First Report of Tomato Fruit Blotch Virus Infecting Tomatoes in Brazil

Erich Y. T. Nakasu,¹ Tatsuya Nagata,² and Alice K. Inoue-Nagata^{1,†}

¹ Embrapa Vegetables, Brasília, DF – Brazil

² Department of Molecular Biology, University of Brasília, Brasília, DF – Brazil

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Recently, a new blunervirus, tomato fruit blotch virus (ToFBV), was reported in tomatoes with fruit chlorotic lesions. It was found associated with tomato fruit blotch disease in Italy and Australia, even though Koch's postulates were not fulfilled and no viral particles were seen in leaf dips under an electron microscope (Ciuffo et al. 2020). In December 2019, symptoms of circular or irregular chlorotic blotches were observed in tomato fruits in an organic farm in Distrito Federal, Brazil. Five cultivars (2,100 plants of Sweet Grape, 1,700 of Giacomo, 560 of Grazianni, 160 of Tropical, and 160 of DRC 5640) were grown in two greenhouses, and all presented symptoms in at least one fruit, particularly in older fruits. No virus-like symptoms were observed in young or middle leaves; old leaves were not examined because they were removed as a routine farming practice and also because of a moderate infestation of the tomato russet mite *Aculops lycopersici*, which is associated with leaf and stem necrosis. No viral particles were observed in an electron microscope analysis of symptomatic fruit tissues. Sap inoculation and stem grafting produced no symptoms in indicator plants. Two young asymptomatic plants with the first fruits in development were moved from another greenhouse of the farm to our greenhouse, but neither typical blotch symptoms in the fruits nor necrosis symptoms in the leaves appeared. Serological tests of all leaf and fruit samples using antibodies produced in-house against common tomato-infecting tospoviruses and potyviruses were negative, as was a PCR detection test for begomoviruses (Rojas et al. 1993). Total RNA from a newly collected symptomatic fruit and five asymptomatic leaves from distinct plants were individually extracted using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and pooled for next generation sequencing. A library was constructed using TruSeq Stranded Total RNA with Ribo-Zero

Plant (Illumina, San Diego, U.S.A.) and sequenced at Macrogen (Seoul, South Korea) in an Illumina Novaseq6000 platform. The 4,621,977,958 reads obtained were trimmed using Trimomatic 0.35 (Bolger et al. 2014), and contigs were assembled using Velvet (Zerbino and Birney 2008). After tblastx analysis on Geneious 9.1.8 (Biomatters Ltd.) and BLAST on NCBI (Altschul et al. 1990), seven contigs matching tomato chlorosis virus (ToCV) and five matching ToFBV were identified. Sequences of the four ToFBV genome components (MK517477 to MK517480) in databases were used as references using the map to reference function in Geneious. A total of 338,402, 78,039, 555,302, and 461,474 reads were mapped to genome components one to four, respectively, each with >99% coverage. Four final consensus sequences in NCBI BLAST analyses presented 97 to 99.7% nucleotide identity with those used for mapping. The sequences were deposited in GenBank as isolate MAL (nos. MW546267 [RNA 1, 5,770 nt], MW546268 [RNA 2, 3,612 nt], MW546269 [RNA 3, 2,826 nt], MW546270 [RNA 4, 1,950 nt]). Primer pair Bluner1F (5'-ATTCTGTTCCTTCGGATAAACTCGT-3') and Bluner1R (5'-CACACGTGCAGGAAATGGAAAGA-3') directed to RNA 1 was used to specifically detect the virus. Three leaf and two fruit samples from different plants with typical symptoms tested positive for ToFBV and negative using ToCV-specific primers in RT-PCR (Dovas et al. 2002). This confirmed that while some plants pooled in the HTS library were infected with ToCV, chlorotic blotch was clearly associated with the presence of ToFBV. Also, a ~0.5-kbp amplicon for ToFBV-specific primers from a random sample was sequenced with both primers; it shared 100% nt identity with the RNA 1 of ToFBV isolate Fondi2018 from Italy (MK517477). Then, the virus was detected in surface, but not in internal tissue of another fruit, suggesting a superficial infection. These findings are of high phytosanitary significance, given the strong symptoms associated with tomato fruit blotch disease and the identification of ToFBV in tomato from Brazil.

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The author(s) declare no conflict of interest.

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[†]Indicates the corresponding author.

A. Inoue-Nagata; alice.nagata@embrapa.br