New Disease Reports

First report of natural infection of beetroot with *Beet soil-borne virus*

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Beetroot (*Beta vulgaris* subsp. *vulgaris*) is becoming increasingly popular in Germany with an increase in field production from 1,205 ha in 2013 to 1,826 ha in 2018 (Behr, 2019). It is estimated that EU-wide 24,000 ha of beetroot were produced in 2018 (Behr, 2019). In contrast, sugarbeet was produced on a substantially larger scale with 413,900 ha in Germany in 2018 (Kemper *et al*., 2019).

A symptomatic beetroot sample was collected in October 2018 from a small field (c. 200 m^2) in Rhineland-Palatinate, Germany, where several plants displayed virus-like symptoms. The sample submitted displayed necrosis, reduced size and in particular root proliferation (bearding) resembling the symptoms of rhizomania (Fig. 1). However, immunosorbent electron microscopy (ISEM) examination using various antibodies raised against the following beet viruses was not successful in identifying any causal agent: *Beet black scorch virus*, *Beet curly top virus*, *Beet necrotic yellow vein virus*, *Beet mosaic virus*, *Beet oak-leaf virus*, *Beet soil-borne virus* (BSBV), *Beet soil-borne mosaic virus*, *Beet virus Q*, *Beet western yellows virus*, *Beet yellows virus* and *Tobacco rattle virus*.

Total RNA was extracted from the infected beetroot sample using innuPREP RNA Mini Kit (Analytik Jena AG, Germany). Ribosomal RNA was depleted using RiboMinus Plant Kit for RNA-Seq (ThermoFisher Scientific, USA). A library was prepared using TrueSeq Stranded mRNA kit (Illumina, USA). The sequencing was done on a NextSeq 500 platform (2×150). The generated data was analysed on Geneious Prime (2019.1.1). The raw reads were quality-trimmed then *de novo* assembled using SPAdes assembler (3.10.1) (Bankevich *et al*., 2012). The contigs were compared against a local virus reference database using tBlastx. Twenty-one contigs showed only similarities to BSBV and *Beet cryptic virus-2* (BCV-2), respectively; no other virus sequences were found in the data.

BSBV is a member of the *Pomovirus* genus (family *Virgaviridae*) (Adams *et al*., 2017). The virus is widely distributed in sugar beet growing areas causing yield losses. BCV-2, a member of the *Deltapartitivirus* genus (family *Partitiviridae*), is a symptomless virus that is also common in sugar beet (Antoniw *et al*., 1990; Vainio *et al*., 2018). For confirmation of BSBV infection, total RNA was re-extracted from the infected beetroot sample and RT-PCR was performed using two specific primer pairs targeting the RNA-dependent RNA polymerase and movement protein regions of BSBV, respectively (HZ772 5'-GTTGGTGTTGGTCAGTTGGC-3'/HZ773 5'-TGGTCAACGGCGAAATCAGA-3' and HZ774 5'-GAGGGGTAAGACACAGCGAC-3'/HZ775 5'-CACTTCGTCCTCCTGGTCAC-3'). Two bands of the expected sizes (923 and 766 bp, respectively) were produced.

The almost complete genomes of BSBV and BCV-2 were assembled by Geneious mapping using reference sequences (BSBV: Genbank Accession Nos NC_003518-NC_003520 and BCV-2: NC_038845-NC_038847). The sequences of the beetroot BSBV and BCV-2 isolates were submitted to Genbank (MK731954-MK731959). Sequence analysis revealed that the BSBV isolate shares 97.3-98.5% nucleotide identity with the reference genome (German isolate NC_003518-NC_003520). The BCV-2 isolate shares 98.7-99.7% nt nucleotide identity with the reference genome (Hungarian isolate NC_038845-NC_038847).

This work provides the first suggestion that BSBV naturally infects beetroot. This identification exposes the limit of diagnostic methods such as ISEM, possibly due to low titre or the existence of a divergent virus isolate, and also highlights the strength of high throughput sequencing to rapidly and accurately diagnose plant viruses. Furthermore, these findings demonstrate that high value crops such as beetroot might be affected by pathogens of major crops and therefore should be considered in crop rotations.

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Figure 1

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