGAMP, P. – STERK, P. – STOESSER, G. – TULI, M.A. 2000. The EMBL Nucleotide Sequence Database. In *Nucleic Acids Research*, vol. *28*, no. 1, pp. 19−23. DOI: 10.1093/nar/28.1.19.

VINCENT, J.R. – LISTER, R.M. – LARKINS, B.A. 1991. Nucleotide sequence analysis and genomic organization of the NY-RPV isolate of barley yellow dwarf virus. In *Journal of General Virology,* vol. *72,* pp. 2347−2355. DOI: 10.1099/0022-1317-72-10-2347.

ZAMURRAD, M. – SHAHID, H. – KAMRAN, S. – UL HAQUE, I. – KAUSAR, S. 2014. Phylogenetic analysis of coat protein gene of CYDV-RPV strain from wheat. In *Advancements in Life Sciences,* vol. *2,* no. 1, pp. 16−22. DOI: www.als-journal.com/214-14.

Received: September 9, 2020
Accepted: December 14, 2020