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Occurrence of a closely-related isolate to Maize yellow striate virus in wheat plants

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SUMMARY

In Argentina, wheat fields have exhibited virus-like symptoms, such as chlorotic streaking, dwarfing, yellowing and empty ears since 2007. Symptomatic plants and leaves samples were collected in Marcos Juárez (2007) and Río Cuarto (2008 and 2013), both of them in Córdoba province. The virus was experimentally transmitted from symptomatic wheat plants to wheat cv. Baguette 10 and cv. BIOINTA 3005 using the vector *Delphacodes kuscheli* Fennah (Delphacidae); symptoms of chlorotic streaking, dwarfing and yellowing appeared in the inoculated cereals at 10–15 days post-inoculation. Virus presence was confirmed by electron microscopy and RT-PCR using degenerated primers, which amplified a conserved region of the plant rhabdovirus polymerase (L) gene. Sequence comparison showed 98% nucleotide identity with Maize yellow striate virus C. Caroya (JQ715419) isolated from corn in Argentina. To our knowledge, this is the first report of the occurrence of Maize yellow striate virus in wheat in Argentina.

Keywords: *Triticum aestivum, Cytorhabdovirus*, polymerase gene, *Delphacodes kuscheli.*

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RESUMEN

En la Argentina, desde 2007 se han observado síntomas similares a virus en campos de trigo, tales como rayas cloróticas, enanismo y amarillamiento. Se tomaron muestras de plantas y hojas sintomáticas de Marcos Juárez (2007) y de Río Cuarto (2008 y 2013), ambas localidades de la provincia de Córdoba. El virus fue transmitido experimentalmente a partir de plantas de trigo sintomáticas a los cultivares Baguette 10 y BIOINTA 3005, utilizando el vector *Delphacodes kuscheli* Fennah (Delphacidae); los síntomas de rayas cloróticas, enanismo y amarillamiento en los cereales inoculados se reprodujeron a los 10-15 días posinoculación. La presencia de virus se confirmó por microscopía electrónica y RT-PCR usando cebadores degenerados, que amplifican una región conservada del gen de la polimerasa (L) de los rhabdovirus de plantas. La comparación de

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secuencias mostró 98% de identidad de nucleótidos con el Maize yellow striate virus C. Caroya (JQ715419) aislado de maíz en Argentina. Hasta el momento, este es el primer reporte de la presencia del Maize yellow striate virus en trigo en la Argentina.

Palabras clave: *Triticum aestivum, Cytorhabdovirus*, gen de la polimerasa, *Delphacodes kuscheli.*

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important winter cereal in Argentina, with the cropping area covering 3.9 million ha (Asociación Argentina Pro Trigo, 2014). This crop is affected by several viruses worldwide, some of which have also been reported in Argentina: *Barley yellow dwarf virus* (*Luteoviridae*: *Luteovirus*, BYDV-PAV, BYDV-MAV, BYDV-SGV and BYDV-RMV), *Cereal yellow dwarf virus* (*Luteoviridae*: *Polerovirus*, CYDV-RPV), *Wheat streak mosaic virus* (*Potyviridae*: *Tritimovirus*, WSMV), *Barley stripe mosaic virus* (*Hordeiviridae*: *Hordeivirus*, BSMV), *Mal de Río Cuarto virus* (*Reoviridae*: *Fijivirus*, MRCV), and *Soil-borne wheat mosaic virus* (*Togaviridae: Furovirus*, SBWMV) (Truol, 2009). In addition, the *Cytorhabdovirus Barley yellow striate mosaic virus* (*Rhabdoviridae*, BYSMV) was detected by our group in wheat field plants in 2006 (Dumón *et al*., 2011).

Virus-like symptoms have been observed in wheat fields in several provinces of Argentina since 2007. The symptoms include mild chlorotic streaking on the leaves, dwarfing, yellowing and the presence of empty and deformed ears (Fig. 1). Except for deformed ears and a relatively more severe yellowing observed in these plants, all the other symptoms are consistent with symptoms previously described for BYSMV (Dumón *et al*., 2011).

Based on the similarity of the observed symptoms to those characteristic of cereal rhabdoviruses (Shtein-Margolina, 2002), as well as on the presence of rhabdovirus vectors (*Toya propinqua* and *Delphacodes kuscheli*) (Jackson *et al*., 2005; Dumón *et al*., 2011) in the infected fields where samples were collected, we proposed that an unknown rhabdovirus is affecting wheat plants. The aim of this study was to identify the causal agent of this novel wheat disease.

MATERIALS AND METHODS

Sample collection

Samples of symptomatic wheat plants were collected from two localities of Córdoba province (Argentina) situated in the production area, in November and December of different years. Wheat cv. BIOINTA 1003 was collected from Marcos Juarez in 2007 (MJ 2007) and cvs. Baguette 10 and Biointa 3005 were collected from Río Cuarto during 2008 (RC 2008) and 2013 (RC 2013). Some symptomatic leaves were selected and kept at -80 ºC for molecular studies.

Experimental transmission and electron microscopy

Experimental transmissions were performed using as vector third-instars' nymphs of *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae) reared in our laboratory. This species was selected because was mentioned as a principal vector of another plants virus in Argentina (Truol *et al*., 2001; Dumón *et al*., 2011). Transmission tests were performed from symptomatic diseased wheat plants (MJ 2007 and RC 2013) to wheat cv. Baguette

10 and cv. BIOINTA 3005, grown under controlled greenhouse conditions of temperature: 24 ± 1 ºC and a photoperiod of 16:8 (L:D) h. Acquisition, latency and inoculation periods of 2, 10 and 1 day, respectively, were considered. Three insects per plant were used to inoculate wheat seedlings. Three replicates of 20 plants were conducted for this assay. To test pathogenesis on other cereal species and cultivars, transmissions were performed to another wheat cultivar (*Triticum aestivum* L., ProINTA Federal), oat (*Avena sativa* L., cv. Bonaerense Payé) and barley (*Hordeum vulgare* L., cv. Goldie). One week after inoculation, plants were observed daily for the occurrence of symptoms.

Symptomatic samples were collected from inoculated plants 20 days after transmission for electron microscopy observations. Leaf-dip analyses were performed using the protocol proposed by Truol *et al.* (2009) and samples were examined under a Jeol JEM EXII transmission electronic microscope (TEM) (Jeol, Tokyo, Japan). In order to determine the size of the virions, 100 viral particles were measured on digital micrograph at magnifications of 250000x. Measurements were taken on isolated particles after negative staining.

Reverse transcription polymerase chain reaction (RT-PCR) and sequence analysis

Wheat cv. BIOINTA 1003 leaves from fields and wheat leaves from experimentally transmitted plants were processed. Total RNA was purified using Nucleospin RNA plant kit (Macherey-Nagel). RT-PCR was performed using degenerate primers targeting a conserved region of plant rhabdovirus polymerase (L) gene (Lamprecht *et al*., 2009). Amplification products of about 900 bp were cloned into pCR4-TOPO vector (Invitrogen), sequenced and deposited in GenBank database. Nucleotide sequences were compared with sequences available at the National Center for Biotechnology Information (NCBI) using BLAST. Alignments were performed with Clustal W (Thompson *et al*., 1994). Sequence comparisons between ours different isolates of wheat cytorhabdovirus and other available cereal rhabdovirus (*Barley yellow striate mosaic virus*, *Northern cereal mosaic virus*, *Maize mosaic virus* and *Iranian maize mosaic nucleorhabdovirus*) were performed using a sequence identity matrix in the BioEdit program (Hall, 1999).

RESULTS AND DISCUSSION

Chlorotic streaking, dwarfing and marked yellowing symptoms observed in plants experimentally inoculated with *D. kuscheli* were identical to those observed in the fields. The earliest symptoms were observed at 10-15 days post-inoculation in both tested cultivars, Baguette 10 and Biointa 3005 (Fig. 2a). *D. kuscheli* transmitted this virus with an efficiency of 45% in both cultivars. This value is lower than the BYSMV transmission efficiency reported by Dumón *et al.* (2011) (80%) and higher than MRCV transmission efficiency, in experimental assays (30%) (Truol *et al.,* 2001).

In pathogenicity tests, RC 2013 isolate was successfully transmitted to another wheat cultivar (ProINTA Federal), as well as to oat (cv. Bonaerense Payé) and barley (cv. Goldie). Symptoms were similar among the tested species. In wheat and oat, symptoms started with fine chlorotic lines and then progressed to chlorotic striations, yellowing and dwarfing (Fig. 2b,c). By contrast, barley showed marked chlorotic striation in the first 10 days post-transmission, and then the striation became more tenuous. In some cases, this cereal showed leaves with wavy edges (Fig. 2d).

In addition to the result mentioned recently, the same bacilliform particles characteristic of plant rhabdoviruses were observed under electron microscopy in plants collected in the fields as well as in plants inoculated with *D. kuscheli* at 20 days post-transmission (Fig. 3a). Fig. 3 (b,c) shows the size distribution of particles in wheat (length and width, respectively). Particles were (350–420) x (65–75) nm, with a mean size of 380 x 70 nm. These particles closely resemble those detected by our group in ultrathin sections of BYSMV (Dumón *et al*., 2011) although the latter are shorter in length (about 300 nm). However, this difference may be due to the different preparation techniques used for other rhabdoviruses (Herold, 1972; Conti & Appiano 1973; Martelli *et al*., 1975).

Partial sequences of MJ 2007, RC 2008 and RC 2013 isolates were obtained by conventional RT-PCR using degenerate primers, which amplified a conserved region of the L gene (Lamprecht *et al*., 2009). BLAST analysis confirmed that the cloned inserts had viral RNA origin (polymerase gene) and were most closely related to sequences of cytorhabdovirus polymerase gene. The sequences obtained were submitted to GenBank (Accession N. MJ 2007: KM097989, RC 2008: KF430632 and RC 2013: KM009143).

The 900-bp sequenced fragments obtained from the three Argentine isolates were aligned with the L gene of known plant rhabdoviruses available at GenBank. A high sequence identity (98%) of MJ 2007, RC 2008 and RC 2013 isolates with Maize yellow striate virus (Accession No. JQ715419) iso-

Fig. 1: Virus-like symptoms observed in wheat growing under field conditions (cv. BIOINTA 3005): chlorotic streaking and yellow and empty ears (left). Healthy plant, leaf and ear (right).

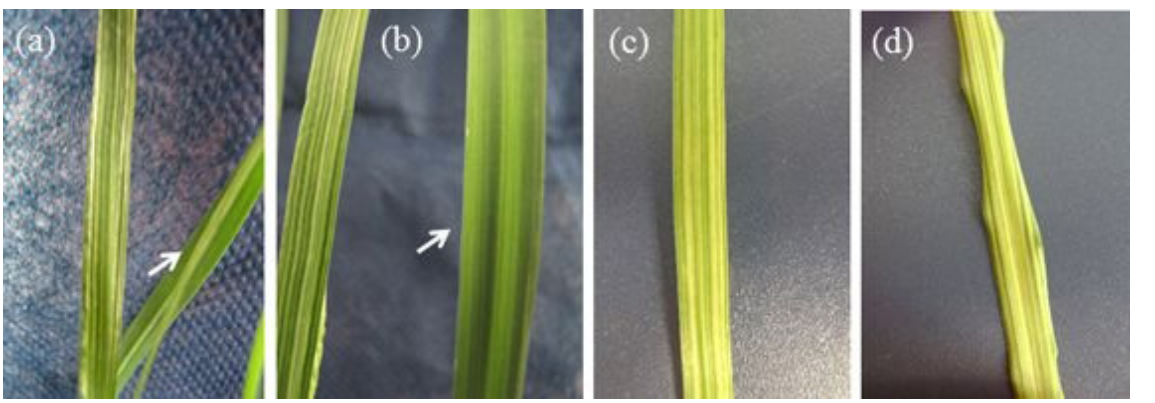

Fig. 2: Virus-like symptoms on several monocot species after 15 days of *D. kuscheli* transmission. (a): Wheat cv. BIOINTA3005; (b): wheat cv. ProINTA Federal; (c): oat cv. Bonaerense Payé and (d): barley cv. Goldie. White arrows indicate non-infected leaves.

lated from corn was observed, whereas identity with *Barley yellow striate mosaic virus* Zanjan-1 isolate (BYSMV; FJ665628) was 73% and with *Northern cereal mosaic virus* (NCMV; AB030277) was 71%. In addition, Table 1 shows that MJ 2007, RC 2008 and RC 2013 wheat isolates had high identity values with Maize yellow striate virus (*Cytorhabdovirus*), suggesting that the virus isolated from wheat in this work belongs to the same species as the one isolated from corn. When nucleorhabdovirus were included in the comparison, *Iranian maize mosaic nucleorhabdovirus* (NC011542) and *Maize mosaic virus* (NC005975), identity decreased to values ranging from 0.44 to 0.49.

Maize yellow striate virus proposed as a new viral species, was reported in 2012 on maize fields in Argentina and was experimentally transmitted using a planthopper, *Peregrinus maidis* (Ashmead) (Maurino *et al*., 2012). In the present work, symptoms produced using *D. kuscheli* as a vector for winter cereals were similar to those previously described for maize (Maurino *et al*., 2012). Different planthopper species are known to transmit viruses from winter cereals to corn crops in Argentina, such as *D. kuscheli*, transmitting *Mal de Río Cuarto virus* (MRCV, *Fijivirus*) (March *et al*., 1995). This phenomenon would increase the chances a rapid spread of this new cytorhabdovirus from wheat to corn, highlighting the importance of this vector in the epidemiology of the disease.

In conclusion, the results presented here based on a partial L polymerase gene sequence indicate that most probably the three Argentine wheat isolates and the Maize yellow striate virus (C. Caroya

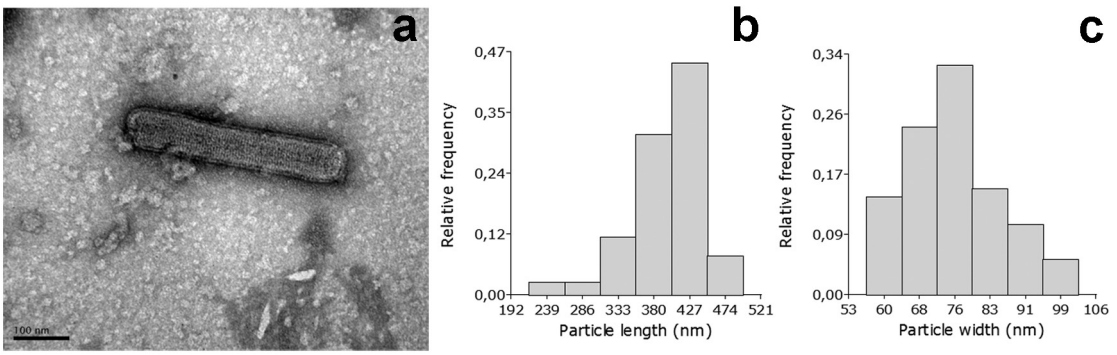

Fig. 3: Electron micrograph of virus particles and their size distribution. (a) Bacilliforms particles, typical of rhabdovirus, observed under electronic microscope on dip preparations, using negative staining with uranyl acetate. Bar: 100 nm; Magnification 250000x (b) Particle length distributions. (c) Particle width distributions.

Table 1. Sequence identity matrix showing nucleotide identity of the three studied wheat cytorhabdovirus isolates with other cytorhabdoviruses deposited at GenBank.

	Sequences	1	2	3	4	5	6	7	8
1	RC 2008 (KF430632)	ID							
2	MJ 2007 (KM097989)	0.985	ID						
3	RC 2013(KM009143)	0.985	0.984	ID					
4	Maize yellow striate virus C. Caroya isolate (JQ715419)	0.981	0.986	0.979	ID				
5	*Northern cereal mosaic virus* (AB030277)	0.676	0.68	0.683	0.681	ID			
6	*Barley yellow striate mosaic virus* Zanjan-1 isolate (FJ665628)	0.725	0.727	0.726	0.726	0.676	ID		
7	*Iranian maize mosaic nucleorhabdovirus* (NC011542)	0.492	0.492	0.486	0.490	0.487	0.488	ID	
8	*Maize mosaic virus* (NC005975)	0.454	0.459	0.451	0.455	0.440	0.473	0.654	ID

isolate) belong to the same rhabdovirus species. However, further molecular studies and comparison with the complete nucleotide sequences of *Cytorhabdovirus* members are needed to confirm this point.

Our results also suggest that the symptoms observed in field-collected plants correspond to a new disease in wheat, caused by cytorhabdovirus, as well as that the planthopper *D. kuscheli* might be a vector of this cytorhabdovirus. *D. kuscheli* is also the vector of two viruses that cause important cereal crop diseases: *Mal de Río Cuarto virus* and *Barley yellow striate mosaic virus* (Ornaghi *et al*., 1999; Dumón *et al*., 2011). Therefore, the possible occurrence of double or triple-infected plants should be a concern.

This is the first report of an occurrence of a closely-related isolate to Maize yellow striate virus in wheat plants in Argentina, and it makes an important contribution to the epidemiology of diseases.

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REFERENCES

Asociación Argentina Pro Trigo, 2014. <http://www.aaprotrigo.org/desarrollo_noticia.php> Verified 04/07/2014.

Conti, M. and A. Appiano, 1973. *Barley yellow striate mosaic virus* and associated viroplasms in barley cells. J. Gen. Virol 21:315-322.

Dumón, A.D.; E.B. Argüello-Caro, V.M. Alemandri, C. Bainotti, M.F. Mattio, S.M. Rodríguez, M. del Vas y G. Truol, 2011. Identificación y caracterización biológica del *Barley yellow striate mosaic virus* (BYSMV): Nueva enfermedad del trigo en Argentina. Trop. Plant Pathol 36:374-382.

Hall, T.A., 1999. BioEdit: a user-friendly biological se-

quence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser 41:95-98.

Herold, F., 1972. *Maize mosaic virus*. CMI/AAB Descriptions of Plant Viruses. 94.

Jackson, A.O.; R.D. Dietzgen, M.M. Goodin, J.N. Bragg and M. Deng, 2005. Biology of plant rhabdoviruses. Annu. Rev. Phytopathol 43:623-660.

Lamprecht, R.L.; G. Pietersen, G.G.F. Kasdorf and L.H. Nel, 2009. Characterization of a proposed *Nucleorhabdovirus* new to South Africa. Eur. J. Plant Pathol 123:105-110.

March, G.J.; M. Balzarini, J.A. Ornaghi, J.E. Beviacqua and A. Marinelli, 1995. Predictive model for "Mal de Río Cuarto" disease intensity. Plant Dis 79:1051-1053.

Martelli, G.P.; M. Russo and G. Malaguti, 1975. Ultra structural aspects of maize mosaic virus in the host cells. Phytophatol. Mediterr 14:140.

Maurino, M.F.; I. Laguna, F. Giolitti, C. Nome and M.P. Giménez Pecci, 2012. First Occurrence of a Rhabdovirus infecting maize in Argentina. Plant Dis 96:1383.

Ornaghi, J.A.; G.J. March, G.T. Boito, A. Marinelli, J.E. Beviacqua, J. Giuggia and S.L. Lenardon, 1999. Infectivity in natural populations of *Delphacodeskuscheli*vector of Mal de Río Cuarto virus. Maydica 44:219-223.

Shtein-Margolina, V.A, 2002. Cytopathology of plants infected with viruses. Ultraestructure of the leaf cells of cereals affected by cereal rhabdoviruses. Biol. Bull 29:12-23.

Thompson, J.D.; D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673-4680.

Truol, G.A.; T. Usugi, J. Hirao, J. Arneodo, M.P. Giménez Pecci e I. Laguna, 2001. Transmisión experimental del virus del Mal de Río Cuarto (MRCV) por *Delphacodes kuscheli*. Fitopatol. Bras. 26:39-44.

Truol, G., 2009. Enfermedades virales asociadas al cultivo de trigo en Argentina: reconocimiento, importancia, formas de transmisión y manejo. Córdoba, Argentina, Ediciones INTA.

Truol, G.; C. Nome, M. Sagadin, V. Alemandri, V. Conci y E. Cafrune, 2009. Microscopía electrónica, principios fundamentales, técnicas de microscopía electrónica empleadas en la identificación de virus de plantas. In: Laguna, I.G.; V. Conci, G. Rodríguez Pardina, G. Truol, M. Fiorona y L. Di Feo (eds.): Procedimientos empleados en la identificación de organismos fitopatógenos. Córdoba, Argentina, Ediciones INTA, pp 25-30.