

1 First Report of Zucchini Lethal Chlorosis Virus in Argentina Infecting 2 Squash Crops

3 E A.Pozzi¹, C.E. Luciani^{1,2}, M.G. Celli¹, V.C.Conci^{1,2}, M.C.Perotto^{1,2†}

4 ¹Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CCT, Córdoba
5 X5020ICA, Argentina

6 ²Instituto de Patología Vegetal (IPAVE-CIAP-INTA), Camino 60 cuadras km 5,5, Córdoba
7 X5020ICA, Argentina.

8 Virus species of the genus *Orthospovirus* are among the most economically important plant
9 pathogens in the world because they cause severe crop losses; mainly, in ornamental and
10 horticultural crops (Pappu et al. 2009). They are exclusively transmitted by thrips. Several
11 species of *Orthospovirus* have been reported infecting cucurbits: *Watermelon silver mottle*
12 *virus*, *Zucchini lethal chlorosis virus* (ZLCV), *Watermelon bud necrosis virus*, *Melon yellow spot*
13 *virus*, *Melon severe mosaic virus* and *Groundnut ringspot virus* (Ciuffo et al. 2017, Spadotti et
14 al. 2014). The symptoms caused by ZLCV infection can include chlorosis and systemic necrosis
15 on leaves, apical upward leaf curl, reduction of leaf blade and fruit malformation (Fig. S1)
16 (Giampan et al. 2007). The collection of 90 symptomatic leaves of squash from Salta and Jujuy
17 provinces was carried out during early 2016. For an initial assessment of the presence of ZLCV
18 a plate trapped antibody enzyme-linked immunosorbent assay (PTA-ELISA) (Lommel et al.
19 1982) with antiserum against ZLCV, kindly provided by Jorge A. M. Rezende from the
20 Universidade de São Paulo, Brazil, was performed. Fifty four of the 90 samples reacted
21 positively to ZLCV-specific antiserum, being 18 and 11 positive plants of *Cucurbita maxima* var.
22 *zapallito redondo del tronco*, var. *zapallo plomo* respectively, 11 positive plants of *C. pepo*, 11
23 positive plants of *C. ficifolia*, var. "cayote" and 3 positive plants of *C. moschata*. Ultrathin
24 sections of leaf samples of naturally infected plants were examined by transmission electron
25 microscopy and presumable orthospovirus particles were observed (Fig. S2). To confirm the
26 identity of the virus, a one-step reverse transcription-polymerase chain reaction (RT-PCR)
27 assay was carried out on the RNA extracts from Squash plants, using ZLCV-specific primers
28 designed to direct amplification of nucleotides (ZLCV-F ATCATGCTGTCCAGTCTCCT and ZLCV-
29 RCCACATTTTGCATTGCGA), of the nucleocapsid gene region. The RT-PCR reaction for ZLCV
30 detection consisted of reverse transcription at 46°C for 30 min, followed by denaturation at
31 94°C for 3 min, and 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and
32 extension at 72°C for 45 s. Amplicons of the two ZLCV isolates MK680830 and MK680831 were
33 Sanger sequenced. The consensus sequences were aligned using clustalW aligned and
34 compared with other ZLCV sequences in the public domain using Mega 7 (Kumar et al. 2016).
35 Alignments of the N gene sequences of these ZLCV isolates displayed nucleotide sequence
36 identity above 94% with other ZLCV isolates available at the GenBank database. In addition,
37 the amino acid sequence demonstrated above 97% identity with equivalent regions of S
38 segment of Brazilian ZLCV isolate from *Cucumis sativus* and squash. The phylogenetic analysis
39 of the identified sequence of ZLCV and other related sequences from the GenBank, showed a
40 cluster of argentine isolates, close to ZLCV-DF isolate obtained from *Cucumis sativus*
41 (KU681011). This is the first report of ZLCV outside of Brazil. Although we have not observed
42 presence of *Frankliniella zucchini* in the field, which was identified and described as vector of

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

46 **Acknowledgments** This work was supported by PNPV 1135022.

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

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

50 **References:**

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

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

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

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

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

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

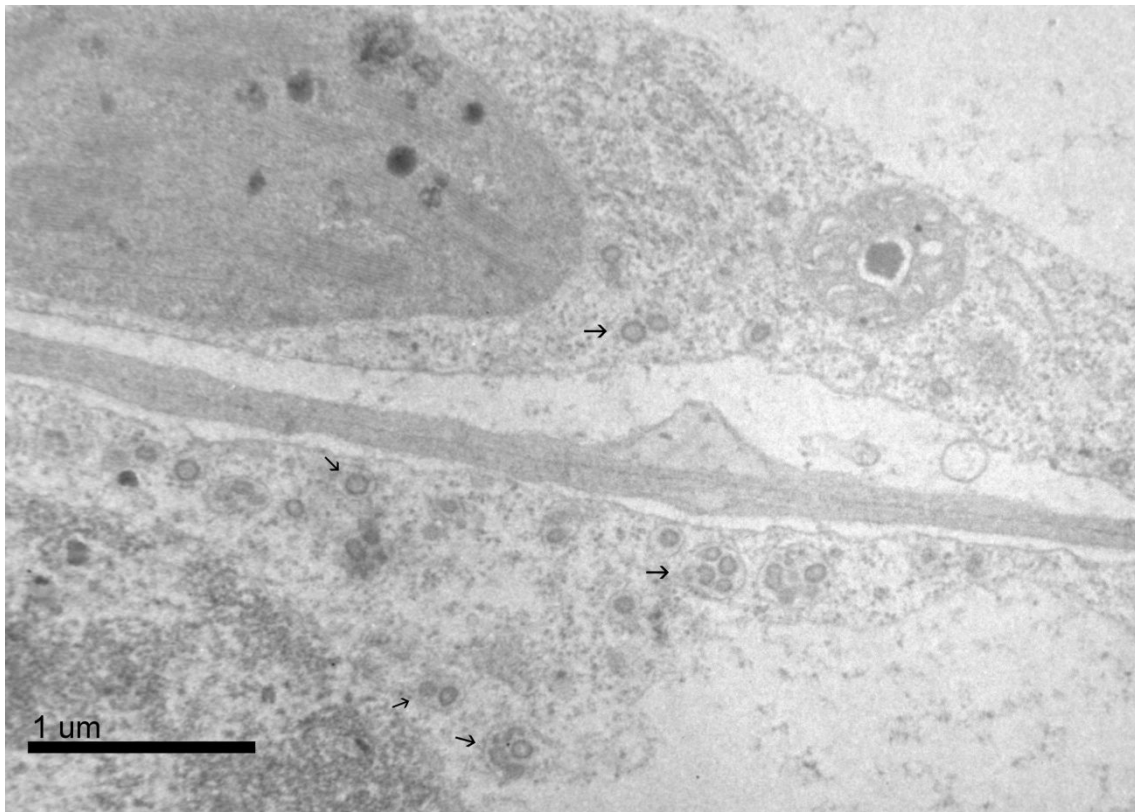
57 Riley, D.G., et al. 2011. **J. Integ. Pest Mngmt.** 1: 11; DOI: 10.1603/IPM10020.

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

59



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.