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Detection and Discrimination of Barley- and Wheat-specific Forms of *Wheat Dwarf Virus* in Poland

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Wheat dwarf virus (WDV) is one of the most common viruses on cereal crops in Poland. Studies were undertaken aiming at molecular characterization of Polish isolates of the virus. The presence of two main groups, WDV-barley- and WDV-wheat-specific forms, in field samples has been confirmed. Detection and differentiation of WDV isolates was conducted using immuno-capture polymerase chain reactions. The studies were carried out on a set of 68 samples collected from different parts of the country. Obtained results demonstrated that WDV-wheat-specific form can infect all tested cereals: wheat, triticale, rye and barley while WDV-barley-specific form was identified mainly in barley and in rare cases in wheat plants. Comparative analysis of coat protein gene was performed using 16 WDV isolates originated from different hosts revealed high (>98%) nucleotide sequence identity. Moreover, WDV-wheat-specific (Pol-WDV-W) and WDV-barley-specific (WDV-B) isolates were fully sequenced. Based on nucleotide sequence similarity, Pol-WDV-W should be classified as WDV-E and WDV-B as WDV-F strains. This is the first report of the complete sequence of WDV isolates from Poland.

Keywords: WDV, wheat, barley, IC-PCR

Introduction

Wheat dwarf virus (WDV) was initially reported in wheat plants, in the former Czechoslovakia (Vacke 1961). The next studies led to the isolation and description of quasi-isometric twinned viral particles about 20 × 30 nm (Lindsten et al. 1980). The virus is transmitted by *Psammotettix alienus* (Dahlb.) in persistent manner (Vacke 1962). WDV presence was confirmed in large parts of Europe: Bulgaria (Stephanow and Dimov 1981), Hungary (Bisztray et al. 1989), France (Lindsten and Lindsten 1993), Romania (Jilaveanu and Vacke 1995), Germany (Huth and Lessemann 1994), Finland (Lemmetty and Huusela-Veistola 2005), Spain (Achon et al. 2006), Ukraine (Snihur et al. 2007), Austria, United Kingdom (Shubert et al. 2014) and also in Asia: Turkey (Köklü et al. 2007), and China (Xie et al. 2007) as well as in Near East: Iran (Behjatnia et al. 2011), Syria (Ekzayez et al. 2011), and in North Africa: Tunisia (Najar et al. 2000), and Zambia (Kapooria and Ndunguru 2004).

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WDV infects plants of the family Poaceae, especially cereals: wheat (*Triticum aestivum* L.), barley (*Hordeum vulgaris* L.), rye (*Secale cereale* L.) and triticale (×*Triticosecale* Wittm. ex A.Camus). The main symptoms caused by WDV are leaf mosaic and dwarfing. Yield losses may reach as much as 80% (Lindbland et al. 1999). WDV has a ssDNA genome of 2.7 kb which encodes four proteins: the movement protein (MP), the coat protein (CP) on the viral sense strand and two proteins associated with replication (Rep and RepA) on the complementary strand. Two non-coding regions: longer (LIR) and shorter (SIR) contain sequence elements necessary for viral replication and transcription (Vacke et al. 2004).

Lindsten and Vacke (1991) divided WDV isolates into: wheat- and barley-adapted forms (WDV-W and WDV-B strains), respectively. The next survey revealed that WDV-B is serologically similar to WDV-W but the strains differ in nucleotide sequence (84% identity) and in the size of the genome: 2749–2750 nt for WDV-W and 2734 nt for the WDV-B (Köklü et al. 2007). Wheat strains of the virus have a high level of identity (>98%), while the barley strains are more variable (>94% identity) (Tóbiás et al. 2011). The phylogenetic analysis of both WDV forms showed theirs separation into strains A to E (Muhire et al. 2013) and recently into A to F (Wu et al. 2015). However, the sequence similarity and phylogenetic relationship results confirmed significant separation of WDV-wheat- and WDV-barley- specific groups. A and F strains were mainly originated from barley and B-E were mainly from wheat (Wu et al. 2015).

In Poland WDV was found for the first time in 1999 (Jeżewska 2001). Next studies, based on ELISA results, confirmed WDV infection of wheat, triticale and barley plants in different parts of Poland (Jeżewska et al. 2010). The literature data do not contain any information on differentiation of WDV-wheat- and WDV-barley- adapted forms in Poland. Furthermore, there is no sequence data for Polish isolates. Therefore, the aims of this study were developing a molecular protocol using immuno-capture polymerase chain reaction (IC-PCR) to detect and discriminate both forms of WDV and subsequently evaluation of their distribution in the country. The differentiation surveys were supported by sequencing method. Thus, the paper contains first information on the complete genome sequence of two WDV isolates from Poland.

Material and Methods

Plant material and virus detection

The studies were conducted in 2012–2016 seasons. A total of 408 samples, containing 270 barley, 112 wheat, 21 triticale and 5 rye plants with yellowing and stunting symptoms were collected from northern (Pomerania and Warmia-Masuria provinces), central (Lubusz, Greater Poland, Lodzkie, Lublin provinces) and southern (Lower Silesia, Opole, Silesia, Lesser Poland provinces) parts of Poland. Preliminary screening tests were performed by ELISA (Clark and Adams 1977) with commercial kit (Loewe, Sauerlach, Germany). A sample was considered positive when its optical density (OD) value was at least

three-times higher than the average OD of negative control (healthy plants). Positive samples were selected for further discrimination studies by molecular techniques.

Immuno-capture-polymerase chain reaction (IC-PCR)

First, thin-wall polypropylene PCR tubes were coated with a 20 µl mixture containing 10-fold diluted polyclonal anti-WDV antibody with commercial coating buffer (Loewe) and incubated for 1 h at 37 °C. After that time, tubes were washed three times in commercial washing buffer (Loewe). Next, the plant tissue was homogenized using a mortar and pestle in commercial conjugate buffer (Loewe). The coated reaction tubes were incubated with 50 µl plant sap for 1 h at 37 °C to allow WDV particles to attach to the tube walls. Following washing in washing buffer, the PCR tubes were used for PCR analysis. Specific genome regions were amplified using Allegro*Taq* polymerase (Novazym, Poznań, Poland) with originally designed primer pairs: WDV-H-F (CAAGGGGCGAGATCACACA) / WDV-H-R (CCACAACTACTACAACAGCC) and WDV-T-F (CGAGTAGTTGATGAATGACTCG) / WDV-T-R (GGCTGTTTCAACTCCAGGTCG) targeting fragments of short intergenic region (SIR) and replicase gene (RepB) – in genome region of differentiating barley- and wheat-specific groups of WDV (Kundu et al. 2009). The primers were designed using the Primer3 software (http://frodo.wi.mit.edu/) (Rosen and Skaletski 2000) based on alignment of full nucleotide sequence of two WDV groups. The PCR reactions were performed in a final volume of 10 µl containing: 0.5 µl of forward and 0.5 µl reverse primers (10 µM); 1 µl of buffer Allegro*Taq* pH 8.6; 0.4 µl of dNTP Mix (10 mM), 0.1 µl of Allegro*Taq* polymerase DNA. The reactions were performed in thermal profile 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 cycle of 7 min at 72 °C. PCR products were separated by 1% agarose gel electrophoresis and stained with Midori Green DNA Stain (NIPPON genetics Europe GmbH, Düren, Germany) for UV light visualization.

DNA extraction, PCR and sequencing

Total DNA was isolated using NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. PCR reactions were performed with a set of published (Kvarnheden et al. 2002) and originally designed primers covering complete genome sequence of WDV (Table 1). The primers were designed using the Primer3 software based on full nucleotide sequence of CZWDV-W (FJ546188) and WDV-HE (FM999833). The reactions were conducted in thermal conditions as described above. The obtained PCR products of expected size were excised from agarose gel and purified using Wizard®SV Gel and PCR Clean-Up System (Promega Corp., Madison, WI, USA), according to the manufacturer's instructions. At least three samples of each amplicon were subsequently sequenced by Genomed S.A. (Warsaw, Poland) with specific primers. The nucleotide sequences were analyzed using BlastN online tool, and then compiled and edited in the BioEdit software (Hall 1999).

Table 1. The primers used for amplification of WDV genome sequence

Primer	Primer sequence	5 Position	NCBI accession	Amplicon size (bp)
WDV1-F*	CTTACGGAGTAGAGATGTTC	1876	FJ546188	1567
WDV2-R*	AACAGAGTGTAAGCAAGCCA	309		
WDVCP-F	GAGGACCGAGGAAATTGGTT	371	FJ546188	988
WDVCP-R	CGGACGGCGTACAGTTTCTA	1338		
WDVJ1-F	GATCAATACCAGGCCCCTTAC	446	FM999833	635
WDVJ1-R	GGCAGCAGATTCCAAGGCATC	1061		
WDVJ2-F	CTATGACATACAACACACTC	1505	FM999833	686
WDVJ2-R	GTTAGCACTTACACACCTGAG	2171		
WDVP1-F	CCTTCGGTTTGCTAATAGCC	277	FJ546188	509
WDVP1-R	CTCTGCATCATAGACTAACC	767		
WDVP2-F	CGAAATACAACACCCTGAG	1307	FJ546188	751
WDVP2-R	CCTCAGCATGGTTTGCAATCG	2038		
WDVP3-F	GTACATTGAGGATATGTTAG	2440	FJ546188	404
WDVP3-R	CCATCTTCCACGAAAGTTC	77		

*Primers from the literature.

Results

The studies confirmed common distribution of WDV in Poland. The virus was detected in all analyzed locations: 2 in northern, 7 in central and 8 in southern parts of the country. Detailed ELISA results were presented in Table 2. The incidence of WDV in triticale, wheat and barley plants ranged 90.5%, 50% and 34.8%, respectively. Moreover, for the first time in Poland, the virus was detected in 4 out of 7 tested rye plants. In 2015, when barley yellow dwarf occurred at high incidence in almost all parts of Poland (Jeżewska

Table 2. Detection of WDV infection by ELISA test
(number of infected plants/number of tested plants are given)

Date	Wheat	Rye	Triticale	Barley	Average (percent of positive samples)
2012	7/14	–*	3/5	3/8	13/27 (48.1)
2013	10/49	1/2	–	10/17	21/68 (30.9)
2014	18/20	–	–	–	18/20 (90.0)
2015	12/20	2/3	–	33/163	47/186 (25.3)
2016	9/9	–	16/16	27/82	52/107 (48.6)
Total	56/112	3/5	19/21	73/270	151/408 (37.0)

*Not tested samples.

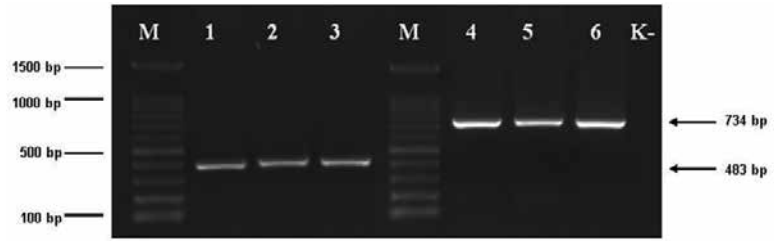

Figure 1. Electrophoretic mobility of IC-PCR products with WDV-T-F /WDV-T-R (734 bp) and WDV-H-F/ WDV-H-R (483 bp) on 1% agarose gel. M – marker DNA Nova 100 bp DNA ladder (Novazym), 1–6 studied samples, K – negative control

Table 3. Detection and discrimination of barley- and wheat-specific strains of WDV by IC-PCR (number of infected plants/number of tested samples are given)

Date	WDV-barley-specific strain				WDV-wheat-specific strain			
	Wheat	Triticale	Rye	Barley	Wheat	Triticale	Rye	Barley
2012	1/1	0/2	–*	2/2	0/1	2/2	–	0/2
2013	0/4	–	0/2	2/3	4/4	–	1/2	1/3
2014	0/4	–	–	–	4/4	–	–	–
2015	0/5	–	0/3	2/15	4/5	–	2/3	8/15
2016	0/3	0/4	–	17/20	3/3	3/4	–	0/20
Total	24/68				32/68			

*Not tested samples.

and Trzmiel 2016), mixed infections of WDV and *Barley yellow dwarf virus-MAV* (BYDV-MAV) or *Barley yellow dwarf virus-PAV* (BYDV-PAV) occurred commonly.

Molecular detection and discrimination of two main WDV forms using IC-PCR were performed for 68 samples. The IC-PCR products of expected size 483 bp and 734 bp, for WDV-barley-specific and WDV-wheat-specific strains, respectively, were visualized on 1% agarose gel. Partial results were presented in Fig. 1. Detailed IC-PCR results are listed in Table 3.

The results indicated the presence of both forms of WDV in Poland with a slight predominance of WDV-wheat-specific strain. WDV-barley-specific-strain was frequently detected in barley and in only one case in wheat plant while infections with WDV-wheat-specific-strain were often confirmed in wheat, triticale, rye and barley plants. The PCR using WDVCP-F/WDVCP-R primers generated specific (988 bp in size) amplicons for all randomly selected probes from 12 locations (data not shown). Obtained DNA was purified and 16 samples, were sent for sequencing. Received data were consistent with the results of WDV division by IC-PCR.

The sequence analysis of CP gene were carried out for 16 isolates, originating from: barley, wheat, rye and triticale (Table 4). Additionally, one isolate of WDV-wheat-specific

Table 4. Description of WDV isolates sequenced in this study

Isolate name	Geographical origin	Host	Collection date	Accession No.
WDV-Ant	Antoniny	wheat	04/2014	KY781933
WDV-Bol	Bolesławiec	barley	04/2015	KY781933
WDV-Dl	Dłużec	barley	05/2016	KY781934
WDV-Gl	Głubczyce	wheat	04/2015	KY781935
WDV-Kndr1	Kondratowice	wheat	05/2013	KY781936
WDV-Kndr2	Kondratowice	triticale	05/2013	KY781937
WDV-Kob	Kobierzyce	wheat	06/2016	KY781938
WDV-Lip	Lipsko	barley	05/2015	KY781939
WDV-Luk	Łuków	barley	04/2016	KY781940
WDV-Sos	Sośnicowice	barley	04/2016	KY781941
WDV-Sz1	Szelejewo	barley	05/2012	KY781942
WDV-Sz2	Szelejewo	wheat	05/2012	KY781943
WDV-Sz3	Szelejewo	wheat	04/2013	KY781944
WDV-Sz4	Szelejewo	triticale	05/2012	KY781945
WDV-Wp	Wieprz	barley	05/2015	KY781946
WDV-Wtr	Wiatrowo	rye	05/2015	KY781947
WDV-B	Szelejewo	barley	04/2012	KM079155
Pol-WDV-W	Łagiewniki	barley	04/2012	KM079154

form (Pol-WDV-W) and one isolate of WDV-barley-specific form (WDV-B) were fully sequenced and deposited in the NCBI GenBank database with following accession numbers (KM079154 and KM079155).

Comparative analysis of CP gene of WDV-wheat- and WDV-barley-specific indigenous isolates obtained from different hosts revealed their high (>98%) nucleotide sequence identity (data not shown). Our results are consistent with previous findings (Ramsell et al. 2008). Little differences observed within WDV-barley- and WDV-wheat-specific groups were caused by point mutations. Most of them represent synonymous point mutations, ranging from 2 to 9 in WDV-barley-specific isolates and from 1 to 11 in WDV-wheat-specific isolates while non-synonymous mutations were less frequently demonstrated (up to: 9 in WDV-barley- and 2 in WDV-wheat-specific groups) (Table 5).

Obtained nucleotide sequences of CP gene were also compared with corresponding fragments of other known WDV isolates from the GenBank database. The analysis confirmed their high similarity, from 100% (with German isolates e.g. KJ473703-05, AM296022-23 and Czech isolate e.g. FJ546186) to 97% (with Chinese isolates e.g. KJ536104-13, JQ647500-06) and from 99% (with German isolates e.g. HG422312-13, AM942044-45, Czech isolate e.g. FJ546193, Hungarian isolate e.g. AM747816) to 95% (with Turkish isolate e.g. AJ783960) for WDV-wheat-specific and WDV-barley-specific

Table 5. A summary of non-synonymous (NS) and synonymous (S) mutations in nucleotide sequence of CP gene within WDV-barley-specific and WDV-wheat-specific groups of Polish isolates

Point mutations					
WDV-barley-specific isolates	NS	S	WDV-wheat-specific isolates	NS	S
	WDV-B (KM079155)			Pol-WDV-W (KM079154)	
WDV-Dl	0	2	WDV-Ant	0	4
WDV-Luk	0	6	WDV-Bol	0	3
WDV-Sos	0	9	WDV-Gl	0	6
WDV-Sz1	0	6	WDV-Knd1	1	3
WDV-Sz2	0	4	WDV-Knd2	0	3
WDV-Wp	9	9	WDV-Kob	0	1
–	–	–	WDV-Lip	2	11
–	–	–	WDV-Sz3	0	2
–	–	–	WDV-Sz4	1	2
–	–	–	WDV-Wtr	0	1

forms, respectively. The comparison of different geographically WDV isolates confirmed that this genome part is very stable (Kundu et al. 2009; Schubert et al. 2014).

The nucleotide sequences comparison of whole genome of Polish to other WDV isolates from the GenBank database showed that WDV-B is the most similar (>99% identity) to the group of German (e.g. SXA24-AM296024, SXA57-AM942044, Aschersleben3-HG422314); Hungarian (e.g. HE-FM999833, DOI-FM999832, H07-FM210034) and Czech CZWDV-B (FJ546193) isolates while Pol-WDV-W share highest identity (>99%) with groups of German (e.g. SxA23- AM296023, Winter rye 102- KJ473699, Spelt8-KJ473695, Leutewitz, clone24- HG422310); Czech (e.g. CZWDV-W- FJ546188, CZ1841- FJ546191) as well as with Hungarian WDV-B- AM040732 isolates.

Discussion

The analysis of 68 viral isolates originated from different cereal species allowed to present the first molecular characterization of WDV from Poland. The paper presents IC-PCR technique which allows the simultaneous detection and discrimination of two major WDV forms based on different length PCR products. The advantage of IC-PCR is that it is faster than ELISA with monoclonal antibodies (Rabenstein et al. 2005) and less laborious than standard PCR (Commandeur and Huth 1999) and PCR-RFLP (Kundu et al. 2009) or RCA-RFLP (Schubert et al. 2007), because using an immuno-captured virus in the optimized protocol eliminates the time and the costs associated with DNA extraction and the use of restriction enzymes. Therefore, proposed assay is a useful tool for studying the prevalence and distribution of WDV.

Our studies are consistent with those of Áy et al. (2008) who reported that WDV is one of the most dangerous and most frequently identified cereal viruses in Hungary. Described results indicated the presence of both forms of WDV in Poland. Available literature data contain contradictory reports on whether the WDV-wheat-specific strain can infect barley, and whether the WDV-barley-specific strain can infect wheat in nature. Mehner et al. (2003) detected WDV-barley-form in barley, oat, maize and various grass species while WDV-wheat-form was confirmed in wheat whereas triticale was found infected with both forms of the virus. The results presented by Tóbiás et al. (2009) and by Kundu et al. (2009) revealed that WDV-barley-strain was limited to barley, however WDV-wheat-strain can infect both wheat and barley plants. Our results demonstrate that WDV-wheat-specific form can infect all tested cereals: wheat, triticale, rye and barley while WDV-barley-specific form was identified mainly in barley and in rare cases in wheat plants. The findings consist of the results of German researchers (Schubert et al. 2014).

Classification of WDV-barley- and WDV-wheat-specific forms was recently changed. According to the WDV division, proposed by Muhire et al. (2013), Hungarian isolate (WDV-B, AM040732) was considered as a reference isolate for WDV-E strain. Based results presented in this study results Polish isolate (Pol-WDV-W, KM079154) should be classified as WDV-E strain. Last studies on sequence similarity and phylogenetic relationship led to the division of WDV-barley-specific isolates into two strains: A and F (Wu et al. 2015). A new strain has included mainly German as well as Hungarian and Czech isolates among which are those most similar to Polish isolate (WDV-B, KM079155). According to this information, Polish barley-specific isolate should be categorized as WDV-F strain.

The data on the global population of WDV demonstrated that MP and LIR were the crucial regions for WDV division (Wu et al. 2015). In order to explore WDV diversity in Poland the studies aiming to obtain complete genome of next WDV isolates should be undertaken.

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References

Achon, M.A., Serrano, L., Ratti, C., Rubies-Autonell, C. 2006. First detection of *Wheat dwarf virus* in barley in Spain associated with an outbreak of Barley Yellow Dwarf. Plant Dis. **90**:970.

Áy, Z., Kerényi, Z., Takács, A., Papp, M., Petróczi, I.M., Gáborjányi, R., Silhavy, D., Pauk, J., Kertész, Z. 2008. Detection of cereal viruses in wheat (Triticum aestivum L.) by serological and molecular methods. Cereal Res. Commun. **36**:215–224.

Behjatnia, S., Afsharifar, A., Tahan, V., Motlagh, M.A., Gandomani, O.E., Niazi, A., Izadpanah, K. 2011. Widespread occurrence and molecular characterization of Wheat dwarf virus in Iran. Aust. Plant Pathol. **40**:12–19.

Bisztray, G., Gáborjányi, R., Vacke, J. 1989. Isolation and characterization of wheat dwarf virus found for the first time in Hungary. J. of Plant Dis. Prot. **96**:449–454.

Clark, M.F., Adams, A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. of General Virol. **34**:475–483.

Commandeur, U., Huth, W. 1999. Differentiation of strain of wheat dwarf virus in infected wheat and barley plants by means of polymerase chain reaction. J. of Plant Dis. Prot. **106**:550–552.

Ekzayez, A.M., Kumari, S.G., Ismail, I. 2011. First report of Wheat dwarf virus and its vector (*Psammotettix provincialis*) affecting wheat and barley crops in Syria. Plant Dis. **95**:76.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows95/98/NT. Nucleic Acids Symposium Series **41**:95–98.

Huth, V.W., Lessemann, D.E. 1994. Evidence of Wheat dwarf virus in Germany. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes **46**:105–106.

Jeżewska, M. 2001. First report of *Wheat dwarf virus* occurring in Poland. Phytopathologica Polonica **21**:93–100.

Jeżewska, M., Cajza, M., Buchowska-Ruszkowska, M. 2010. Monitoring i diagnostyka molekularna wirusów zbóż. In: Sosnowska, D. (ed), Ograniczanie strat w plonach roślin uprawnych z zachowaniem bezpieczeństwa żywności. Instytut Ochrony Roślin–PIB, Poznań, pp. 157–180. [Monitoring and molecular diagnostics of cereal viruses. In: Reducing losses in crop yields with food safety].

Jeżewska, M., Trzmiel, K. 2016. Masowe wystąpienia żółtej mozaiki jęczmienia na zbożach ozimych w Polsce w sezonie 2014/2015. Progress in Plant Protection 56(3): 296–301. [Outbreak of barley yellow dwarf in winter cereals in Poland in the season 2014/2015.]

Jilaveanu, A., Vacke, J. 1995. Isolation and identification of wheat dwarf virus (WDV) in Romania. Probleme de Protectia Plantelor **23**:51–62.

Kapooria, R.G., Ndunguru, J. 2004. Occurrence of viruses in irrigated wheat in Zambia. EPPO Bulletin **34**:413–419.

Köklü, G., Ramsell, J.N.E., Kvarnheden, A. 2007. The complete genome sequence for a Turkish isolate of *Wheat dwarf virus* (WDV) from barley confirms the presence of two distinct WDV strains. Virus Genes **34**:359–366.

Kundu, J.K., Gadiou, S., Červená, G. 2009. Discrimination and genetic diversity of *Wheat dwarf virus* in the Czech Republic. Virus Genes **38**:468–474.

Kvarnheden, A., Lindbland, M., Lindsten, K., Valkonen, J.P.T. 2002. Genetic diversity of *Wheat dwarf virus*. Archives of Virology **147**:206–216.

Lemmetty, A., Huusela-Veistola, E. 2005. First report of WDV in winter wheat in Finland. Plant Dis. **89**:912.

Lindbland, M., Sandgren, M., Sigvald, R. 1999. Epidemiology and control of wheat dwarf. In: Proc. VIIth Int. Plant Virus Epidemiology Symp. Aguadulce (Almeria), Spain. p. 114.

Lindsten, K., Lindsten, B., Abdelmoeti, M., Juntti, N. 1980. Purification and some properties of wheat dwarf virus. In: Proc. 3rd Conf. on Virus Diseases of Gramineae in Europe. Rothamsted Experimental Station, Harpenden, Herts. pp. 27–31.

Lindsten, K., Lindsten, B. 1993. Occurrence and transmission of Wheat dwarf virus (WDV) in France. In: A.N.P.P. Third Int. Conf. on Pests in Agriculture. Montpellier, France. pp. 41–48.

Lindsten, K., Vacke, J. 1991. A possible barley adapted strain of *Wheat dwarf virus* (WDV). Acta Phytopathologica et Entomologica Hungarica **26**:175–180.

Mehner, S., Manurung, B., Grüntzig, M., Habekuß, A., Witsack, W., Fuchs, E., 2003. Investigations into the ecology of the *Wheat dwarf virus* (WDV) in Saxony-Anhalt, Germany. J. of Plant Dis. Prot. **110**:313–323.

Muhire, B., Martin, D.P., Brown, J.K., Navas-Castillo, J., Moriones, E., Zerbini, F.M., Rivera-Bustamante, R., Malathi, V.G., Briddon, R.W., Varsani, A. 2013. A genome-wide pairwise-identity-based proposal for the classification of the viruses in the genus *Mastrevirus* (family *Geminiviridae*). Archives of Virology **158**:1411–1424.

Najar, A., Makkouk, K.M., Kumari, S.G. 2000. First record of *Barley yellow striate mosaic virus*, *Barley stripe mosaic virus*, and *Wheat dwarf virus* infecting cereal crops in Tunisia. Plant Dis. **84**:1045.

Rabenstein, F., Sukhacheva, E., Habekuß, A., Schubert, J. 2005. Differentiation of *Wheat dwarf virus* isolates from wheat, triticale and barley by means of a monoclonal antibody. In: Proc. Xth Conf. on Viral Diseases of Gramineae in Europe. Louvain-la-Neuve, Belgium. p. 60.

Ramsell, J.N.E., Lemmetty, A., Jonasson, J., Andersson, A., Sigvald, R., Kvarnheden, A. 2008. Sequence analyses of Wheat dwarf virus isolates from different hosts reveal low genetic diversity within the wheat strain. Plant Pathol. **57**:834–841.

Rosen, S., Skaletski, H.J. 2000. Primer3 on the WWW for general use and for biologist programmers. In: Krawetz, S. Misenes, S. (eds), Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press. Totova/New Jersey, USA. pp. 365–386.

Schubert, J., Habekuß, A., Kazmaier, K., Jeske, H. 2007. Surveying cereal-infecting geminiviruses in Germany – Diagnostic and direct sequencing using rolling circle amplification. Virus Res. **127**:61–70.

Schubert, J., Habekuß, A., Wu, B., Thieme, T., Wang, X. 2014. Analysis of complete genomes of isolates of the Wheat dwarf virus from new geographical locations and descriptions of their defective forms. Virus Genes **48**:133–139.

Snihur, H., Polischuk, V., Kastirr, U. 2007. Dissemination of viruses of cereal crops in agrocoenosises of Ukraine. In: 10th International Plant Virus Epidemiology Symp. ICRISAT. Hyderabad, India. p. 107.

Stephanov, J., Dimov, A. 1981. Bolestta vdjudjavanje po spenittsata Bolgaria. Rasteniev Nauki **18**:1274–128. [The Wheat dwarf disease in Bulgaria.] Tóbiás, I., Kiss, B., Bakardjieva, N., Palkovics, L. 2009. The nucleotide sequence of barley strain of of *Wheat Dwarf Virus* isolated in Bulgaria. Cereal Res. Commun. **37**:237–242.

Tóbiás, I., Shevchenko, O., Kiss, B., Bysov, A., Snihur, H., Polischuk, V., Salanki, K., Palkovics, L. 2011. Comparison of the nucleotide sequences of *Wheat Dwarf Virus* (WDV) isolates from Hungary and Ukraine. Polish J. of Microbiol. **60**:125–131.

Vacke, J. 1961. Wheat dwarf virus disease. Biologia Plantarum (Praha) **3**:228–233.

Vacke, J. 1962. Some new findings on what dwarf virus. In: Plant Virology, Proc. 5th Conference of the Czechoslovak Plant Virologists. Publishing House of the Czechoslovak Academy of Sciences. Prague, Czechoslovakia. pp. 331–334.

Vacke, J., Kvarnheden, A., Lindbland, M., Lindsten, K. 2004. Wheat Dwarf. In: Lapierre, H., Signoret, P.A. (eds), Viruses and Virus Diseases of *Poaceae* (*Gramineae*). INRA. Paris, France. pp. 590–593.

Wu, B., Shang, X., Shubert, J., Habekuß, A., Elena, S.F., Wang, X. 2015. Global-scale computational analysis of genomic sequences reveals the recombination pattern and coevolution dynamics of cereal-infecting geminiviruses. Scientific Reports **5**:e 8153.

Xie, J., Wang, X., Liu, Y., Peng, Y., Zhou, G. 2007. First report of the occurrence of *Wheat dwarf virus* in wheat in China. Plant Dis. **91**:111.