First Report of Zucchini Lethal Chlorosis Virus in Argentina Infecting Squash Crops

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Virus species of the genus *Orthotospovirus* are among the most economically important plant pathogens in the world because they cause severe crop losses; mainly, in ornamental and horticultural crops (Pappu et al. 2009). They are exclusively transmitted by thrips. Several species of *Orthotospovirus* have been reported infecting cucurbits: *Watermelon silver mottle virus*, *Zucchini lethal chlorosis virus* (ZLCV), *Watermelon bud necrosis virus*, *Melon yellow spot virus*, *Melon severe mosaic virus* and *Groundnut ringspot virus* (Ciuffo et al. 2017, Spadotti et al. 2014). The symptoms caused by ZLCV infection can include chlorosis and systemic necrosis on leaves, apical upward leaf curl, reduction of leaf blade and fruit malformation (Fig. S1) (Giampan et al. 2007). The collection of 90 symptomatic leaves of squash from Salta and Jujuy provinces was carried out during early 2016. For an initial assessment of the presence of ZLCV a plate trapped antibody enzyme-linked immunosorbent assay (PTA-ELISA) (Lommel et al. 1982) with antiserum against ZLCV, kindly provided by Jorge A. M. Rezende from the Universidade de São Paulo, Brazil, was performed. Fifty four of the 90 samples reacted positively to ZLCV-specific antiserum, being 18 and 11 positive plants of Cucurbita *maxima* var. *zapallito redondo del tronco*, var. *zapallo plomo* respectively, 11 positive plants of *C. pepo*, 11 positive plants of *C. ficifolia*, var. “cayote” and 3 positive plants of *C. moschata*. Ultrathin sections of leaf samples of naturally infected plants were examined by transmission electron microscopy and presumable orthotospovirus particles were observed (Fig. S2). To confirm the identity of the virus, a one-step reverse transcription-polymerase chain reaction (RT-PCR) assay was carried out on the RNA extracts from Squash plants, using ZLCV-specific primers designed to direct amplification of nucleotides (ZLCV-F ATCATGCTGTCCAGTCTCCT and ZLCV-RCCCACATTTTGCACTTGCGA), of the nucleocapsid gene region. The RT-PCR reaction for ZLCV detection consisted of reverse transcription at 46°C for 30 min, followed by denaturation at 94°C for 3 min, and 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s. Amplicons of the two ZLCV isolates MK680830 and MK680831 were Sanger sequenced. The consensus sequences were aligned using clustalW aligned and compared with other ZLCV sequences in the public domain using Mega 7 (Kumar et al. 2016). Alignments of the N gene sequences of these ZLCV isolates displayed nucleotide sequence identity above 94% with other ZLCV isolates available at the GenBank database. In addition, the amino acid sequence demonstrated above 97% identity with equivalent regions of S segment of Brazilian ZLCV isolate from *Cucumis sativus* and squash. The phylogenetic analysis of the identified sequence of ZLCV and other related sequences from the GenBank, showed a cluster of argentine isolates, close to ZLCV-DF isolate obtained from *Cucumis sativus* (KU681011). This is the first report of ZLCV outside of Brazil. Although we have not observed presence of *Frankliniella zucchini* in the field, which was identified and described as vector of

ZLCV (Riley et al. 2011), as well as virus distribution be limited to Brazil (Nakahara and Monteiro, 1999); It would be important to consider the presumable entry of *F. zucchini* in Argentina.

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Compliance with ethical standards All Authors in this manuscript have read and approved the current version of the article.

Conflict of interest No conflict of interest exists in the submission of this manuscript.

References:

Ciuffo, M., et al. 2017. **Arch. Virol.** 162: 1419. doi:10.1007/s00705-017-3237-0

Giampan, J.S., et al. 2007. **Sci. Hort.** 114: 129.

Kumar, S., et al. 2016. **Mol. Biol. Evol.** 33: 1870.

Lommel, S.A., et al. 1982. **Phytopathology** 72: 1018.

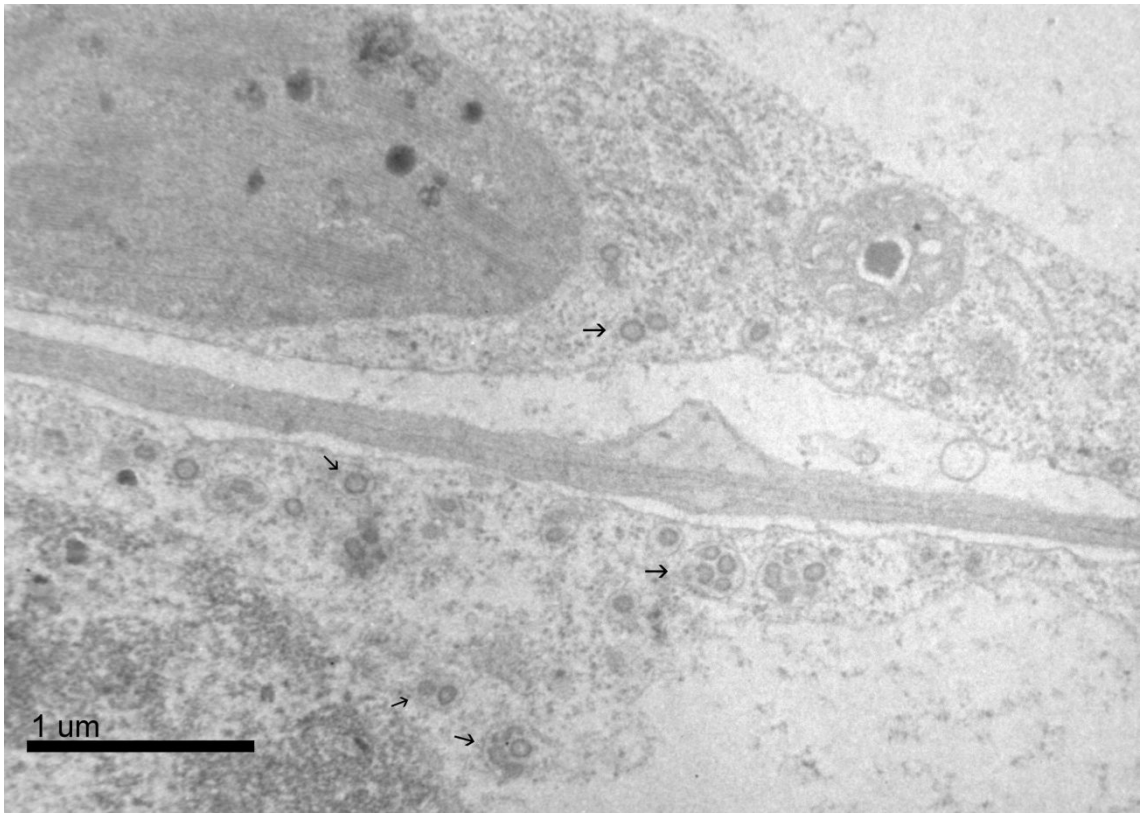
Nakahara, S., and Monteiro, R.C. 1999. **Proc. Entomol. Soc. Wash.** 101:290.

Pappu, H.R., et al. 2009. **Virus Res.** 141: 219. doi:10.1016/j.virusres.2009.01.009.

Riley, D.G., et al. 2011. **J. Integ. Pest Mngmt.** 1: I1; DOI: 10.1603/IPM10020.

Spadotti, D.M.A., et al. 2014. **New Dis. Rep.** 29:25.

Supplemental figure S1 (A) severe chlorosis of leaves and apical upward leaf curl due to ZLCV infection. (B) Malformed fruits.



Supplemental figure S2 Electron micrograph of ultrathin sections of squash leaves (*Cucurbita maxima*) infected by Zucchini lethal chlorosis virus (ZLCV). The arrows indicate presumable virions.