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Wheat streak mosaic virus detected in winter wheat in Lithuania

Laima URBANAVIČIENĖ, Donatas ŠNEIDERIS, Marija ŽIŽYTĖ

Institute of Botany, Nature Research Centre
Žaliųjų Ežerų 49, Vilnius, Lithuania
E-mail: laima.urbanaviciene@botanika.lt

Abstract

Wheat streak mosaic virus (WSMV) causing viral diseases in graminaceous plants worldwide has been isolated in Lithuania from natural infected winter wheat (*Triticum aestivum* L.) plants. The diseased wheat plants with pronounced symptoms (chlorotic mottling, streaks of leaves and stunting) were found in various localities of Vilnius and Kaunas regions. Virus isolates were investigated by serology, electron microscopy (EM) and reverse transcription-polymerase chain reaction (RT-PCR) methods. The identification of the virus was based on the results of symptomology on host-plants, positive DAS-ELISA (double-antibody sandwich enzyme-linked immunosorbent assay) test, particle morphology (presence of flexuous, filamentous virus particles ~15 nm in diameter and 700 nm in length) and a specific amplification fragment size (750 bp) of virus RNA (ribonucleic acid) in RT-PCR. Isolate (WSMV-1313) of WSMV from wheat plants was characterized by a coat protein gene sequence analysis. Comparison with other WSMV isolates from the GenBank database showed that the Lithuanian WSMV isolate shares the highest sequence similarity to WSMV isolates from Russia (98.3%) and Italy (98.1%). This is the first report of wheat as a natural host for WSMV in Lithuania.

Key words: identification, phylogenetic analysis, *Wheat streak mosaic virus*, winter wheat.

Introduction

Wheat streak mosaic virus (WSMV) is the type species of the recently established genus *Tritimovirus* of the family *Potyviridae* (Stenger et al., 1998; Rabenstein et al., 2004). The virus was first found in wheat in the USA by McKinney in 1937 (McKinney, 1937). WSMV is an important pathogen in wheat that is widely distributed in the United States, Canada, Europe, the Middle East, North Africa, Australia and Asia (Šutic et al., 1999).

The WSMV is characterized by flexuous rod-shaped particles 700 nm in length and 15 nm in width. The genome of WSMV contains 9,384 nucleotide (nt) single stranded sense RNA that encodes a polyprotein of 3,035 amino acid residues (Stenger et al., 1998). The virus is serologically related to *Brome streak mosaic tritimovirus* and *Oat necrotic mottle tritimovirus*. WSMV was previously assigned with other mite-transmitted viruses to the genus *Rymovirus* but sequence comparisons reveal that the genera *Tritimovirus* and *Rymovirus* are quite divergent and reassigned to the genus *Tritimovirus* (Fauquet, Mayo, 1999; Sivamani et al., 2002; French, Stenger, 2004). WSMV has been reported to infect 116 species of 34 genera of the family *Poaceae* (Edwardson, Christie, 1991). It infects all varieties of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), several grasses, and certain cultivars of maize (*Zea mays* L.) and millet (*Panicum* L., *Setaria* P. Beauv. and *Echinochloa* P. Beauv. spp.) (Brakke, 1971; Brunt et al., 1996; French, Stenger, 2002; Ellis et al., 2004). WSMV can cause significant damage to spring and winter wheat. Outbreaks of WSMV have great potential for destruction in wheat crops, causing yield losses approaching 100% (Christian, Willis, 1993; French, Stenger, 2002; Hadi et al., 2011). The virus is transmitted from infected to healthy wheat plants by the wheat leaf curl mite *Aceria tosichella* Keifer (Slykhuis, 1955; French, Stenger, 2002; Navia et al., 2013), and low rates of seed transmission have been reported (Jones et al., 2005; Lanoiselet et al., 2008). Symptoms develop at temperatures above 10°C, and include mottling and chlorotic streaking in leaves, stunted growth, reduced tillering, and sterile or partially filled heads (Brakke, 1971). Symptoms severity also depends on wheat cultivar, strain of the virus, time of infection, temperature and environmental conditions (Divis et al., 2006).

This study was aimed to isolate, identify and characterize the causal agent of wheat virus disease, expressing leaf mottling, leaf streaking and stunting symptoms, in Lithuania.

Materials and methods

Diseased winter wheat (*Triticum aestivum* L.) plant samples were observed in Vilnius and Kaunas regions in 2012–2013. Plant samples were collected by visual screening of grown fields for the presence of

symptoms of viral etiology. A total of 33 plant samples were collected with leaf mottling, leaf streaking and stunting symptoms from field areas.

Double-antibody sandwich enzyme-linked immunosobent assays (DAS-ELISA). Collected plant samples were tested for the presence of *Wheat streak mosaic virus* (WSMV) using direct DAS-ELISA with specific antibodies (DSMZ Plant Virus Collection, Germany) according to described procedures (Clark, Adams, 1977). Tests were considered to be positive if the ultraviolet (UV) absorbance at 405 nm wave length of investigated sample was equal or greater than three times the absorbance of the negative control.

Electron microscopy (EM). Virus particles were examined in leaf dip preparations negatively stained with 2% uranyl acetate by electron microscope "Jeol Jem-100S" (Japan) electron microscope at the instrumental magnification of 25 000 (Dijkstra, de Jager, 1998).

Purification of ribonucleic acid (RNA). Total RNA extraction was performed with CTAB (hexadecyltrimetylamoniumbromid) based method (Chang et al., 1993). The resulting RNA was dissolved in 30 µl diethyl pyrocarbonated (DEPC)-treated water.

Reverse transcription-polymerase chain reaction (RT-PCR) and sequence analysis. Extracted RNA was used for virus detection and identification by RT-PCR. Extracts from healthy plants were used as negative control. RT-PCR was accomplished using primers designed for WSMV coat protein gene. The forward WS-8166F (5'-GAG AGC AAT ACT GCG TGT ACG-3') and the reverse WS-8909R (5'-GCA TAA TGG CTC GAA GTG ATG-3') primers were selected, resulting in 750 bp amplification product (Kudela et al., 2008). Amplification was performed in Professional Thermocycler ("Biometra", Germany) using double-step RT-PCR method. First strand synthesis of WSMV was carried out using 2 µl of reverse primer (20 µM) with 3 µl of total RNA and 6 µl of DEPC-treated water. The mixture was incubated for 5 min at 70°C and 5 min at 4°C. After denaturation, 11 µl RNA solution was added to the PCR mixture containing 4 µl of RT 5 × buffer, 1 µl of 40 U μl^{-1} RNAse inhibitor, 2 µl 10 mM dNTPs and 1 µl 200 U μl^{-1} RevertAid™ M-MuLV reverse transcriptase (for one sample) ("Thermo Scientific", Lithuania). The synthesis of cDNA was carried out at 42°C for 60 min and 70°C for 10 min.

Deoxyribonucleic acid (DNA) amplification reaction was performed in mixtures containing (per sample) 2 µl of each primer (25 µM), 1µl of 10 mM dNTP mix, 5 µl of 10 × *Taq* reaction buffer without detergents, 3 µl of 25 mM $MgCl_2$, 0.25 µl 5 U μl^{-1} recombinant *Taq* DNA polymerase ("Thermo Scientific") 4 µl cDNA and DEPC water up to a total volume of 50 µl. The initial denaturing was performed at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 1 min, and the final extension of amplification products for 7 min at 72°C.

Amplified DNA fragments were analysed by electrophoresis through 1.5% agarose gel, stained with ethidium bromide, and visualized in "BioDocAnalyze" ("Biometra") gel documentation system using 50 bp DNA ladder marker "GeneRuler™" ("Thermo Scientific"). Specific 750 bp length PCR products were excised from gel and purified using a DNA extraction kit ("Thermo Scientific") according to the instruction of manufacturer and submitted for sequencing at "Macrogen" (South Korea). To reduce the impact of sequencing errors, sequencing of each isolate was carried out twice in each direction. Obtained nucleotide sequences were compared with sequences available in the GenBank (http://www.ncbi.nlm.nih.gov) using basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments were performed and phylogenetic trees were constructed by using computer programmes *DNASTAR7* and *MEGA4.1* (Tamura et al., 2007). Phylogenetic analysis was carried out by neighbour-joining method with bootstrap values based on 1000 pseudoreplicates.

Results and discussion

During the 2012–2013 period, 33 samples of wheat plants with symptoms of possible virus infection were collected from fields of two administrative districts (Vilnius and Kaunas) of Lithuania.

In the first stage of the study, the collected plant samples were tested for the presence of WSMV using direct DAS-ELISA. Out of the 33 wheat samples WSMV was detected in four samples collected from different locations. Four tested plants gave clearly expressed positive reaction confirming WSMV infection. WSMV isolates (WSMV-1206, WSMV-1306, WSMV-1310 and WSMV-1313) obtained from naturally infected winter wheat plants exhibiting leaf mottling, leaf streaking (Fig. 1 A) and stunting (Fig. 1 B) symptoms were selected as material for further investigation.

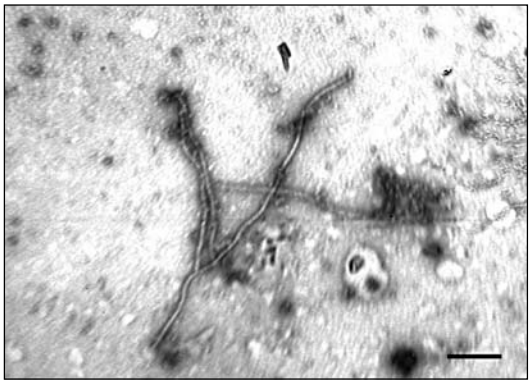
A B

Figure 1. Symptoms on winter wheat naturally infected with *Wheat streak mosaic virus* (WSMV): chlorotic mottling and streaking (A) and stunting (B)

Electron microscopy examination of leaf dip preparations of naturally infected host plant tissue revealed the presence of flexuous, filamentous virus particles, about 700 nm long (Fig. 2), characteristic of viruses within the family *Potyviridae*.

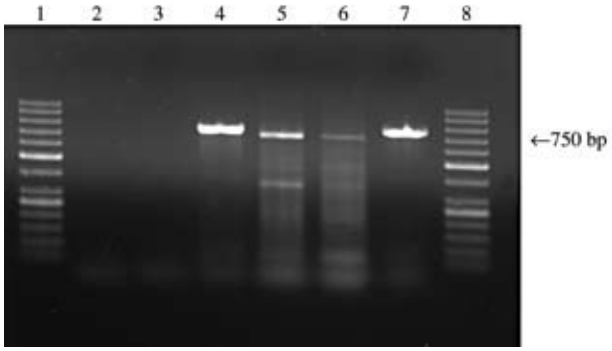
WSMV identification by EM and DAS-ELISA test was verified in RT-PCR using four virus isolates from diseased winter wheat. The WSMV-specific PCR products were obtained with investigated isolates, but not with negative controls (PCR water and healthy plant). Specific bands in agarose gel of analyzed products at a position corresponding to the expected size of amplification product of 750 bp were obtained, confirming WSMV identity (Fig. 3).

Based on the disease symptoms, presence of the wheat curl mite, positive reaction with WSMV-specific antiserum in the DAS-ELISA test, particle morphology and RT-PCR results it was ascertained that viral infections causing disease in wheat was WSMV. The results presented in this article and literature data



Note. Bar represents 100 nm.

Figure 2. Electron micrograph of *Wheat streak mosaic virus* (WSMV) particles



Lanes: 1, 8 – DNA size standard GeneRuler™ 50 bp DNA ladder, fragment sizes (from top to bottom): 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100 and 50 bp.; 2 – healthy wheat plant, 3 – water control, 4 – isolate WSMV-1313, 5 – isolate WSMV-1206, 6 – isolate WSMV-1306, 7 – isolate WSMV-1310

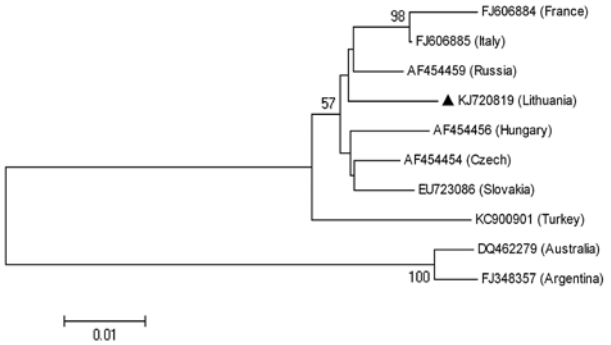
Figure 3. *Wheat streak mosaic virus* (WSMV) specific 750 bp RT-PCR products of four virus isolates from winter wheat

(Brunt et al., 1996; Šutic et al., 1999; French, Stenger, 2004) indicate that virus detected in wheat is the WSMV from the genus *Tritimovirus*. This is the first report of the identification and characterization of WSMV in *Triticum aestivum* in Lithuania.

Specific PCR product obtained from isolate WSMV-1313 was purified and submitted for sequencing. Obtained nucleotide sequence was deposited in GenBank with accession number KJ720819. This sequence contains larger part of WSMV coat protein gene. Only the most distinct nucleotide sequences of WSMV coat protein gene from GenBank were included in the phylogenetic tree presented in Figure 4.

Philogenetic analysis of CP gene sequence showed that Lithuanian WSMV-1313 (accession No. KJ720819) isolate shares the highest sequence similarity to WSMV isolates from Russia (accession No. AF454459.1) and Italy (accession No. FJ606885.1) 98.3% and 98.1%, respectively. While the identity of WSMV-1313 isolate was just 90.2% with isolate from Australia (accession No. DQ462279.1) and 89.9% with isolate from Argentina (accession No. FJ348357.1). In general, Lithuanian WSMV seems to cluster in one group with WSMV isolates from other European countries (Fig. 4).

This research illustrates that WSMV is spread in the Eastern and Central part of Lithuania. Literature



Note. Lithuanian isolate WSMV-1313 marked with a black triangle.

Figure 4. Phylogenetic tree of *Wheat streak mosaic virus* (WSMV) based on coat protein full gene sequences, constructed with *MEGA4.1* using neighbour-joining algorithm

indicates that symptoms of WSM on wheat vary widely and are affected by temperature, cultivar, environment and strain of WSMV (Montana et al., 1996). WSMV ranks as economically damaging, especially for winter wheat when early infections of WSMV in the autumn immediately follow plant emergence (Šutic et al., 1999). Plants infected in the spring have a milder mosaic and stunting is reduced. Since WSMV-infected plants often develop sterile heads, the effects of WSMV on yield can be severe. Yield losses can be as high as 100% depending on the environmental conditions (French, Stenger, 2004). Wheat is the main host of WSMV, but the virus also infects oat, barley, triticale, some cultivars of maize and many wild grasses (Šutic et al., 1999). It would be appropriate to continue research on the detection and characterization of WSMV in the other regions of Lithuania.

Conclusions

1. The causal agent of winter wheat (*Triticum aestivum* L.) disease found in Lithuania was identified as *Wheat streak mosaic virus* (WSMV) from *Tritimovirus* genus on the basis of electron microscopy, serological and molecular methods.

2. Philogenetic analysis based on coat protein gene sequence showed the highest similarity of Lithuanian WSMV isolate to isolates from Russia and Italy.

3. The results confirmed the presence of WSMV in wheat-growing areas of Vilnius and Kaunas regions.

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Kviečių dryžuotosios mozaikos virusas aptiktas žieminiuose kviečiuose Lietuvoje

L. Urbanavičienė, D. Šneideris, M. Žižytė

Gamtos tyrimų centro Botanikos institutas

Santrauka

Pasaulyje miglinius augalus pažeidžiantis kviečių dryžuotosios mozaikos virusas (*Wheat streak mosaic virus*, WSMV) Lietuvoje buvo išskirtas iš natūraliai infekuotų žieminių kviečių. Sergančių kviečių augalai su ryškiais ligos požymiais (chlorotiniu margumu, dryžuotumu ir žemaūge) buvo aptikti Vilniaus ir Kauno rajonų laukuose. Išskirti viruso izoliatai buvo tirti imunofermentinės analizės (DAS-ELISA), elektroninės mikroskopijos (EM) ir atvirkštinės transkripcijos polimerazinės grandininės reakcijos (AT-PGR) metodais. Pagal augaluose šeimininkuose nustatytus specifinius ligos požymius, teigiamus DAS-ELISA testo duomenis, virionų morfologiją (maždaug 15 nm pločio bei 700 nm ilgio) ir AT-PGR tyrimo rezultatus (viruso specifinio cDNR fragmento (750 bp) dydžio amplifikaciją) buvo identifikuotas kviečių dryžuotosios mozaikos virusas (WSMV), priskiriamas *Tritimovirus* genčiai. Sekoskaitos metu gauta WSMV-1313 izoliato apvalkalo baltymo geno seka buvo panaudota filogenetinei analizei. Gauta nukleotidų seka buvo palyginta su kitomis WSMV izoliatų atitinkamomis sekomis, gautomis iš tarptautinės Genų banko duomenų bazės. Apvalkalo baltymo geno sekos analizė parodė, kad Lietuvoje aptiktas WSMV izoliatas pagal seką panašiausias į izoliatus, aptiktus Rusijoje (98,3 %) ir Italijoje (98,1 %). Tai pirmasis Lietuvoje kviečiuose aptikto WSMV tyrimas.

Reikšminiai žodžiai: identifikacija, filogenetinė analizė, kviečių dryžuotosios mozaikos virusas, žieminiai kviečiai.