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ORIGINAL ARTICLE

Occurrence of *Wheat dwarf virus* and *Barley yellow dwarf virus* species in Poland in the spring of 2019

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Abstract

In the spring of 2019, many plants, mainly winter wheat, were observed to have dwarfism and leaf yellowing symptoms. These plants from several regions of Poland were collected and sent to the Plant Disease Clinic of the Institute of Plant Protection – National Research Institute in Poznań to test for the presence of viral diseases. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) results showed numerous cases of *Wheat dwarf virus* (WDV) and a few cases of plant infections caused by *Barley yellow dwarf viruses* (BYDVs). WDV was detected in 163 out of 236 tested winter wheat plants (69.1%), in 10 out of 27 tested winter barley plants (37%) and in 6 out of 7 triticale plants (85.7%) while BYDVs were found, respectively, in 9.7% (23 out of 236) and in 18.5% (5 out of 27) of tested winter forms of wheat and barley plants. Infected plants came mainly from the regions of Lower Silesia and Greater Poland. Furthermore, individual cases of infections were also confirmed in the following districts: Lubusz, Opole, Silesia, Kuyavia-Pomerania and Warmia-Masuria. Results of Duplex-immunocapture-polymerase chain reaction (Duplex-IC-PCR) indicated the dominance of WDV-W form in wheat and WDV-B form in barley plants. Moreover, results of reverse transcription – polymerase chain reaction (RT-PCR) connected with restriction fragment length polymorphism (RFLP) analysis, performed for 17 BYDVs samples, revealed 8 BYDV-PAS, 4 BYDV-MAV and 2 BYDV-PAV as well as the presence of two mixed infections of BYDV-MAV/-PAS and one case of BYDV-MAV/-PAV. Next, RT-PCR reactions confirmed single BYDV-GAV infection and the common presence of BYDV-SGV. To the best of our knowledge, in 2020 the viruses were not a big threat to cereal crops in Poland.

Keywords: *Barley yellow dwarf virus*, double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), Duplex-immunocapture-polymerase chain reaction (Duplex-IC-PCR) reverse transcription-polymerase chain reaction connected with restriction fragment length polymorphism analyses (RT-PCR-RFLP), *Wheat dwarf virus*

Introduction

In the spring of 2019, many plants, mainly winter forms of wheat (*Triticum aestivum* L.), some winter barley (*Hordeum vulgare* L.) and triticale (x *Triticosecale* Wittm. ex A. Camus), were observed with dwarfism and leaf yellowing symptoms. The problem concerned primarily the southwestern part of Poland. The symptoms may indicate viral infection, such as leaf discoloration and plant growth inhibition which are characteristic of barley yellow dwarf disease (BYD) and they may be induced by *Wheat dwarf virus* (WDV).

BYD is the most important and widespread viral disease of cereal crops. It is caused by a group of related monopartite single-stranded (+ss)RNA spherical viruses transmitted by about 25 aphid species (Domier 1995; Halbert and Voegtlin 1995). This viral disease is induced by *Barley yellow dwarf virus* species

(BYDV)-kerII, -kerIII, -MAV, -PAS, -PAV, -GAV; *Cereal yellow dwarf virus* (CYDV)-RPV, -RPS and *Maize yellow dwarf virus*-RMV (MYDV-RMV) as well as BYDV-SGV and -GPV, which are members of the genus *Luteovirus*, *Polerovirus* and unassigned members in family Luteoviridae, respectively (Domier 2012). In Poland, BYD was first detected in oat (Hoppe *et al.* 1983) and subsequently in winter wheat and barley (Jeżewska 2003) and in maize plants (Trzmiel and Lubik 2011). The results of studies conducted in 2006–2010 revealed different levels of severity of BYD in Poland (Jeżewska *et al.* 2010). The first outbreaks of BYD on winter cereals were observed in 2001/2002 (Jeżewska 2003) and next in 2014/2015 growing seasons (Jeżewska and Trzmiel 2016; Trzmiel 2017). BYD is a threat especially for winter forms of cereals when infected aphids spread virus infection during the growth of young plants. Yield losses caused by BYD are dependent on many factors and can be as much as several dozen percent (Thackray *et al.* 2005).

Wheat dwarf virus (WDV) is the second most important pathogen which infects main cereal crops. The virus has a monopartite single-stranded circular (ss) DNA genome and it is classified as a member of the genus *Mastrevirus* in the family Geminiviridae (Vacke *et al.* 2004). WDV is transmitted only by the leafhopper species, *Psammotettix alienus* (Dahlb.) (Vacke 1962) and *P. provincialis* (Ekzayez *et al.* 2011) in a persistent manner, by both the larval and the imago stages. The intensity of disease symptoms depends on the growth phase of infected plants and is the strongest in the one-leaf stage. WDV causes yield losses which can be as much as 80% (Lindbland *et al.* 1999). In Poland WDV was found for the first time in 1999 (Jeżewska 2001). The next studies confirmed WDV infections of wheat, barley and triticale plants in different parts of Poland (Jeżewska *et al.* 2010) as well as the presence of both WDV-B and WDV-W forms (Trzmiel 2018).

This study was undertaken to evaluate the prevalence and diversity of viruses infecting cereal crops in the spring of 2019 in Poland.

Materials and Methods

Plant sources

The plant samples of winter forms, including barley (27), triticale (7) and wheat (239) with yellowing and stunting symptoms, were collected in the spring of 2019 (from March to June), from commercial fields in 40 different locations. Samples originated from northern [Pomerania (1), Kuyavia-Pomerania (2), Warmia-Masuria (1)], central [Lubusz (1), Greater Poland (10) and Lublin (1)] as well as southern [Lower Silesia (20), Opole (2) and Silesia (2)] regions of Poland.

ELISA tests

Screening tests using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977) with commercial polyclonal antibodies (Loewe, Sauerlach, Germany) specific for BYDV-MAV/BYDV-PAV and WDV were done. Samples were considered positive when their optical density (OD) values were at least three-times higher than the average OD of the negative control (healthy plants from a greenhouse). Some positive samples (17 BYDVs and 22 WDV) were selected for further studies by molecular techniques.

Duplex-immunocapture-polymerase chain reaction (Duplex-IC-PCR)

In order to discriminate barley- and wheat-specific forms of wheat dwarf virus (WDV-B and WDV-W, respectively) Duplex-IC-PCR was carried out for 16 winter wheat and 6 winter barley samples. The reactions were performed with WDV-H-F (CAAGGG GCGAGATCACACA)/WDV-H-R(CCACAACTACT ACAACAGCC) and WDV-T-F (CGAGTAGTTGA TGAATGACTCG)/WDV-T-R(GGCTGTTTCAACT CCAGGTCG) primer pairs according to (Trzmiel 2018). Firstly, PCR tubes were coated with 20 μl mixture of 10-fold diluted polyclonal anti-WDV antibody with commercial coating buffer (Loewe). Next, the coated reaction tubes were incubated with 50 μl plant sap for 1 h at 37°C. The reactions were carried out using 5 μl of Dream Taq Green PCR Master Mix (2X) (Thermo Fisher Scientific), 0.2 μM Primer Mix (10 $\mu mol \cdot \mu l^{-1}$ each) and sterile Milli-Q water for a final volume of 10 μl. The reactions were performed under thermal conditions as follows: initial denaturation at 94°C for 2 min, 35 cycles of 30 s at 94°C, 30 s at 55°C, 60 s at 72°C and a final elongation at 72°C for 7 min. PCR products were separated by electrophoresis using 1% TAE agarose gel and stained with Midori Green DNA Stain (Nippon Genetics Europe GmbH, Düren, Germany) for UV light visualization.

RNA isolation

Total RNA was extracted from 17 symptomatic plant samples (13 winter wheat and 4 winter barley samples, originating from different locations) using Total RNA Purification Kit (Novazym, Poznań, Poland) according to the manufacturer's instructions. The concentration of total RNA was measured with a NanoDrop 2,000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and stored at –20°C.

Reverse transcription-polymerase chain reaction connected with restriction fragment length polymorphism analyses (RT-PCR-RFLP)

First-strand cDNA was synthetized using Super-Script IV Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA) with BYcp-R (CCG GTGTTGAGGAGTCTACC) (Kundu 2008) following the manufacturer's instructions. PCR reaction was carried out using 1 µl of RT mixture, 5 µl of Dream Taq Green PCR Master Mix (2X) (Thermo Fisher Scientific), 0.2 µM Primer Mix (10 µmol · μl^{-1} each) BYcp-F (CCACTTAGAGAGGTGGTGAATG) and BYcp-R (CCGGTGTTGAGGAGTCTACC) (Kundu 2008) and sterile Milli-Q water for a final volume of 10 µl. Amplification was performed under thermal conditions as proposed by Kundu *et al.* (2009). PCR products (641 bp in size) were analyzed by electrophoresis as before. In the next step, RFLP analyses were done to discriminate BYDV species. PCR amplicons were digested by *Hpa* II endonuclease (Thermo Fisher Scientific) at 37°C for 3 h (Kundu *et al.* 2009). Digested PCR products were separated in 2% TAE agarose gel and stained as before.

Reverse transcription-polymerase chain reaction (RT-PCR)

The reactions, using the OneStep RT-PCR Kit (Qiagen, Hilden, Germany), were carried out with SGVL2 (ACCAGATCTTAGCCGGGTTT)/SGVR2 (CTGGACGTCGACCATTTCTT) (Deb and Anderson 2008) and GAV2-F (CAACCCCCTATCAGCAGA GGGA)/GAV3-R(TCCAGCGTTAAGCACCAAACCA) (Trzmiel 2017). The reactions were performed in a final volume of 10 µl according to the manufacturer's instructions. The cycling parameters were as follows: 30 min at 50°C (reverse transcription), 15 min at 95°C (PCR activation), 40 cycles of 30 s at 95°C, 30 s at 55 or 58°C, 1 min at 72°C and 7 min at 72°C (final extension). RT-PCR products for BYDV-SGV (237 bp) and BYDV-GAV (856 bp) were analyzed by electrophoresis as before.

Results

DAS-ELISA results confirmed the presence of viruses in most of the tested samples (76.7%) (Table 1). The symptoms of virus-free samples may have been caused by other pathogens e.g. fungi or may have been the result of some phytotoxicity.

Preliminary diagnoses using DAS-ELISA tests revealed a common presence of WDV in the south-western region of Poland (Fig. 1). With the exception of Pomerania and Lublin, infected plants with WDV were confirmed in almost all of the 40 tested locations. WDV incidence was 69.1% in tested winter wheat plants, 37% in winter barley plants and 85.7% in triticale plants (Table 2). Molecular diagnostic results revealed the presence of WDV-B and WDV-W forms. In Duplex-IC-PCR specific amplicons, of the expected size for WDV-B (483 bp) and for WDV-W (734 bp), were obtained (Fig. 2). Moreover, the obtained data indicated numerical superiority of WDV-W over WDV-B in infected plants (63.6 and 36.4%, respectively). WDV-W was dominant in wheat and WDV-B was dominant in barley plants (Table 3).

Table 1. Detection of BYDV-MAV/-PAV and WDV by DAS-ELISA in the spring of 2019 in particular locations

Location (district)	Number of infected plants/number of tested samples	
	BYDVs	WDV
Pomerania	2/8	0/8
Warmia-Masury	0/2	2/2
Kuyavia-Pomerania	0/1	1/1
Lubusz	0/2	1/2
Great Poland	14/83	61/83
Lublin	0/5	0/5
Lower Silesia	8/157	111/187
Opole	2/7	2/7
Silesia	0/5	2/5

BYDVs – *Barley yellow dwarf viruses*; WDV – *Wheat dwarf virus*

Fig. 1. Locations of *Wheat dwarf virus* (WDV) infections in Poland in the spring of 2019

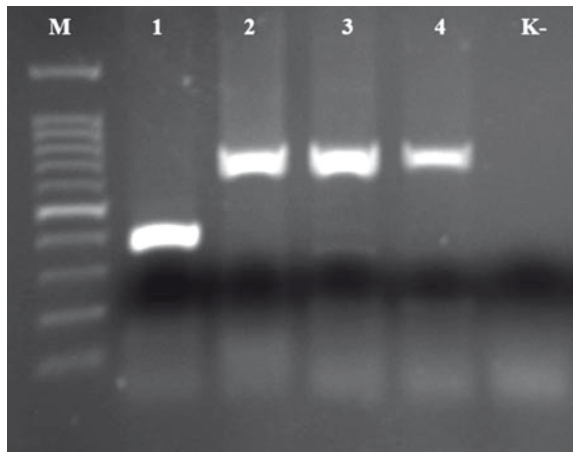

Fig. 2. Detection of WDV-W (734 bp) and WDV-B (483 bp) in plant samples by Duplex-IC-PCR. Amplified products were analyzed in 1% TAE agarose gel. Lanes: M – 100-bp DNA ladder (Novazym); 1 – tested barley sample; 2–4 – tested wheat samples; K- – no-template control

Table 2. Detection of *Wheat dwarf virus* (WDV) and BYDV-MAV/-PAV infections by DAS-ELISA test (number of infected plants/number of tested plants are given)

Virus	Wheat	Barley	Triticale	Average (percent of positive samples)
BYDV-MAV/-PAV	23/236	5/27	0/7	28/270 (10.37%)
WDV	163/236	10/27	6/7	179/270 (66.29%)

Table 3. Detection and discrimination of barley- and wheat-specific forms of *Wheat dwarf virus* (WDV) by Duplex-IC-PCR (number of infected plants/number of tested plants are given)

WDV-barley-specific form (WDV-B)		WDV-wheat-specific form (WDV-W)	
Barley	Wheat	Barley	Wheat
5/6	3/16	1/6	13/16

On the other hand, DAS-ELISA results showed a limited presence of BYDV species. BYDV-MAV/-PAV infections were confirmed in plants collected from Greater Poland, Lower Silesia, Opole and Silesia (Table 1). Moreover, the rate of infected plants was much lower: 9.6 and 18.5% for tested winter forms of wheat and barley plants, respectively (Table 2). Furthermore, RT-PCR-RFLP results demonstrated the presence of single infections caused by BYDV-MAV, BYDV-PAV and additionally by BYDV-PAS. Moreover, mixed infections with BYDV-MAV/BYDV-PAS and BYDV-MAV/BYDV-PAV were reported. RFLP results of the positive samples showed five different patterns after digestion of PCR products by *Hpa* II as follows: (1) four samples with the BYDV-MAV pattern, (2) eight samples with the BYDV-PAS pattern, (3) two

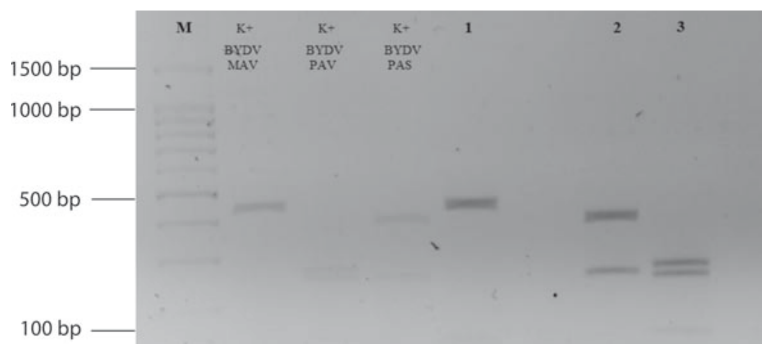

Fig. 3. Restriction profiles of *Barley yellow dwarf virus* (BYDV) species. PCR products were digested by *Hpa* II endonuclease and separated on 2% TAE agarose gel. Lanes: M – 100-bp DNA ladder (Novazym); K+ BYDV-MAV – positive control of BYDV-MAV; K+ BYDV-PAV – positive control of BYDV-PAV; K+ BYDV-PAS – positive control of BYDV-PAS; 1 – tested BYDV-MAV sample; 2 – tested BYDV-PAS sample; 3 – tested BYDV-PAV sample

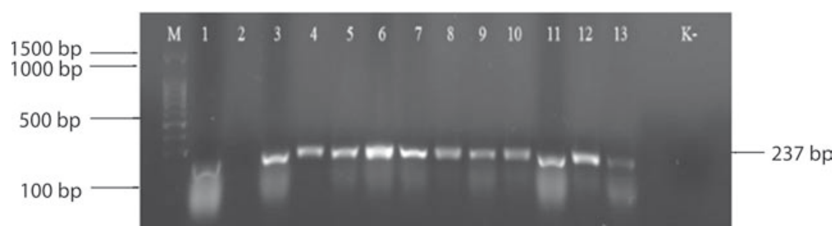

Fig. 4. Detection of *Barley yellow dwarf virus* (BYDV)-SGV (237 bp) in plant samples by RT-PCR. Amplified products were analyzed in 1% TAE agarose gel. Lanes: M – 100-bp DNA ladder (Novazym); 1–13 – tested samples; K- – no-template control

samples with the BYDV-PAV pattern, (4) two samples with mixed infections BYDV-MAV/BYDV-PAS, and (5) one sample with mixed infections BYDV-MAV/BYDV-PAV. Partial results are presented in Figure 3. Additionally, RT-PCR using specific primer pairs revealed single infection with BYDV-GAV in Greater Poland (data not shown) and the common presence (12 samples) of BYDV-SGV (Fig. 4).

Discussion

Information about the presence and diversity of virus species in local fields is very important. It can be useful for forecasting outbreaks of viral diseases in the future. In Poland the last epidemical incidence of BYDV species was recorded in 2015 (Jeżewska and Trzmiel 2016). Published results indicated the dominance of BYDV--MAV and BYDV-PAV, which as before (Jeżewska *et al.* 2010), were the most frequently identified in field samples. Moreover, the presence of BYDV-PAS, -SGV and -GAV have been confirmed for the first time in the country (Trzmiel 2017). As in the past (Paliway 1982), the prevalence of a virus species can vary from year to year. The results presented herein revealed the common presence of BYDV-SGV in tested samples. Our data, in comparison to a previous report (Trzmiel 2017) may suggest a slight spreading of the virus to other locations. Moreover, RT-PCR-RFLP results indicated numerous examples of BYDV-PAS among tested samples. This data could be interesting in view of the results presented by Jarošová *et al.* (2013) from the Czech Republic who reported that BYDV-PAS was the most common among BYDV species.

WDV is one of the most common viruses on cereal crops in Poland. The results of previous studies conducted in 2012–2016 revealed an average rate of infection in relation to tested plants at the level of 37% (Trzmiel 2018). Moreover, in 2015 when an outbreak of BYD on winter wheat and barley was observed, mixed infection of BYDV-MAV or BYDV-PAV and WDV was common but the rate of WDV infection was definitely lower (Trzmiel 2018). The results obtained in this study indicated the opposite situation. Mixed infection of BYDVs and WDV were not confirmed. Moreover, WDV was revealed as the main causal agent of viral infections among tested cereal samples and BYDVs were less severe. The average infection rate was 65.57% and 10.26%, respectively. These data are comparable with the results of Kroutil and Markytánová (2005) and Raus (2008), who reported that WDV epidemics prevailed in the Czech Republic. Furthermore, molecular diagnostics showed an unchanging dominance of WDV-W over WDV-B in Poland (Trzmiel 2018). Our results demonstrated barley and wheat infections by both WDV-W and WDV-B, which is comparable with the results of Schubert *et al.* (2014).

In conclusion, the presented results showed a new tendency, namely, the common presence of BYDV-PAS and BYDV-SGV in a group of BYDV species and an increase of WDV infection in cereal crops in Poland. Such information on the risk of potential WDV and BYDVs outbreaks should be taken into account while developing disease management strategies.

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