Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2013, Article ID 983414, 6 pages
http://dx.doi.org/10.1155/2013/983414

Research Article

Identification and Characterization of *Wheat Streak Mosaic Virus* Isolates in Wheat-Growing Areas in Brazil

Talita Bernardon Mar,[1] Douglas Lau,[2] Jurema Schons,[1] Paulo Roberto Valle da Silva Pereira,[2] and Alan Johnny Carminatti[1]

[1] *Universidade de Passo Fundo, BR 285, Km 292, 99001-970 Passo Fundo, RS, Brazil*
[2] *Embrapa Trigo, BR 285, Km 294, 99001-970 Passo Fundo, RS, Brazil*

Correspondence should be addressed to Talita Bernardon Mar; talitabernardon@gmail.com

Received 12 July 2013; Accepted 13 September 2013

Academic Editor: Kent Burkey

Wheat streak mosaic virus (WSMV—*Potyviridae*, *Tritimovirus*), transmitted by the eriophyid mite *Aceria tosichella* Keifer (*Acari*: *Eriophyidae*), was considered a quarantine pest in South America. Since the first report of virus in Argentina, the vector has been found in Argentina, Uruguay and Brazil. The objective of this work was to determine the occurrence of WSMV in Brazil and characterize isolates from wheat-growing areas. Between 2009 and 2011, a total of 40 samples collected in wheat (*Triticum aestivum*) growing areas, where the presence of the mite was previously related, were tested by RT-PCR for virus detection. Six isolates of WSMV were obtained and characterized by sequencing. Two of them had their host range determined. The Brazilian WSMV isolates clustered in clade D are closely related to the Argentine isolate Arg2 (FJ348359). As expected, isolates were unable to infect dicotyledons plants. They caused mosaic in *Triticum aestivum*, *Hordeum vulgare*, *Secale cereale*, ×*Triticosecale*, and *Zea mays*, and they were also able to infect *Avena sativa*. The presence of WSMV in wheat-growing areas of the Brazilian state Rio Grande do Sul was confirmed, possibly having been introduced from a common source and/or direct vector bearing virus migration from Argentina.

1. Introduction

Wheat streak mosaic virus (WSMV) is the type species of genus *Tritimovirus* in the family *Potyviridae* [1]. First reported, in 1929, in the Central Great Plains of the USA [2], currently it is common in most major wheat-growing regions of the world. WSMV is transmitted by eriophyid mite *Aceria tosichella* Keifer (*Acari*: *Eriophyidae*) [3], and low rates of seed transmission have been reported [4–6].

The virus infects several plant species of the family *Poaceae* [7, 8], causing severe mosaic, stunting, and necrosis. WSMV is considered the most important virus disease of North America, Europe, Oceania, and Middle East wheat-growing regions [3]. Annual production losses were frequently observed, in some instances severe local WSMV infection could result in yield losses up to 100% [5, 9, 10]. Divergent strains of WSMV occur in the USA, Mexico and Eurasia [7, 11, 12], and WSMV isolates, similar at those found in American Pacific Northwest (APNW), were detected in the southern hemisphere [5, 8, 13–15].

Since a decade ago the complex *Aceria tosichella* and WSMV had not been found in South America, being considered a quarantine pest [3]. However, in 2002, the WSMV was detected in the central area of Cordoba province in Argentina [14], and in 2004 the vector, *A. tosichella*, was found associated with WSMV infected plants in Argentina [16]. In 2006, *A. tosichella* was detected in Brazil infecting wheat-growing regions in four municipalities in northwestern the state Rio Grande do Sul [17], and, in 2007, the vector was also reported in Uruguay [18].

Since the first report of the mite in Brazil, results of the surveys have indicated a wider distribution of *A. tosichella* in the northern and western municipalities of Rio Grande do Sul [3]. The presence of the mite in Argentina, Uruguay, and Brazil and the virus occurrence in Argentina indicated a

Table 1: Samples of wheat (*Triticum aestivum* L.) showing mosaic symptoms collected in ten counties from the state of Rio Grande do Sul.

Sample	Latitude	Longitude	Geographic origin	Year	CP gene amplification*
329	−28 24′57,20000″	−55 00′29,60000″	São Luiz Gonzaga	2009	—
358	−27 29′49,60000″	−52 54′07,40000″	Nonoai	2009	—
363	−27 17″10,20000″	−52 43′25,60000″	Nonoai	2009	—
451	−28 24′57,20000″	−55 00′29,60000″	São Luiz Gonzaga	2009	—
485	−28 13′49,89000″	−52 24′15,99000″	Passo Fundo (Greenhouse)	2010	—
486	−28 13′49,89000″	−52 24′15,99000″	Passo Fundo (Greenhouse)	2010	948 bp
763	−27 19′36,23677″	−52 46′18,55250″	Nonoai	2010	—
765	−27 19′36,23677″	−52 46′18,55250″	Nonoai	2010	—
817	−28 06′35,02305″	−52 54′29,57809″	Alm. Tamandaré do Sul	2010	—
823	−27 45′57,35304″	−53 27′29,49793″	Palmeira das Missões	2010	—
837	−28 23′59,00000″	−54 39′52,00000″	São Miguel das Missões	2010	—
839	−28 24′57,22000″	−55 00′29,41628″	São Luiz Gonzaga	2010	—
852	−28 23′00,40000″	−54 02′56,00000″	Ijuí	2010	—
862	−27 45′57,35304″	−53 27′29,49793″	Palmeira das Missões	2010	—
879	−28 24′57,22908″	−55 00′29,41628″	São Luiz Gonzaga	2010	—
910	−28 13′31,15000″	−52 24′19,31000″	Passo Fundo	2010	—
911	−28 14′10,94000″	−52 24′26,67000″	Passo Fundo	2010	—
912	−28 13′49,89000″	−52 24′15,99000″	Passo Fundo (Greenhouse)	2010	948 bp
913	−28 13′33,99000″	−52 23′23,75000″	Passo Fundo	2010	—
914	−28 13′33,99000″	−52 23′23,75000″	Passo Fundo	2010	—
915	−28 13′49,89000″	−52 24′15,99000″	Passo Fundo (Greenhouse)	2011	948 bp
1211	−28 06′35,02305″	−52 54′29,57809″	Alm. Tamandaré do Sul	2011	—
1212	−28 06′59,00000″	−52 57′02,00000″	Alm. Tamandaré do Sul	2011	—
1213	−28 01′08,80966″	−53 05′52,31161″	Chapada	2011	—
1214	−27 58′44,00000″	−53 08′53,00000″	Chapada	2011	—
1215	−27 57′16,00000″	−53 14′58,00000″	Palmeira das Missões	2011	—
1216	−27 51′59,00000″	−53 19′31,00000″	Palmeira das Missões	2011	—
1217	−27 45′57,35304″	−53 27′29,49793″	Palmeira das Missões	2011	—
1220	−27 52′52,94114″	−54 26′02,50637″	Santa Rosa	2011	—
1226	−28 29′35,59530″	−54 33′37,15222″	São Miguel das Missões	2011	—
1227	−28 23′59,00000″	−54 39′52,00000″	São Miguel das Missões	2011	—
1228	−28 24′29,00000″	−54 42′15,0000″	São Miguel das Missões	2011	—
1229	−28 25′02,00000″	−54 49′21,0000″	São Luiz Gonzaga	2011	—
1230	−28 25′40,00000″	−54 57′57,00000″	São Luiz Gonzaga	2011	—
1231	−28 30′05,00000″	−54 57′08,00000″	São Luiz Gonzaga	2011	—
1233	−28 24′57,22908″	−55 00′29,41628″	São Luiz Gonzaga	2011	948 bp
1234	−28 29′03,0000″	−55 13′12,00000″	Sto. Antônio das Missões	2011	—
1251	−28 13′31,15000″	−52 24′19,31000″	Passo Fundo	2011	—
1254	−28 13′33,99000″	−52 23′23,75000″	Passo Fundo	2011	948 bp
1256	−28 13′49,89000″	−52 24′15,99000″	Passo Fundo (Greenhouse)	2011	948 bp

* Amplification of a "948 pb" or "—" PCR negative samples.

high level of probability that the complex might be in other places in South America. Due to the economic importance of WSMV, the expansion of the vector to new areas and the presence of the virus in a neighbor country, the objective of this work was to determine the occurrence of WSMV in wheat growing areas of the Brazilian state Rio Grande do Sul and to characterize the WSMV isolates.

2. Materials and Methods

Between 2009 and 2011 a total of 40 wheat (*Triticum aestivum* L.) samples were collected in ten counties from the state of Rio Grande do Sul, Brazil, where the presence of mite was previously confirmed by [3, 17]. From field areas (Table 1) 35 samples were collected with mosaic symptoms. In addition to field areas, five samples of wheat with mosaic and typical

Table 2: WSMV isolates used in sequence comparisons.

WSMV isolate	Accession number	Geographic origin	Reference
486	KC020196	Passo Fundo*, Brazil	This study
912	KC152462	Passo Fundo*, Brazil	This study
915	KC152463	Passo Fundo*, Brazil	This study
1233	KC152464	São Luiz Gonzaga, Brazil	This study
1254	KC152465	Passo Fundo, Brazil	This study
1256	KC152466	Passo Fundo*, Brazil	This study
Arg2	FJ348359	Argentina	Stenger and French, 2009 [15]
Czech	AF454454	Czech	Rabenstein et al., 2002 [22]
El Batán3	AF285170	Mexico	Choi et al., 2001 [11]
H95S	AF511614	Kansas, USA	Stenger and French, 2009 [15]
H98	AF511615	Kansas, USA	Stenger and French, 2009 [15]
ID96	AF511618	Idaho, USA	Stenger and French, 2009 [15]
ID99	AF511619	Idaho, USA	Stenger and French, 2009 [15]
Iran	AF454458	Iran	Rabenstein et al., 2002 [22]
Mon96	AF511630	Montana, USA	Stenger and French, 2009 [15]
Sidney81	AF057533	Nebraska, USA	Stenger et al., 1998 [1]
TK1	AF454455	Turkey	Rabenstein et al., 2002 [22]
Type	AF285169	Kansas, USA	Choi et al., 2001 [11]
WA94	FJ348358	Washington, USA	Stenger and French, 2009 [15]

*From Embrapa Trigo, Passo Fundo greenhouses.

symptoms caused by *A. tosichella* (leaf curling or leaf rolling) were also collected in greenhouse at Passo Fundo, RS, Brazil.

Total RNA (40 samples), extracted using RNeasy Kit (QIAGEN, Hilden, Germany), was used for the first strand cDNA synthesis with *ImProm-II* Reverse Transcription System Kit (PROMEGA, Madison, Wisconsin, USA), according to the manufacturer's instructions. Two sets of primers that amplify different regions of WSMV genome were used. The first one, WSMVF (5′TCGAGTAGTGGAAGCACTCA3′) and WSMVR (5′CCTCACATCATCTGCATCAT3′), designed based on WSMV GenBank sequences, targeting approximately 948 bp of the coat protein (CP) gene. The second one, WSMVL2 and WSMVR2, targets approximately 198 bp of VPg-NIa gene [19].

The PCR mixture contained 2 μL of first strand cDNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.4 μM of each primer and 0.625 U of *GoTaq* Flexi DNA Polymerase Kit (Promega, Madison, Wisconsin, USA) in a 25 μL reaction volume. The PCR conditions were set to 95°C for 2 min as initial denaturation temperature followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, respectively, and a final elongation step at 72°C for 10 min. PCR products were analyzed by electrophoresis in 1.5% (w/v) agarose gel by ethidium bromide staining (10 mg mL^{-1}). Amplified PCR products were cloned in pGEM-T easy vector (PROMEGA Madison, Wisconsin, USA), according to the manufacturer's instructions.

Sequencing reactions were carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3700 DNA sequencer (Applied Biosystems, Foster City, CA, USA). After proofing the sequence quality, sequence identities to both regions were verified by nucleotide BLAST National Center for Biotechnology information (NCBI, National Library of medicine, Bethesda, USA) search program [20].

Six sequences of 828 bp from CP gene (nucleotide 8368 to 9196 comparing with NC_001886), obtained in samples collected in Passo Fundo and São Luiz Gonzaga (Table 2), were aligned on CLUSTAL W program (European Bioinformatics Institute, EBI, Cambridgeshire, UK) and compared with 13 WSMV isolates available in the GenBank (Table 2) and an isolate of *Oat necrotic mottle virus* (ONMV; GenBank accession number AY377938), the most closely related species from the genus *Tritimovirus* species [21]. Neighbor-joining analysis was performed using CLUSTAL X (EBI, Cambridgeshire, UK) with 1,000 bootstrap iterations, gaps were excluded from the analysis, and all other parameters were set to default values. The phylogenetic tree was visualized using the TreeView program rooted with ONMV designated as the Outgroup.

The biological characterization consisted of a host range study of two isolates (915 and 1256) which caused symptoms in previous sap inoculation on wheat (cv. BRS Guabiju). The study was performed using *Chenopodium amaranticolor* Coste and Reyn, *Nicotiana tabacum* Linnaeus, oat (*Avena strigosa* Schreb.), barley (*Hordeum vulgare* Linnaeus), maize (*Zea mays* Linnaeus), rye (*Secale cereale* Linnaeus cv. BRS Serrano), three wheat cultivars (*Triticum aestivum* Linnaeus) Embrapa 16, BRS Guabiju and BRS Timbaúva, and two of triticale (×*Triticosecale* Wittmack) BRS Saturno and BRS Ulisses. Each isolate was sap inoculated using 0.2 M potassium phosphate buffer (pH 7) in four plants of each host, and two negative controls were maintained (mock-inoculated and not inoculated). All the plants were kept in a greenhouse, and

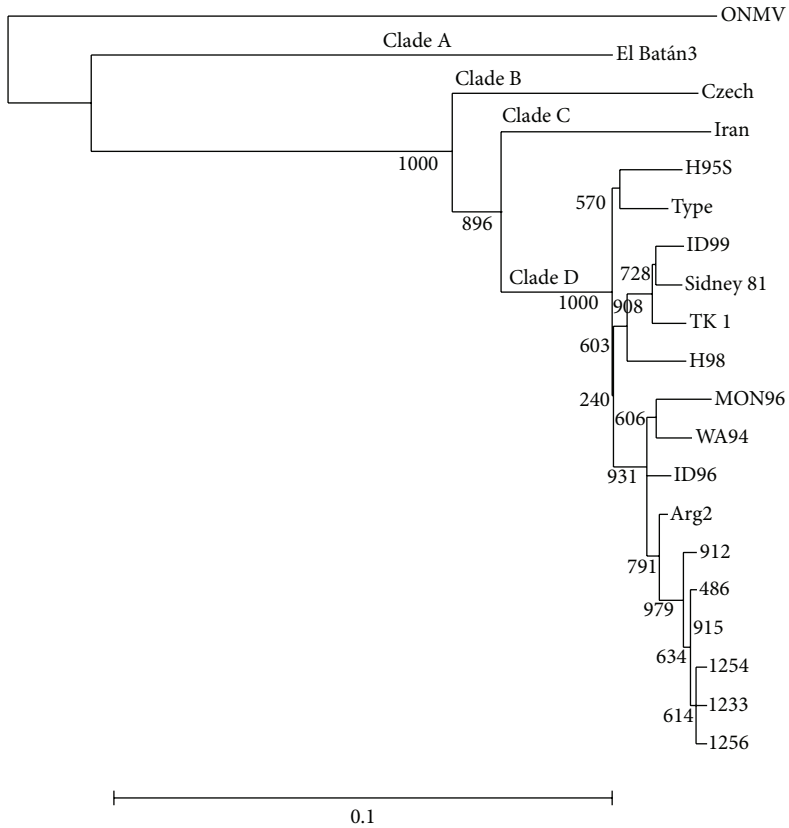

Figure 1: Phylogenetic relationships among 828 bp corresponding to coat protein gene of 19 WSMV submitted in Table 1. Presented is a neighbor-joining tree based on 1,000 bootstrap iterations and rooted with the sequence of ONMV designated as outgroup. Bootstrap values are indicated on branches basal to each node. WSMV Clades A to D are labeled at the respective basal branch defining each clade. Branch lengths are proportional to genetic distances; length of scale bar at lower left corresponds to a genetic distance of 0.1.

symptoms were assessed 15 days after inoculation (dai). The presence of virus was confirmed by RT-PCR 20 dai.

The possible back-inoculation to wheat was also tested for the isolate 915. Wheat plants were sap inoculated using extract (0.2 M potassium phosphate buffer, pH 7) from each host plants (except from *N. tabacum*) inoculated previously with virus isolate 915. The reinfection was confirmed by RT-PCR.

3. Results and Discussion

We confirmed the occurrence of WSMV in wheat-growing areas of Rio Grande do Sul, Brazil, where the presence of the vector has been previously reported. Two infected field samples were identified at Passo Fundo and São Luíz Gonzaga counties, these areas correspond to the first points of occurrence of the vector in Brazil [3, 17]. We also found WSMV in four greenhouse samples at Passo Fundo (Table 1).

Despite the geographic expansion of *A. tosichella* in Brazil at the moment, the populations found in the field are still small [3, 17]. On the other hand, in greenhouse conditions high populations have been found. In this condition, the symptoms caused by mite infestation were evident [17]. This may explain why few field samples were positive for the virus while the majority of greenhouse samples were positive.

The six Brazilian WSMV isolates found in this work (Table 2) are closely related and are similar to the Argentine isolate Arg2 (Figure 1). The sequences of CP gene and of VPg-NIa gene of WSMV isolates from Brazil exhibited 99% to 100% of nucleotide identity among themselves and showed 99% of nucleotide identity, differing at most by eight nucleotides to WSMV Arg2 isolate (FJ348359), from Argentina, recently reported [14]. The WSMV may have been introduced by a common source (seeds) and/or direct migration, through its vector, from Argentina.

Phylogenetic analyses of the CP sequences can indicate the relationship between virus and the genus *Tritimovirus* [15, 22]. The CP sequences of Brazilian isolates present between 97% and 99% of nucleotide identity with sequenced isolates from American Pacific Northwest clustered with clade D, including Argentinean isolates (Figure 1).

The clade D shares about 96% nucleotide sequence identity and includes species from North America, Australia, New Zealand, and two from Turkey [5, 12–15, 22, 23], and the Brazilian isolates (Figure 1) differ by 8% from the Iranian isolate that represents Clade C [12, 22], which differ from Clade D genotypes by 8% [15]. The Brazilian isolates also differ by 9-10% from Czech isolates that represent isolates from Central Europe and Russia comprising Clade B [12, 22] and differing in nucleotide sequence from Clade C and D genotypes by 10% [15].

Table 3: Host range of two Brazilian WSMV isolates through mechanical inoculation and symptoms.

Host	WSMV isolate 915		WSMV isolate 1256	
	Symptoms[1]	Infected/inoculated (%)[2]	Symptoms[1]	Infected/inoculated (%)[2]
Chenopodium amaranticolor Coste and Reyn	—	0	—	0
Nicotiana tabacum Linnaeus	—	0	—	0
Avena strigosa Schreb.	—	0	—	0
Hordeum vulgare Linnaeus	M	40	M	47,6
Zea mays Linnaeus	M	14,3	—	0
Triticum aestivum Linnaeus (cv. Embrapa 16)	M	100	M	100
Triticum aestivum Linnaeus (cv. BRS Guabiju)	M	100	M	100
Triticum aestivum Linnaeus (cv. BRS Timbaúva)	M	100	M	100
Secale cereale Linnaeus (cv. BRS Serrano)	M	25	M	47,4
×*Triticosecale* Wittmack. (cv. BRS Saturno)	M	47,6	M	41,7
×*Triticosecale* Wittmack. (cv. BRS Ulisses)	M; LN	100	M	95,8

[1]M: mosaic; LN: necrotic local lesions.
[2]Number of infected plants/number of inoculated plants, verified by observation of symptoms.

The Passo Fundo WSMV isolates 915 and 1256 were not able to infect dicotyledonous plants but did infect wheat, triticale, barley, rye, oat, and, at least, one maize plant (Table 3). According to [7], WSMV infects several plant species of the family *Poaceae* and no species of dicotyledons. Wheat is the main host of WSMV [13, 24], but the virus also infects rye, oat, barley, triticale, and some cultivars of maize [7].

All wheat cultivars exhibited symptoms that began as chlorotic streaks that later fused to form a mosaic. The mosaic was also observed in ×*Triticosecale*, but in a lower frequency in cv. BRS Saturno and with necrotic local lesions in cv. BRS Ulisses (Table 2). Similar mosaic symptoms, expressed on wheat, were also observed on rye and barley, but they were milder and with lower frequency. All these systemic infections were confirmed by RT-PCR and back-inoculation on heath wheat plants (Table 3).

Necrotic symptoms have been described in wheat [13, 24], oat, and barley [7]. But among the hosts tested and evaluated for up to 15 dai, only triticale cv. BRS Ulisses showed necrotic lesions. Oat was an asymptomatic host, because no symptoms were observed, but virus RNA was detected and the virus could be back-transmitted from this host to healthy wheat plants, which expressed mild symptoms.

The hybrid maize tested presented low frequency of mild symptoms but the virus could not be mechanically back-transmitted to wheat, similar to what is found in other studies [7]. Other varieties could be susceptible to Brazilian isolates. Maize genotypes resistant and susceptible to WSMV have been reported in other studies [7].

The results confirmed the presence of WSMV in wheat-growing areas of Brazilian state Rio Grande do Sul. The six WSMV isolates from Passo Fundo and São Luíz Gonzaga analyzed in this study are closely related, belong to Clade D, and are similar to the Argentinean isolate Arg2 (FJ348359). The WSMV may have been introduced from a common source and/or direct vector bearing virus migration from Argentina.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank CAPES for scholarship, CNPq PROC 578646/2008-0, and Embrapa for financial support.

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