



Complete Genome Sequence of a Novel Recombinant *Citrus Tristeza Virus*, a Resistance-Breaking Isolate from Uruguay

María José Benítez-Galeano,^a Thomas Vallet,^b Lucía Carrau,^b Lester Hernández-Rodríguez,^c Ana Bertalmío,^c Fernando Rivas,^c Leticia Rubio,^c Diego Maeso,^c  Marco Vignuzzi,^b Gonzalo Moratorio,^b  Rodney Colina^a

^aLaboratory of Molecular Virology, University of the Republic, Salto, Uruguay

^bInstitut Pasteur, Viral Populations and Pathogenesis, Centre National de la Recherche Scientifique UMR 3569, Paris, France

^cNational Institute of Agricultural Research, Salto, Uruguay

ABSTRACT We report here the complete genome sequence of a *Citrus tristeza virus* (CTV) from Uruguay, sequenced by using Illumina and Sanger sequencing technology. This CTV DSST-17 genome clustered within genotype resistance breaking (RB) and presents two recombination events.

Citrus crops are among the most important commercial fruit crops worldwide. *Citrus tristeza virus* (CTV) (*Closterovirus*, *Closteroviridae*) is one of the most destructive pathogens that affects citrus trees around the world and has been responsible for the loss of over 100 million trees in the past 70 years (1). Depending on the viral strain and on the species or scion-rootstock combination, CTV may cause three distinct host reactions, named seedling yellows, quick decline, and stem pitting, of which the last two are significant problems for citrus cultivation (2).

The CTV genome, the largest plant virus reported so far, is a single-stranded positive-sense RNA molecule of approximately 19.3 kb in length, containing 12 open reading frames (ORFs) that encode at least 19 proteins (3). Genetic studies of different strains of CTV revealed the existence of seven distinct genetic lineages or genotypes worldwide, known as VT, T3, T30, T36, T68, resistance breaking (RB), and NC (4, 5). The RB genotype, described for the first time in New Zealand by Dawson and Mooney, is the only CTV-infecting genotype capable of overcoming the trifoliolate-rootstock resistance due to the ability of replication and systemic movement throughout *Poncirus trifoliata* (6). Last year, Hernández-Rodríguez and coworkers reported a New Hall sweet orange tree infected with the CTV-RB genotype in Uruguay, but only partial sequences were available (7).

In the present study, subisolate DSST-17, obtained by single aphid transmission from a field sample collected in 2014 in Salto, Uruguay, from a Navelina sweet orange, was subjected to Illumina sequencing technology. Total RNA was extracted using the RNeasy plant minikit (Qiagen) and submitted to RNA library preparation with a NEBNext Ultra II RNA library prep kit (Illumina). The library was sequenced using the NextSeq 500 system platform (Illumina). Reads were trimmed (quality limit 0.02; Phred score ≥ 30) and assembled with CLC Genomics Workbench version 11. After trimming, reads with an average length of 150 nucleotides (nt) were used to generate through *de novo* assembly a long contig of 19,269 nt. The complete genome obtained was compared with all available full genomes of the GenBank database using MEGA 6 (8). Phylogenetic analysis grouped the DSST-17 isolate within the RB genotype with a genome nucleotide identity ranging from 94.1% to 99.6%. Strikingly, the highest similarity was with isolate B390-5 (GenBank accession number KU883265) from South Africa, which has weather conditions similar to those of Uruguay. A recombination analysis using the Recombination Detection program version 4 and SimPlot program version 3.5.1 was performed

Received 19 April 2018 Accepted 24 April 2018 Published 31 May 2018

Citation Benítez-Galeano MJ, Vallet T, Carrau L, Hernández-Rodríguez L, Bertalmío A, Rivas F, Rubio L, Maeso D, Vignuzzi M, Moratorio G, Colina R. 2018. Complete genome sequence of a novel recombinant *Citrus tristeza virus*, a resistance-breaking isolate from Uruguay. *Genome Announc* 6:e00442-18. <https://doi.org/10.1128/genomeA.00442-18>.

Copyright © 2018 Benítez-Galeano et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Rodney Colina, rodneycolina1@gmail.com.

G.M. and R.C. contributed equally to this work.

(9, 10). The analysis revealed that isolate DSST-17 is a recombinant genome with at least two recombination events. For the first recombinant region, a T36-like fragment goes from positions 1 to 3616. The second event seems to be with an NC-like isolate (HA16-5) from base 14586 to the end of the genome. In addition, PCR amplification and Sanger sequencing were performed to confirm these recombination events. As far as we know, this is the first report from South America of a complete genome of CTV belonging to the RB genotype.

Accession number(s). The genomic sequence for isolate CTV DSST-17 was deposited in GenBank under accession number [MH186146](https://www.ncbi.nlm.nih.gov/nuclseq/MH186146).

ACKNOWLEDGMENTS

We thank Stephanie Beaucourt for invaluable technical advice.

This work was funded by the National Institute of Agricultural Research from Uruguay (INIA-FPTA-310). M.J.B.-G. received a travel scholarship from the University of the Republic, Uruguay (PEDECIBA and CSIC), for an internship at the Institut Pasteur and is the recipient of a PhD scholarship from the Academic Postgraduate Commission, University of the Republic, Uruguay.

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

1. Roistacher CN, da Graça JV, Müller GW. 2010. Cross protection against Citrus tristeza virus—a review, p 1–27. *In* Brlansky RH, Lee RF, Timmer LW, (ed), Proceedings of the 17th Conference of the International Organization of Citrus Virologists. IOCV, Riverside, CA.
2. Moreno P, Ambrós S, Albiach-Martí M, Guerri J, Peña L. 2008. *Citrus tristeza virus*: a pathogen that changed the course of the citrus industry. *Mol Plant Pathol* 9:251–268. <https://doi.org/10.1111/j.1364-3703.2007.00455.x>.
3. Karasev AV, Boyko VP, Gowda S, Nikolaeva OV, Hilf ME, Koonin EV, Niblett CL, Cline K, Gumpf DJ, Lee RF, Garnsey SM, Lewandowski DJ, Dawson WO. 1995. Complete sequence of the Citrus tristeza virus RNA genome. *Virology* 208:511–520. <https://doi.org/10.1006/viro.1995.1182>.
4. Harper SJ. 2013. *Citrus tristeza virus*: evolution of complex and varied genotypic groups. *Front Microbiol* 4:1–18. <https://doi.org/10.3389/fmicb.2013.00093>.
5. Benítez-Galeano MJ, Rubio L, Bertalmío A, Maeso D, Rivas F, Colina R. 2015. Phylogenetic studies of the three RNA silencing suppressor genes of South American CTV isolates reveal the circulation of a novel genetic lineage. *Viruses* 7:4152–4168. <https://doi.org/10.3390/v7072814>.
6. Dawson TE, Mooney PA. 2000. Evidence for trifoliolate resistance breaking isolates of Citrus tristeza virus in New Zealand, p 69–76. *In* Yokomi RK, Lee RF, Da Graça JV (ed), Proceedings of the 14th Conference of the International Organization of Citrus Virologists. IOCV, Riverside, CA.
7. Hernández-Rodríguez L, Bertalmío A, Arruabarrena A, Rubio L, Rivas F, Benítez-Galeano MJ, Colina R, Maeso D. 2017. First report of the *Citrus tristeza virus trifoliolate* resistance-breaking (RB) genotype in 'Newhall' sweet orange in South America. *Plant Dis* 101:1063. <https://doi.org/10.1094/PDIS-04-16-0430-PDN>.
8. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>.
9. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. 2015. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol* 1:vev003. <https://doi.org/10.1093/ve/vev003>.
10. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC. 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 73:152–160.