

## Notas Científicas

### Interception of *Wheat mosaic virus* (WMoV) in Brazil in maize seeds from the United States

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**Abstract** – The objective of this work was to evaluate the phytosanitary aspect of two accessions of maize (*Zea mays*) seeds from the United States introduced to Brazil, regarding the presence of *Wheat mosaic virus* (WMoV). Two to three weeks after sowing, symptomatic leaves were tested by Elisa using specific antiserum to WMoV. The reaction was positive, and leaf samples were analyzed by real-time PCR and amplified PCR products were sequenced. The WMoV isolates had 99 to 100% nucleotide identity with isolates from Australia and the United States. Until now, there is no report of the presence of this virus in Brazil. According to the federal law on plant protection, the plants were burned to avoid the introduction of this exotic pest in the country. The obtained results show WMoV interception in Brazil.

**Index terms:** *Zea mays*, exotic pest, *High plains virus*, plant quarantine, phytosanitary analysis.

### Interceptação do *Wheat mosaic virus* (WMoV) no Brasil em sementes de milho provenientes dos Estados Unidos

**Resumo** – O objetivo deste trabalho foi avaliar o aspecto fitossanitário de dois acessos de sementes de milho dos Estados Unidos introduzidas no Brasil, quanto à presença do *Wheat mosaic virus* (WMoV). Duas a três semanas após a semeadura, folhas sintomáticas foram testadas por Elisa, tendo-se utilizado antissoro específico para WMoV. A reação foi positiva, e as amostras foliares foram analisadas por PCR em tempo real e os produtos de PCR amplificados foram sequenciados. Os isolados de WMoV apresentaram 99 a 100% de identidade nucleotídica com isolados da Austrália e dos Estados Unidos. Até o momento, não há relato da presença desse vírus no Brasil. De acordo com a legislação federal de defesa vegetal, as plantas foram incineradas para evitar a introdução dessa praga exótica no País. Os resultados obtidos mostram a interceptação de WMoV no Brasil.

**Termos para indexação:** *Zea mays*, praga exótica, *High plains virus*, quarentena vegetal, análise fitossanitária.

High Plains disease (HPD) was first described in the United States, in 1993, in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) crops in Nebraska and in other states in the High Plains region, such as Texas, Kansas, Idaho, Colorado, and Utah (Jensen et al., 1996; Stewart et al., 2013). The causal agent is a negative-sense RNA virus in the genus *Emaravirus*, referred to, in the literature, as *High Plains virus* (HPV), *Maize red stripe virus* (MRSV/MRStV), or, more recently, *Wheat mosaic virus* (WMoV) (Stewart et al., 2013). The virus has been found in Israel, Chile

(Jensen, 1999), Argentina (Truol, 2009), and Australia (Coutts et al., 2014), and has a host range that includes economically important plants, such as wheat, maize, barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), and some weeds (Seifers et al., 1998).

HPD symptoms and severity vary considerably from mild to severe, and include mosaic, chlorosis, and necrosis (Jensen et al., 1996). WMoV is transmitted by the eriophyid wheat curl mite *Aceria tosichella* Keifer (Seifers et al., 1997), which is also the vector for *Wheat*

*streak mosaic virus* (WSMV, genus *Rymovirus*, family *Potyviridae*) (Navia et al., 2013). WSMV and WMoV are often found in mixed infection (Skare et al., 2006; Navia et al., 2013). Until now, there is no report of WMoV in Brazil; however, the recent detection of the pathosystem *Aceria tosichella*/WSMV in the country (Mar et al., 2013) has alerted to the threat represented by WMoV introduction. Since cereals are important crops for Brazil, it is essential for Brazilian agriculture to import WMoV-free cereal seeds. Therefore, to prevent the entry of this pest in the country, it is fundamental to perform phytosanitary analyses in imported cereal seeds.

The objective of this work was to evaluate the phytosanitary aspect of two accessions of maize seeds from the United States introduced to Brazil, regarding the presence of WMoV.

A procedure was performed for inspecting plants and testing maize seedlings in quarantine using serological and molecular tests at the plant quarantine laboratory of Embrapa Recursos Genéticos e Biotecnologia, located in Brasília, Brazil. Two maize accessions imported in 2013 (20130100 and 20130234) were received for phytosanitary analyses and placed to germinate. About two and three weeks after sowing, symptomatic seedlings were collected for analysis. Maize leaf samples from each accession were assayed by Elisa using specific antiserum to WMoV (syn. HPV, Product No. 17200, Agdia, Elkhart, IN, USA), according to the manufacturer's instructions.

Specific primers, named HPVFW414 (5' GAG TGC TGG TTT TTC TAA GGA GCA CA 3') and HPVREV565 (5' CTG ACC ATA GGT GCC ACA AGG TCT GA 3'), were designed from the RNA3 HPV sequence (GenBank accession U60141). Total RNA was extracted from leaf samples using the Qiagen RNeasy plant kit (Qiagen, Inc., Valencia, CA, USA), following the manufacturer's instructions. First-strand cDNA was synthesized using the HPV-R1 primer (Lebas et al., 2005) and MMLV-Reverse Transcriptase (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Real-time reactions were monitored using a Rotor-Gene Q 5plex HRM platform (Qiagen Inc., Valencia, CA, USA). For qPCR, the 25  $\mu$ L volume reaction contained 12.5  $\mu$ L of Rotor-Gene SYBR Green RT-PCR Master Mix (Qiagen, Inc., Valencia, CA, USA), 6.5  $\mu$ L H<sub>2</sub>O, 0.5  $\mu$ L at 10  $\mu$ mol L<sup>-1</sup> concentration of each primer, and 5.0  $\mu$ L of cDNA previously diluted 10x.

The cycling profile for the assay consisted of 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 5 s and annealing/elongation at 65°C for 10 s. A melting curve analysis was performed to assure that a homogenous amplification product had been produced. An Argentine isolate was used as a positive control and to construct the recombinant plasmid for the standard curve. Amplified products were analyzed by 1% agarose gel electrophoresis stained with GelRed (Biotium, Inc., Hayward, CA, USA). The expected PCR products of 201 pb were sent for sequencing at MacroGen laboratories (MacroGen Korea, Seoul, South Korea). Blast searches were performed using the Blastn algorithm available at the National Center for Biotechnology Information website (National Center for Biotechnology Information, 2015).

Symptomatic samples (Figure 1 A and 1 B) tested WMoV positive by Elisa, and were confirmed by RT-PCR (Figure 1 C) and sequencing. The WMoV-positive plants were chlorotic, with different degrees of leaf striping (Figure 1 A and 1 B). The amplification profile of real-time PCR showed a cycle threshold of 10 for the positive control (recombinant plasmid diluted to 0.1 ng  $\mu$ L<sup>-1</sup>). The samples (20130100 and 20130234) had cycle threshold of 30.26 and 35.05, respectively, revealing the presence of the virus in low concentrations in both of them. Melting curve analyses showed a single peak around 80°C for samples and positive control, without nonspecific amplification. The specific amplification of real-time PCR products was confirmed by agarose gel electrophoresis. The sequenced fragments showed 99 to 100% identity with WMoV isolates from Australia (KC337341 and KC337342) and from the United States (U60141), obtained from the GenBank.

In South America, the report of *A. tosichella* and its associated viruses is recent. WSMV was first detected in Argentina in 2002 (Truol et al., 2004) and, two years later, *A. tosichella* was also found in the continent in association with WSMV-infected plants (Navia et al., 2006). In 2007, WMoV was detected in Argentina, in mixed infections with WSMV (Truol, 2009). Until recently, neither the associated viruses nor its vector were found in Brazil, but the presence of *A. tosichella* was reported in 2009 (Pereira et al., 2009) and WSMV was detected (Mar et al., 2013). In the present study, Elisa and qPCR allowed efficient virus detection in symptomatic plants.



**Figure 1.** Maize (*Zea mays*) plants showing mild symptoms induced by *Wheat mosaic virus* (A and B), and PCR-amplified products of ca. 200 pb (C). M, 1 Kb Plus Ladder (Thermo Fisher Scientific, Inc., Waltham, MA, USA); C-, negative control; C+, positive control; and 20130100 and 20130234, evaluated maize accessions.

This is the first known and confirmed report of WMoV interception in Brazil. The plants were burned to avoid the introduction of this exotic pest in the country. The expanding distribution of this emerging virus is significant because of its potential to cause additional severe economic impact on two major crops – wheat and corn.

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