Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Short Notes

First report of cereal yellow dwarf virus (CYDV-RPS) on maize in Bosnia and Herzegovina

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Summary. A survey was conducted in Bosnia and Herzegovina in 2023, to investigate the presence of several important viruses affecting cereals, particularly those associated to cereal yellow dwarf viruses (CYDVs) and barley yellow dwarf viruses (BYDVs). Sixty leaf samples were collected, including 47 from maize plants (*Zea mays* L.) and 13 from barley plants (*Hordeum vulgare* L.), from across four grain-producing regions (Odzak, Sarajevo, Gornji Vakuf and Ilidza). Assessments for both groups of viruses, using ELISA and RT-PCR assays, detected CYDV in one maize sample (hybrid BC 418B) out of the 60 samples assessed. Nucleotide sequence analysis of the RT-PCR amplicon (2476 bp) of Bosnian isolate from maize hybrid BC418B (GenBank no. PV476203) showed that the isolate had 99.1% similarity with the CYDV RPS Mexican isolate (RPV-Mex-1; GenBank no. NC002198). This is the first report of the presence of CYDV-RPS in Bosnia and Herzegovina.

Keywords. Maize, barley, yellow dwarf viruses, RT-PCR.

OPEN ACCESS

Citation: Ben Slimen, A., Vukojevic, A., Mujkovic, M., Krajina-Ibrulj, E. & Elbeaino, T. (2025). First report of cereal yellow dwarf virus (CYDV-RPS) on maize in Bosnia and Herzegovina. *Phytopathologia Mediterranea* 64(2): 199-203. doi: 10.36253/phyto-16200

Accepted: July 6, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Arnaud G Blouin, Institut des sciences en production végétale IPV, DEFR, Agroscope, Nyon, Switzerland.

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INTRODUCTION

Yellow dwarf viruses (YDVs) are among the most economically damaging and widespread viruses affecting cereal crops, often leading to significant yield losses (Rybicki, 2015). Some viruses containing "yellow dwarf" in their names do not infect cereals, and conversely, some cereal-infecting viruses lack "yellow dwarf" in their names (e.g., barley virus G). The present study specifically addressed viruses that infect cereals and cause yellow dwarf diseases, particularly viruses within two YDV groups: barley yellow dwarf viruses (BYDV, *Tombusviridae*: *Luteovirus*) and cereal yellow dwarf viruses (CYDV, *Solemoviridae*: *Polerovirus*).

As studies of these two groups have evolved, their classification underwent numerous revisions (Rochow, 1969; Rochow and Muller, 1971; Jin *et*

al., 2004; Zhang *et al*., 2009; Jarosova *et al*., 2013). Taxonomy was initially based on specific virus vectors, clustering all in the *Luteoviridae* which is no longer recognized. BYDV and CYDV complexes included diverse species, *i.e*. the four species that were originally discovered as BYDVs, BYDV-Pav, BYDV-MAV, and BYDV-RMV (now reclassified as maize yellow dwarf virus-RMV; MYDV-RMV), and BYDV-RPV reclassified into CYDV-RPV (Rochow, 1969; Rasochova and Miller, 1997). Further viruses were later identified, including BYDV-GAV, BYDV-SGV, BYDV-KerII and BYDV-KerIII in the United States of America, and BYDV-PAS as a new variant now considered as a diverse species deriving from BYDV-PAV (Wang *et al*., 2001; Zhang *et al*., 2009; Jarosova *et al*., 2013). Similarly, another CYDV virus called CYDV-RPS was separated from the initially found CYDV-RPV (Jarosova *et al*., 2013).

Names for some of these viruses have changed. Formerly known BYDV-GPV is now identified as a wheat yellow dwarf virus under the designation *Polerovirus* WYDVGPV (Cheng *et al*., 1996; Wang *et al*., 1998; Zhang *et al*., 2009). Following sequencing of their genomes, these two groups (CYDVs and BYDVs) were shown to be distantly related. Specifically, CYDV-RPV is more closely related to Potato leafroll virus (PLRV) and Beet western yellows virus (BWYV) than to BYDV-PAV. This led to reclassification of CYDV-RPV and CYDV-RPS into *Polerovirus* (Krueger *et al*., 2013; Delfosse *et al*., 2021), while the BYDVs were assigned to *Luteovirus* (D'Arcy *et al.,* 2000; Ali *et al.,* 2014; Scheets *et al*., 2020).

From 2023, the International Committee on Taxonomy of Viruses (ICTV) implemented a new nomenclature system that reorganized the BYDV-MAV and BYDV-PAV viruses as, respectively, *Luteovirus mavhordei* and *Luteovirus pavhordei*. BYDV-SGV, BYDV-PAS, BYDV-kerII, and BYDV-kerIII have been given the species names of, respectively, *Luteovirus sgvhordei*, *Luteovirus pashordei*, *Luteovirus kerbihordei*, and *Luteovirus kertrihordei*. Similarly, the species name of CYDV-RPS is *Polerovirus CYDV-RPS,* and the species name for CYDV-RPV is *Polerovirus CYDV-RPV* (ICTV, 2024).

BYDVs and CYDVs have +ssRNA genomes, ranging in size from 5.5 to 6 kb, each with five to eight ORFs depending on the genus and isolate (Domier and D'Arcy, 2008). Transmitted by aphids (Lister and Ranieri, 1995), YDVs cause epiphytotic outbreaks in nearly all small grain cereal-producing regions, leading to host symptoms including yellowing, stunting, and\or reddening of leaves, depending on the host (Oswald and Houston, 1953; Zitter, 2001; Ali *et al*., 2018; Trzmiel, 2020). Many of these viruses have been reported in various Eastern European countries, but none have been reported in Bosnia and Herzegovina (BiH) (Jarosova *et al*., 2013; Kakareka *et al*., 2020; Trzmiel and Hasiow-Jaroszewska, 2023).

Because of the economic significance of cereal production in BiH (BHAS, 2014) and potential presence of these viruses in maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.), the present study was conducted to determine virus occurrence in cereal-producing regions of this country.

MATERIALS AND METHODS

Origins of plant material

In the 2023 growing season, a total of 60 leaf samples were collected from four cereal-producing regions of BiH. The sampling locations were in Odzak (29 samples), Butmir (four samples), Otes (11 samples), Bojnik (11 samples) and Gornji Vakuf (five samples). Among the samples, 47 were from maize of five distinct varieties. These were two hybrid varieties for which the specific variety names were unidentified, but the plants were within the maturity classes FAO 400 (11 samples) and FAO 500 (15 samples). Additionally, the sample set comprised hybrid BC678 (five samples), hybrid BC418B (eight samples), and Pajdas (eight samples). Furthermore, 13 barley plants of variety Tuna were also included in the collection. The collected leaves had symptoms indicating virus infections, including yellowing and stunting. After collection, the samples were stored at -80°C for further analyses.

DAS-ELISA, RT-PCR and RT-qPCR assays

All samples were first screened for the presence of BYDVs and CYDVs in a Double-Antibody-Sandwich Assay (DAS-ELISA) (Clark and Adams, 1977), and using polyclonal antibodies to detect the serologically known BYDV-B subgroup (BYDV-PAV) (IgG: Art. No. 140115), the BYDV-F subgroup (BYDV-MAV) (IgG: Art. No. 140215), BYDV-RPV (now CYDV-RPV) (IgG: Art. No. 140615), using the commercial ELISA of BIOREBA AG, Reinach, Switzerland (Derron *et al*., 1986; Ayala *et al*., 2001). The samples were assessed alongside an internal positive control of infected material (BYDV-PAV, Art. No. 140153; BYDV-MAV, Art. No. 140253; CYDV-RPV, Art. No. 140653) and were analyzed using a Multiread 400 Microplate Reader (Biochrom) at 405 nm. Two molecular assays (RT-PCR and RT-qPCR) were subsequently carried out on reverse-transcribed total nucleic acids extracted from leaves, as described by Foissac *et al.* (2001). PCR was carried out using specific prim-

Table 1. List of the specific primer sequences designed and used in RT-PCR for detecting CYDV-RPV and CYDV-RPS in this study.

Virus	Primers Sequence (5′ to 3′)	Amplicon size
CYDV-RPS	RPS-F1: CTCTTGTGACGAGTGAGCACAA RPS-R1: GTCAATCCGAAAGTCATCCCA	1395 bp
	RPS-F2: TGGGATGACTTTCGGATTGAC RPS-R2: GCTCAGTTATCTTTTGTGGTTATGCC	1117 bp
CYDV-RPV	RPV-F1: AAGACATCGAAGACGAGTCGGGAA RPV-R1: ACGTTTCCCAACTTAACTCACCT	794 bp
	RPV-F2: AGGTGAGTTAAGTTGGGAAACGT RPV-R2: ACGCCRGGTACTCGTTGAGCTAA	719 bp

ers for BYDV isolates (BYDV-MAV, BYDV-PAV, MYDV-RMV, BYDV-SGV) and CYDV-RPV (Deb and Anderson, 2008; Balaji *et al.,* 2003). The PCR products were electrophoresed on a 1.2% TAE agarose gel. Amplicons of positive samples were ligated into a pGEM-T Easy vector (Promega) and were transformed into *Escherichia coli* DH5α-competent cells, following the manufacturer's instructions. Three clones containing the expected size of the DNA inserts were sent for sequencing (Eurofins Genomics).

Further RT-PCR assays were carried out using four sets of specific primers targeting two overlapping parts in the RNA-dependent RNA polymerase P1-P2 fusion protein, where CYDV-RPV and CYDV-RPS show most differences. Two primer pairs were designed specifically for CYDV-RPS, based on the alignment of the available sequence isolates retrieved from GenBank, while the other two primer pairs targeted CYDV-RPV using the same approach (Table 1). Following the same procedure as for the previous RT-PCR assays, three clones from each amplification were sent for sequencing (Eurofins Genomics).

RESULTS AND DISCUSSION

DAS-ELISA conducted on barley and maize samples yielded one positive reaction, suggesting the presence of CYDV-RPV in a maize sample (of maize hybrid BC 418B). The RT-PCR and RT-qPCR assays generated positive reactions to CYDVs using the universal primers (RPV-CP-F/RPV-CP-R) (Balaji *et al.,* 2003) from this sample from maize hybrid BC 418B. The nucleotide sequence analysis of the three PCR DNA clones (332 bp) obtained from the infected maize sample showed one sequence type, which in BLASTN analysis had 98.8% similarity with *Polerovirus* isolate CYDV-RPS Mex-1 (AF235168) and 92.8% similarity with *Polerovirus* isolate CYDV-RPV TR-2 (KR005847). Given the slight differences in similarities, this sequence alone was not sufficient to confirm whether the infection was due to CYDV-RPS or CYDV-RPV. The subsequent RT-PCRs were conducted on the same infected maize sample using specific pairs of primers for each of CYDV-RPV and CYDV-RPS. Only RPS1 and RPS2 primers amplified distinct amplicons, in contrast to the specific RPV primers where no amplifications were observed. The three clones obtained for each of the two CYDV-RPS amplicons showed identical sequences and complete alignment; upon merging, they generated a consensus sequence of 2,477 nucleotides in length. BLASTN analysis of the consensus sequence showed that it shared 99.1% nucleotide similarity with CYDV-RPS Mex-1 isolate (AF235168), and 99% similarity at amino acid level to RNA-dependent RNA polymerase P1-P2 fusion protein (AAF62532) of the same CYDV RPS isolate.

When compared to available European CYDV-RPS isolate sequences, the sequence from the single maize sample showed 96.5% similarity to the Estonian isolate Olustvere1-O (MK012664), and 96.2% similarity to the Irish La3a isolate (OQ686645). The newly identified sequence from the present study, named CYDV-RPS BiH isolate, has been deposited in GenBank under accession number PV476203.

In the ELISA test, the CYDV-RPV antibodies used were unable to distinguish between the CYDV-RPV and CYDV-RPS species. A similar limitation was also reported by Miller *et al.* (2002). Consequently, it was necessary to use additional diagnostic assays and sequencing analyses to accurately determine the viral species responsible for the YD infection.

Earlier CYDV-RPS detections, such as those from Mexico (Miller *et al*., 2002) and Iran (Rastgou *et al*., 2005), relied on RT-PCR with CYDV-RPV primers. The recent CYDV-RPS discoveries have mostly been attributed to High-Throughput Sequencing (HTS) techniques, with reports from the United Kingdom (Pallett *et al*., 2010), the United States of America (Malmstrom *et al*.,

2017), the Czech Republic (Singh *et al*., 2020), Estonia (Somera *et al*., 2021), and Ireland (Byrne *et al*., 2024).

This is the first report of a CYDV-RPS in BiH. Further investigations are required to assess the virus's prevalence in this country, as well as its potential correlation to the symptoms observed in the maize hybrid BC 418B.

ACKNOWLEDGEMENT

This study was supported by the project "PHYTO BiH" (AID 011681), funded by the General Directorate for Development Cooperation of the Italian Ministry of Foreign Affairs and International Cooperation.

AUTHOR CONTRIBUTIONS

A.B.S.: writing original manuscript, visualization, validation, methodology, formal analysis, review and editing. A.V.: writing original manuscript, visualization, methodology, review. M.M.: writing, review and editing, visualization, administration. E.K.I.: investigation, visualization, methodology. T.E.: review and editing, visualization, supervision, resources and funding acquisition. All authors read, revised, and approved the final manuscript of this paper.

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