Received: 21 January 2022 | Accepted: 16 February 2022

DOI: 10.1002/ndr2.12065

New Disease Reports Open Access BSPP WILEY

NEW DISEASE REPORT

First report of *Tomato brown rugose fruit virus* in tomato in Switzerland

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KEYWORDS
greenhouse disinfection, Illumina sequencing, mechanical inoculation

In Switzerland, greenhouses for tomato (*Solanum lycopersicum*) production are inspected systematically for *Tomato brown rugose fruit virus* (ToBRFV) in accordance with phytosanitary regulations, by sampling symptomatic plants during routine visits. In July 2021, a suspected viral disease was reported from an 8-hectare greenhouse in the canton of Thurgau in eastern Switzerland, on a single plant in a soil-grown tomato crop (cv. Dubino). This plant exhibited light discoloured spots which were unusual for this variety and were rather reminiscent of the symptoms associated with *Pepino mosaic virus* (PepMV) infection. Transmission electron microscopy observation of leaf-dip preparations showed a mixture of filamentous, potexvirus-like particles likely corresponding to a mild PepMV isolate used for cross-protection a few months earlier, as well as particles with rod-shaped, tobamovirus-like structures (Figure 1).

RT-qPCR analyses using the ToBRFV-specific primer sets CaTa28 and CSP1325 (International Seed Federation, 2020) gave strong positive results with Cq values of 8.4 and 10.8, respectively. The presence of the virus was further confirmed by conventional RT-PCR using the ToBRFV-F/-R primers from Alkowni et al. (2019). Blastn analysis of the amplicon sequence revealed 100% identity with a number of ToBRFV sequences including isolate Tom1-Jo from Jordan (GenBank Accession No. NC_028478.1). Total dsRNA was extracted from infected plant material according to Mahillon et al. (2021) and was subsequently sent for high throughput sequencing on a NovaSeq 6000 platform.

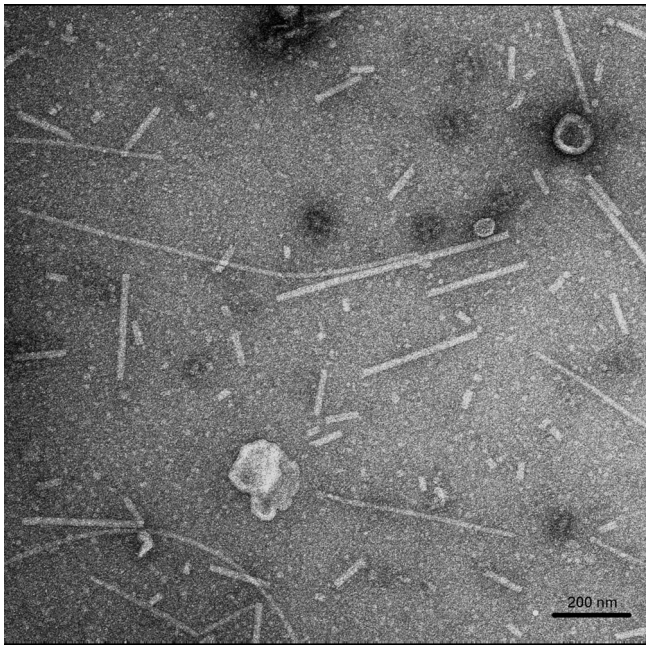

FIGURE 1 Flexuous (potexvirus-like) and rod-shaped (tobamovirus-like) particles observed in leaf-dip preparations by transmission electron microscopy

FIGURE 2 Local necrotic lesions on leaf of *N. tabacum* cv. Xanthi following sap inoculation 10 dpi.

A complete ToBRFV genome (6386 nt; OM305070) was assembled from the obtained reads and shared 99.8% nt identity with ToBRFV-IL (KX619418.1). A complete PepMV genome of 6412 nt was also assembled and shared 99.9% nt identity with PepMV-P12-3G (MK133092.1). No other plant virus was identified. In parallel, sap inoculation of *Nicotiana tabacum* cv. Xanthi induced typical necrotic local lesions visible seven dpi (Figure 2). This is the first report of ToBRFV in Switzerland.

RT-qPCR analysis using the CaTa28 and CSP1325 primer sets of 1670 leaf samples from asymptomatic tomato plants sampled between 12 and 21 July 2021 at their apex (analysed in pools of 10 plants) revealed the presence of ToBRFV in all areas of the greenhouse. Furthermore, four root samples taken from the rows adjacent to the initial symptomatic plant were also positive. After removal of the plants, cleaning of the infrastructure and steam disinfection of the soil, an initial surface analysis using 245 cotton swabs showed high virus titre in samples collected on the fruit packing machines, scissor lifts and overhead heaters. Therefore, an additional cleaning was performed, followed by disinfection with commercial benzoic acid (MENNO Florades) before a second surface analysis. A total of 2% of the soil samples remained weakly positive by RT-qPCR analysis. Consequently, the 2022 production will switch entirely to hydroponics in order to reduce the risk of the outbreak restarting from infected plant debris in the soil. The production site will be monitored for the next two seasons to ensure the effectiveness of the eradication measures implemented.

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How to cite this article: Mahillon, M., Kellenberger, I., Dubuis, N., Brodard, J., Bunter, M., Weibel, J., Sandrini, F., & Schumpp, O. (2022) First report of *Tomato brown rugose fruit virus* in tomato in Switzerland. *New Disease Reports*, 45, e12065. https://doi.org/10.1002/ndr2.12065