New Disease Reports

First report of *Southern tomato virus* in German tomatoes

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Southern tomato virus (STV) is a member of the genus *Amalgavirus* (family: *Amalgaviridae*). It has been identified in tomatoes (*Solanum lycopersicum*) in several countries in Asia, Europe, and North and South America (Sabanadzovic *et al.*, 2009; Candresse *et al.*, 2013; Padmanabhan *et al.*, 2015). Its genome is composed of a dsRNA of ~3.5kb. STV is known to be transmitted through seed at high rates (Sabanadzovic *et al.*, 2009).

In 2019, greenhouse tomatoes from Lower Saxony, Germany, showed symptoms consisting of mottling, yellowing and/or chlorotic spots (Figs. 1-2). Eight samples were sent to the Julius Kuehn Institute for analysis. As an infection with *Tomato brown fruit rugose virus* was suspected, the samples were analysed by high throughput sequencing on a MinION sequencer (Oxford Nanopore Technologies, UK) for rapid diagnosis. Briefly, two samples were pooled, and dsRNA extracted from 100 mg leaf material using the Viral dsRNA Extraction Mini Kit for Plant Tissue (iNtRON, South Korea). Random cDNA was synthesised using ProtoScript II Reverse Transcriptase (NEB, USA) and 8N random primers preceded by a denaturation step at 99°C for two minutes. Second strand synthesis was done using a NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module kit (NEB). The samples were end-repaired using the NEBNext End Repair module (NEB), dA tailed with NEBNext dA-tailing module (NEB) and the four pools barcoded by native barcoding followed by adaptor ligation (Oxford Nanopore Technologies, UK) according to the manufacturers' instructions. All purification steps were performed using a Mag-Bind TotalPure NGS kit (Omega Bio-Tek, USA). The libraries were mixed and loaded to a MinION flow cell and sequenced for 16 hours using a MinION sequencer connected to a computer with MinION software (r18.12.9; ONT).

The reads were basecalled and barcode-splitting was done using the Guppy toolkit (v2.3.7; ONT). *De novo* assembly of reads was done using Canu (v1.8) (Koren *et al.*, 2017). The unassembled reads and assembled contigs were Blastn searched against a local GenBank nt database using Blast+ (v2.9.0) and visualised with Blast Viewer (v5.2.0) (Durand *et al.*, 1997; Camacho *et al.*, 2009). STV sequences were detected in two of the pools, and no other virus sequences were detected. The full genomes were assembled by mapping to STV reference (Genbank Accession no. NC_011591) using mapping to reference tool on Geneious Prime (v2019.1.3). The sequences had 99.9% nt identity to each other and to STV isolate CH_bpo 163 from Switzerland (MF422618).

To confirm the findings in the original samples, total RNA was extracted for the four samples in the positive pools using an innuPREP RNA MiniKit (Analytik Jena AG, Jena, Germany), and RT-PCR was performed using a primer pair (HZ782 5'-CAAGTGGGCCGTTTCTTTGG-3' and HZ783 5'-TGAAGACCGCCTGGAAAGTC-3'). STV infection was confirmed in three samples. The RT-PCR products were purified, and Sanger sequenced at Eurofins Genomics (Germany). The sequences had 100% identity to the sequences from the MinION. The genomes of the two pools were submitted to Genbank (MK948544 and MK948545). To our knowledge, this is the first report of STV infecting tomato in Germany. The study also shows the potential to use MinIon technology for rapid detection and identification of virus sequences.

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Figure 1

Figure 2

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