



First report of wheat stripe mosaic virus in Paraguay

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Abstract

Soil-borne viruses transmitted by *Polymyxa graminis* can lead to major yield losses in wheat crops worldwide. This work describes the identification of a soil-borne virus infecting wheat plants in Paraguay. Based on electron microscopy observations of virus particles in symptomatic leaf and molecular assays the virus was identified as wheat stripe mosaic virus (WhSMV). To our knowledge, this is the first report of WhSMV in Paraguay.

Keywords Wheat · Soil-borne virus · Diagnose

Wheat (*Triticum* spp.) cultivation in Paraguay has experienced a boom in production in the last two decades (FAO 2017). Currently, wheat is the most important crop during the winter season in the southeastern region of the country. In June 2016, randomly irregular patches of wheat plants (var. Itapúa 65) exhibiting streaking mosaic on the leaves and stunting (Fig. 1a, b) were observed in an experimental field at the Paraguayan Institute of Agricultural Technology (IPTA), located in the district of Capitán Miranda, Itapúa Department, Southeast of Paraguay. Negatively stained leaf extract of symptomatic wheat leaves examined in the transmission electron microscopy revealed the presence of rod-shaped like-virus particles (Fig. 2a), similar to those belonging to the genera *Benyvirus* and *Furovirus*, which are transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* (Kanyuka et al. 2003). The presence of resting spores of *P. graminis* in the root of symptomatic wheat plants was confirmed by optical microscopy using the lactophenol

staining technique (Fig. 2b). Total RNA extraction was performed on leaves and roots of six symptomatic wheat plants using Plant RNA purification reagent (Thermo Fisher Scientific, Waltham, USA) following the manufacturer protocol. RT-PCR was performed using specific primers for soil-borne wheat mosaic virus (SBWMV) and wheat spindle streak mosaic virus (WSSMV) (Clover and Henry 1999; Clover et al. 2001), which are found worldwide associated with soil-borne wheat diseases, but no replicons were amplified. Specific primers for wheat stripe mosaic virus (WhSMV), which was recently described associated with soil-borne wheat mosaic disease in South of Brazil (Valente et al. 2019), was also used for RT-PCR. Amplicons of expected size for WhSMV were obtained from total RNA extracted from all symptomatic samples using primers Beny_Pol-F (5'-ATCGATGCTGACGC TTCACTG -3') and Beny_Pol-R (5'-ACGAAACA CCTGTGTCGCTA -3') and primers Beny_CP-F (5'-AAGTGTCGCAAGCTTCGCG-3') and Beny_CP-R (5'-ATCGCACCGACGTAAGAACT-3'), which enables the amplification of genomic regions of 465 bp and 641 bp, corresponding to part of the replicase protein gene and the complete CP gene, respectively (Valente et al. 2019). All amplicons were purified and directly sequenced in both directions at Macrogen Inc. (Seoul, South Korea). The consensus nucleotide sequences were obtained using the Electropherogram quality analysis program (<http://asparagin.cenargen.embrapa.br/phph/>). Deduced amino acid sequences were obtained using ExPASy (<https://web>.

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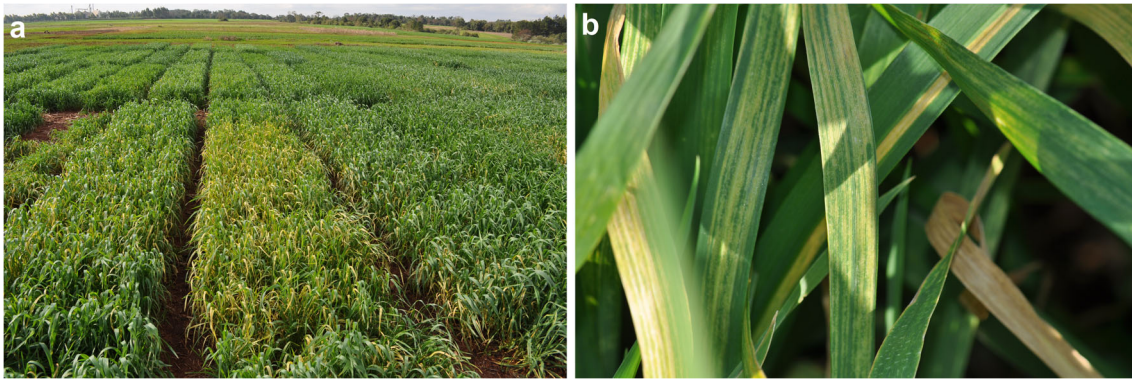


Fig. 1 a Chlorotic patches of infected plants in the experimental field. b Symptoms of *Wheat stripe mosaic virus* on leaves of wheat

expasy.org/translate/) and aligned using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Amino acid sequences of the replicase protein (MK994524) and CP (MK994525) showed 100% and 96.55% to 98.85% identity, respectively, with corresponding protein sequences of WhSMV from Brazil (Replicase protein MH151795 - MH151800; CP MH151801 - MH151805), a putative new member of the family *Benyviridae*. To our knowledge, this is the first report of a virus associated with a soil-borne wheat mosaic disease in Paraguay. Further surveys are necessary to determine the occurrence

and economic impact of WhSMV in commercial wheat crops throughout the country.

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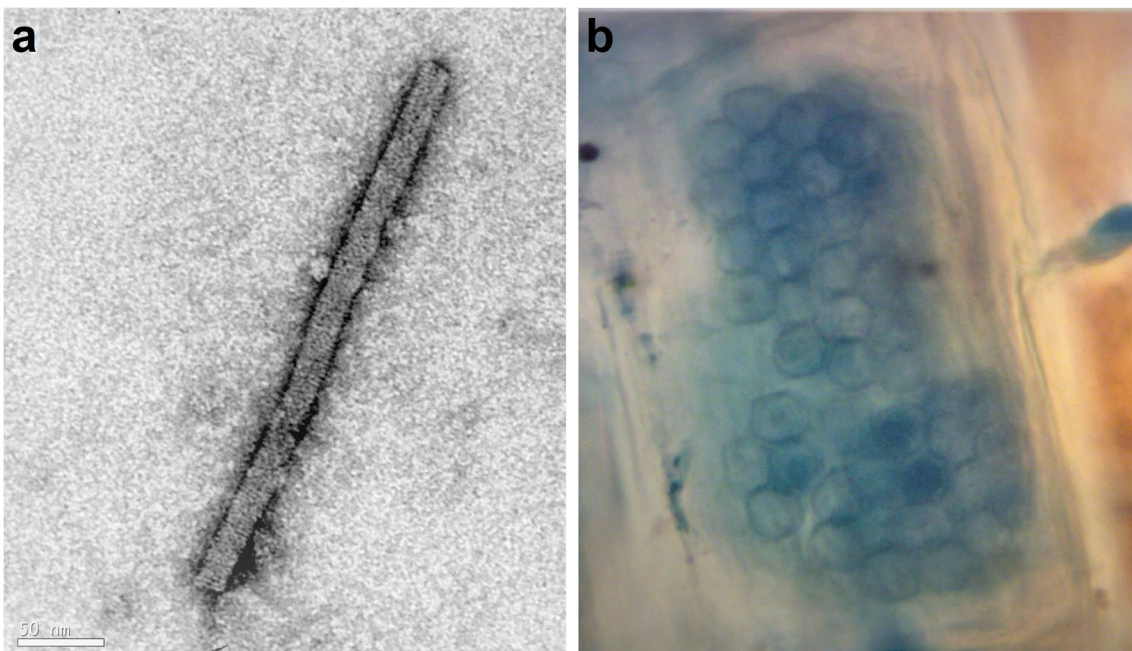


Fig. 2 a Negatively stained leaf extract, showing presumed WhSMV rod-shaped like-virus particle. b Resting spores of *Polymyxa graminis* in roots of symptomatic wheat plants stained with lactophenol

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