Cereal Research Communications 37(2), pp. 237–242 (2009)
DOI: 10.1556/CRC.37.2009.2.10

The Nucleotide Sequence of Barley Strain of *Wheat Dwarf Virus* Isolated in Bulgaria

I. TÓBIÁS[1]*, B. KISS[1], N. BAKARDJIEVA[2] and L. PALKOVICS[3]

[1]Plant Protection Institute, Hungarian Academy of Sciences, P.O. Box 102, H-1525 Budapest, Hungary
[2]Plant Protection Institute, Kostinbrod, Bulgaria
[3]Department of Plant Pathology, Corvinus University of Budapest,
Ménesi út 44, H-1118 Budapest, Hungary

(Received 30 April 2008; accepted 25 September 2008)

Barley-infecting isolates of WDV were collected in the field of near Sofia. The complete genomes of two isolates were amplified by PCR, cloned into pGEM-T plasmid and sequenced. The two clones were the same size and showed complete homology. The WDV-Bg17 clone was compared with *Barley dwarf virus, Oat dwarf virus* and *Wheat dwarf virus* isolates. Based on DNA sequences WDV-Bg17 isolate shows high homology (95 –97%) to *Barley dwarf virus* isolates and differs from *Oat dwarf virus* (71% homology) and *Wheat dwarf virus* (85% homology).

Keywords: *Wheat dwarf virus, Barley dwarf virus, Oat dwarf virus,* barley-infecting isolates, sequence analysis

Introduction

Wheat dwarf virus (WDV) was first described by Vacke (1961) in the former Czechoslovakia and later it has been found in Sweden (Lindsten et al. 1970), Bulgaria (Stephanov and Dimov 1981; Bakardjieva 1998), Hungary (Bisztray et al. 1989), France (Lindsten and Lindsten 1993), Germany (Huth 2000), Turkey (Köklü et al. 2007) and China (Xie et al. 2007). *Wheat dwarf virus* causes a severe disease of cereals, mainly winter barley and wheat. Affected plants show a yellowing of leaves, stunting, reduced root growth, delayed heading, and a reduction in yield. WDV is transmitted by the leafhopper *Psammotettix alienus* Dahlb. (Vacke 1961), therefore the occurrence of diseased plants in the field depends on its vector. The incidence of WDV in crops differs greatly from year to year and between fields. Several important factors of the epidemiology of WDV are still poorly understood, such as alternative hosts in the field and virus variability. Two different forms of WDV exist: a wheat-adapted form (WDV wheat strain) and a barley-adapted form (WDV barley strain) (Lindsten and Vacke 1991; Bendahmane et al. 1995). More recently Schubert et al. (2007) surveyed cereal samples and based DNA sequence differences they proposed the new mastrevirus species *Barley dwarf virus* and *Oat dwarf virus*.

* Corresponding author; E-mail: tobias@julia-nki.hu

0133-3720/$20.00 © 2009 Akadémiai Kiadó, Budapest

Wheat dwarf virus belongs to genus *Mastrevirus* (family *Geminiviridae*) infects monocotyledonous plants. *Mastreviruses* have a monopartite single-stranded genome of circular DNA and the genome encodes four different proteins: movement protein (MP), coat protein (CP) on the viral-sense strand and two replication-associated proteins (Rep and RepA) on the complementary-sense strand (Gutierrez 1999). The presence of an intron in the rep gene makes it possible for WDV to produce the two different forms of the replication protein. The non-coding long intergenic region (LIR) and short intergenic region (SIR) contain sequence elements necessary for viral replication and transcription. The complete genome is known for many wheat strains of WDV but relatively few barley strain of WDV has been determined till 2007.

The aim of this study was to characterise the barley strains of WDV occurring in Bulgaria and compare with the previously sequenced barley and wheat isolates.

Materials and Methods

Virus isolates

The symptoms of viral infection were found during autumn observations carried out in winter barley crops near Sofia. Plants manifesting yellowing of leaves and reduced growth like those described by Stephanov and Dimov (1981) were sampled from the experimental plots of Variety Testing Station, Quarter Negovan. The collected barley plants were replanted into clay pots and placed in an insect-proof isolation net with a healthy barley seedlings and virus-free *Psammotettix alienus* Dahlb. (30 individuals/net) vectors. Six weeks the plants were tested for WDV with ELISA using WDV kit (Bio-Rad No. 31202). Two isolates – WDV-Bg8 and WDV-Bg17 – were selected for further study.

Cloning and sequence analysis of the WDV isolates

Leaves were collected from the selected WDV-positive barley plants. Total nucleic acid was extracted from 100 milligrams of barley leaves using the method of White and Kaper (1989). Ethanol precipitated nucleic acid was redisolved in 40 μl of sterile water, 3 μl was used for polymerase chain reaction (PCR) in 50 μl volume. Primers (WDV-reverse 477-458 5’-GACCCGGGATCGTAAGGGGC-3’ and WDV-forward 468-487 5’-ATCC CGGGTCCTCCGACTAC -3’) were designed to amplify the complete genome of WDV. The reaction mixture contained 20 pmol of primers, 200 pmol of each dNTP, PCR reaction buffer and 2.5 U Taq polymerase (Fermentas). Denaturation at 94 ºC for 4 minutes was followed by 40 cycles of 20 seconds at 94 ºC denaturation, 30 seconds at 56 ºC annealing, 3 minutes and 30 seconds at 72 ºC extension, with a final extension step at 72 ºC for 10 minutes. The amplified DNA fragments were separated in 1% agarose gel and extracted by DNA purification kit (Fermentas). The PCR products were cloned into pGEM-T vector (Promega). The recombinant plasmids were transformed into *Escherichia coli* DH5α (Sambrook et al. 1989).

Clones containing inserts with the expected size of 2.7 kb were sequenced with DyeDeoxyTerminator Kit (Applied Biosystems, Foster, CA, USA) using reverse, univer-

sal (–20) and internal primers. Sequence analysis was performed using University of Wisconsin Genetics Computer Groups (GCG) sequence analysis software package version 9.1.

Results

Virus isolates

The collected barley plants were WDV infected confirmed by ELISA tests. The virus was transmitted by *Psammotettix alienus* Dahlb. and maintained on barley *(Hordeum vulgare)* plants. For total nucleic acid extraction samples were taken from WDV-Bg8 and WDV-Bg17 isolates infected barley plants.

Cloning of the WDV and sequence analysis

The complete genomes were amplified by PCR, separated in 1% agarose gel (Fig. 1), purified, and cloned into pGEM-T (Promega) vector.

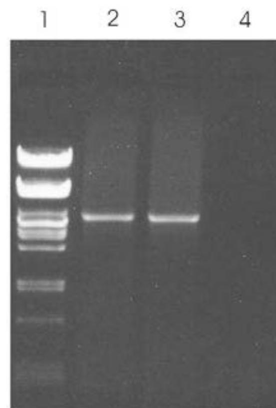

Figure 1. Separation of amplified PCR products of WDV-Bg8 and WDV-Bg17 isolates on a 1% agarose gel stained with ethidium bromide (Lane 1: DNA length marker *Pst* I digested λDNA, Lane 2: WDV-Bg8 isolate, Lane 3: WDV-Bg17 isolate, Lane 4: negative control)

Two clones – WDV-Bg8 and WDV-Bg17 – were sequenced and compared. The sequenced clones were exactly the same size – 2734 nucleotides – and showed complete homology. The nucleotide sequence was deposited into GenBank as WDV-Bg17 (Accession number: AM989927).

The genome contained the four genes (MP, CP, repA, rep) as well as the LIR and SIR like all mastreviruses. The nucleotide sequence of WDV-Bg17 was compared to known barley strain of WDV (Köklü et al. 2007) *Barley dwarf virus* isolates (Schubert et al. 2007). *Oat dwarf virus* (Schubert et al. 2007) and *Wheat dwarf virus* (Tóbiás et al. 2006) (Table 1).

Homology of the complete genome among the barley-infecting WDV isolates were between 94.6% and 97.1%, while to ODV and WDV-F varied around 70% and 84%, respectively.

Table 1. The sequence homology of the complete genome of barley-infecting WDV-Bg17 isolate, *Barley dwarf virus* (BDV), *Oat dwarf virus* (ODV) and *Wheat dwarf virus* (WDV)

	BDV-SxA24	BDV-Cz19	BDV-McP20	BDV-SxA18	BDV-BaW2	BDV-BaW1	WDV-Barl TR	ODV-SxA25	WDV-F
WDV-Bg17	97	96.5	97	97	97.1	97	94.6	71	85.3
BDV-SxA24		98.8	99.2	99.3	99.3	99.1	94.3	69.3	83.7
BDV-Cz19			98.7	98.9	99	98.8	93.7	69.8	83.3
BDV-McP20				99.2	99.2	99	94.2	70.1	83.6
BDV-Sx18					99.6	99.3	94.4	70.1	83.9
BDV-BaW2						99.4	94.4	70.2	83.9
BDV-BaW1							94.2	70	83.7
WDV-Barl TR								70.6	84.7
ODV-SxA25									69.1

Abbreviation and accession numbers: BDV-SxA24: AM296024, BDV-Cz19: AM296019, BDV-McP20: AM296020, BDV-SxA18: AM296018, BDV-BaW2: AM411652, BDV-BaW1: AM411651, WDV-Barl TR: AJ83960, ODV-SxA25: AM296025 and WDV-F: AM040733.

The coding regions – V1 (movement protein, MP), V2 (coat protein, CP) and replication associated proteins (C1/C2) – (replication protein, Rep) – and the non-coding regions, – short intergenic region, SIR and long intergenic region, LIR – of WDV-Bg17 strain was compared with WDV, BDV and ODV strains (Table 2).

Table 2. The sequence homology of the movement protein (V1 gene), coat protein (V2 gene), replication complex (C1/C2 gene), long intergenic region (LIR) and short intergenic region (SIR) of barley-infecting WDV-Bg17 isolate compared to *Barley dwarf virus* (BDV), *Oat dwarf virus* (ODV) and *Wheat dwarf virus* (WDV)

WDV-Bg17	BDV-SxA24	BDV-Cz19	BDV-McP20	BDV-SxA18	BDV-BaW2	BDV-BaW1	WDV-Barl TR	ODV-SxA25	WDV-F
V1 gene	99.6	99.6	99.3	98.9	99.6	99.6	99.3	64.3	85.3
V2 gene	96.9	96.9	97.7	97.7	97.6	97.1	95.4	72.2	82
LIR	92.5	92.8	93.1	93	92	93	93.8	57.7	71.6
SIR	98.3	100	99.4	100	100	99.4	75	67.5	83.5
Replication complex	96.5	95.3	95.9	96.2	96.2	96.3	96.2	73.5	89.5

Abbreviation and accession numbers: BDV-SxA24: AM296024, BDV-Cz19: AM296019, BDV-McP20: AM296020, BDV-SxA18: AM296018, BDV-BaW2: AM411652, BDV-BaW1: AM411651, WDV-Barl TR: AJ83960, ODV-SxA25: AM296025 and WDV-F: AM040733.

The movement protein (V1) shows high homology (above 99%) among the barley strains, but the WDV-Bg17 strain V1 greatly differs from both WDV-F and ODV-SxA25 strains (64% and 85% homology, respectively). The variability of coat protein among the barley strains is higher (between 95% and 97.7%) than in the case of MP, but WDV-Bg17

V2 protein shows less difference (72%) to ODV and almost the same homology (82%) to WDV-F. The replication complex shows almost the same variability as the V2 proteins, 95% and 96.5% among the barley strains 73.5% to ODV and 89.5% to WDV-F. Interestingly the non-coding long intergenetic region (LIR) shows higher sequence divergence (92.5% and 93.8% homology) then the short intergenetic region (SIR) (98.3% and 100% homology) among the barley strains and to comparing to ODV and WDV-F strains, too. The only exception is the WDV-Barley isolate from Turkey which shows around 75% homology to barley strains in SIR (data not shown) and around 93% homology in LIR. The non-coding regions contain several putative regulatory regions, like LIR region for binding of RepA and/or Rep proteins, a TATA-box for directing expression of Rep, and putative regulatory GC-boxes involved in the regulation of the viral-sense promoter (Hughes et al. 1993; Castellano et al. 1999; Missich et al. 2000). The relatively high level of diversity detected in non-coding regions suggests that certain changes can be tolerated.

Acknowledgements

László Palkovics and Balázs Kiss were supported by the Bolyai János fellowship of the Hungarian Academy of Sciences. István Tóbiás visited Bulgaria, supported by Balassi Institute Hungarian Scholarship Board Office. This work was supported by OTKA (grant 61644).

References

Bakardjieva, N. 1998. A viral screening on cereal crops in Bulgaria. Ninth Congress of the Bulgarian Microbiologists, 15–17 October, Sofia. Vol. **2**:531–534. (In Bulgarian)

Bendahmane, M., Schalk, H.J., Gronenborn, B. 1995. Identification and characterization of wheat dwarf virus from France using a rapid method for geminivirus DNA preparation. Phytopathology **85**:1449–1455.

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

Castellano, M.M., Sanz-Burgos, A.P., Gutierrez, C. 1999. Initiation of DNA replication in an eukaryotic rolling-circle replicon: Identification of multiple DNA-protein complexes at the geminivirus origin. J. Mol. Biol. **290**:639–652.

Gutierrez, C. 1999. Geminivirus DNA replication. Cell Mol. Life Sci. **56**:313–329.

Huth, W. 2000. Viruses of Gramineae in Germany – a short overview. J. Plant. Dis. Protect. **107**:406–414.

Hughes, F.L., Rybicki, E.P., Kirby, R. 1993. Complete nucleotide sequence of sugarcane streak Monogeminivirus. Arch. Virol. **132**:171–182.

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

Lindsten, K., Lindsten B. 1993. Occurrence and transmission of Wheat dwarf virus (WDV) in France. In: Proceedings of Third International Conference on Pest in Agriculture. Montpellier, France, 7–9 December 1993, pp. 41–48.

Lindsten, K., Vacke, J. 1991. A possible barley adapted strain of Wheat dwarf virus (WDV). Acta Phytopath. Entomol. Hung. **26**:175–180.

Lindsten, K., Vacke, J., Gerhardson, B. 1970. A preliminary report on three cereal virus diseases new to Sweden spread by *Macrosteles* and *Psammotettix* leafhoppers. Nat. Swedish Inst. Plant Protec. Cent. **14**:285–297.

Missich, R., Ramirez-Parra, E., Gutierrez, C. 2000. Relationship of oligomerization to DNA binding of Wheat dwarf virus RepA and Rep proteins. Virology **273**:178–188.

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular Cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbour Laboratory, Cold Spring Harbour, NY.
Schubert, J., Habekuss, A., Kazmaier, K., Jeske, H. 2007. Surveying cereal-infecting geminiviruses in Germany – Diagnostics and direct sequencing using rolling circle amplification. Virus Research **127**:61–70.
Stephanov, J., Dimov, A. 1981. Bolestta vdjudjavanje po spenittsata Bolgaria. Rasteniev Nauki **18**:124–128.
Tóbiás, I., Kiss, B., Palkovics, L. 2006. The nucleotide sequence of two Hungarian isolates of Wheat dwarf virus. Acta Phytopath. Entomol. Hung. **41**:47–52.
Vacke, J. 1961. Wheat dwarf virus. Biol. Plant. **3**:228–233.
White, J.L., Kaper, J.M. 1989. A simple method for detection of viral satelitte RNA in small tissue samples. J. Virol. Meth. **23**:83–94.
Xie, J., Wang, X., Liu, Y., Peng, Y., Yhou, G. 2007. First report of occurrence of Wheat dwarf virus in wheat in China. Plant Dis. **91**:111.