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

Y. Gaafar 1, A. Sieg-Müller 1, P. Lüddecke 1, J. Hartrick 1, Y. Seide 1, Jürgen Müller 1, C. Maäë 1, S. Schuhmann 1, K.R. Richard-Pöggeler 1, A.G. Blouin 2, S. Massart 1 and H. Ziebell 1*

1 Julius Kuehn Institute, Institute for Epidemiology and Pathogen Diagnostics, Messesweg 11-12, 38104 Braunschweig, Germany; 2 DLR Rheinpfalz, Institute of Plant Protection, Breitenweg 71, 67435 Neustadt an der Weinstraße, Germany

Integrated and Urban Plant Pathology Laboratory Gembloux Agro-Bio Tech, University of Liège, Avenue Maréchal Juin 27, 5030 Gembloux, Belgium

E-mail: heiko.ziebell@julius-kuehn.de

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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, sugar beet 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 m²) in Rhineland-Palatinate, Germany, where several plants displayed virus-like symptoms. The sample submitted displayed necrosis, twisted 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 minuPREP 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 Pomonivirus genus (family Virgaviiridae) (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 and HZ774) and (HZ775 and HZ776) respectively. 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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References


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