First Report of Blackcurrant-Associated Rhabdovirus in Blackcurrants in Latvia

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Blackcurrants (*Ribes nigrum*) are among the most important commercial berry crops in Latvia and, together with redcurrants and gooseberries, have a long history of local cultivation and breeding (Zulge et al. 2018). So far, at least 20 viruses were reported to infect *Ribes* plants (Spak et al. 2021). Blackcurrant-associated rhabdovirus (BCaRV) was previously identified in the U.S.A. by high throughput sequencing (HTS) of blackcurrant germplasm accession introduced from Russia (isolate Veloy) and now serves as an exemplar isolate for BCaRV (Wu et al. 2018). Presence of BCaRV was also confirmed by RT-PCR in blackcurrant germplasm accession of cv. Burga from France during the same study by Wu et al. (2018). Currently, *Blackcurrant betanucleorhabdovirus* is one of the nine species recognized by ICTV in genus *Betanucleorhabdovirus* of family *Rhabdoviridae*, but the impact of BCaRV on the host still remains unknown. Leaf tissue from 12 asymptomatic blackcurrant cv. Mara Eglite plants that negatively tested for blackcurrant reversion virus from Dobeles, Latvia (56°36′31.9″N, 23°18′13.6″E) was collected on 17 May 2019 and used for HTS study of local *Ribes* resistance genes. Total RNA from the leaf tissue of sampled plants was isolated

following a method described by Kalinowska et al. (2012) with minor modifications. Briefly, RNeasy Plant Mini Kit (QIAGEN) was used with RLC lysis buffer being supplemented with 2% PVP and 1% β-mercaptoethanol. Plant rRNA was subsequently removed by a RiboMinus Plant Kit for RNA-Seq (Thermo Fisher Scientific [TFS]) prior to cDNA library construction. HTS libraries were prepared using MGIEasy RNA Directional Library Prep Set for 16 reactions (MGI), following a protocol for 150 bp pair-end reads. According to the manufacturer’s guidelines, libraries were pooled, circularized, and cleaned before being subjected to sequencing on DNBSEQ-G400 (MGI) using PE150 flow cell (MGI). The sequencing run yielded a total of 393,660,492 150-bp long read pairs. Reads were assembled into transcripts using rnaSPAdes v 3.13.1 (Bushmanova et al. 2019) and a 14,424-base long contig with an average coverage of 684× was found to be 99.5% identical (14,358/14,432 identities and 8 gaps in the pairwise alignment) to the previously reported first complete genome of BCaRV (MF543022.1) using EMBL Needle (Madeira et al. 2019). This contig representing the genome of BCaRV isolate Mara Eglite, onto which 66,768 of the raw reads could be mapped, was subsequently deposited at European Nucleotide Archive under accession number OU015520. All 12 individual samples were also tested for the presence of BCaRV by RT-PCR, using Verso cDNA Synthesis Kit with random hexamer primers (TFS) for first strand cDNA synthesis followed by PCR with N protein nested primers BCaRV-N-F (5′-AGATGTGCTTCATC GATGGCTAGTTCTGCT-3′) and BCaRV-N-R (5′-TGCATCCCCACGGG TTAGGAATACATTGGTACT-3′), resulting in a 243-bp long fragment for six of the samples. RT-PCR products from six BCaRV-positive samples were directly sequenced by Sanger-based method using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with BCaRV-N-F and BCaRV-N-R primers. Acquired RT-PCR product sequences matched the corresponding region of BCaRV isolate Mara Eglite genome assembled from HTS data. In this report, we have documented the natural occurrence of BCaRV in Latvia, which makes it the second evidence of the presence of BCaRV in Europe.

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