First report of black raspberry necrosis virus and raspberry leaf mottle virus in Bosnia and Herzegovina

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In May 2018, six raspberry (*Rubus idaeus* L.) plants exhibiting interveinal and net-like leaf chlorosis were collected from commercial orchard in Mrkonjić Grad, Bosnia and Herzegovina (BiH). The RNA extracts of all plants were pooled in a single composite sample and analyzed by a high throughput sequencing (HTS) on Illumina NextSeq 500 platform (Liege University, Belgium). A total of 3,683,482 paired reads were obtained. One of the five contigs represented 7563 bp (accession No. MT156348) was homologous to black raspberry necrosis virus (BRNV) RNA1 (FN908128) showing 91.5% nucleotide identity, while one of four contigs represented 6343 bp (MT156349) has highest identity of 84% to BRNV RNA2 (NC008183). In addition, only one hit with the RLMV reference (RLMV, NC008585) with 92.4% nucleotide identity. A consensus sequence of 17,499 nt (MT156350) was obtained by aligning 1185 reads on the RLMV reference sequences. The presence of both viruses was confirmed in composite sample by RT-PCR using primers BRNV2F (TCAAAGCGCACTGAACCAA) and BRNV2R (CGAGCTCTAACATCCAGGAA

GC) for BRNV RNA2 (Halgren et al. 2007) and CPh F/CPh R (Tzanetakis et al. 2007) for RLMV. BRNV primers were designed in the conserved regions of BRNV RNA2 sequences (DQ344640 and FN908129) resulting in the amplification of 563 bp amplicon including majority of 5'-non-coding region and part of MP gene. The obtained partial sequences of BRNV (MN028391) and RLMV (MN028392) were compared to the sequences in the GenBank database using BLASTN analysis and shared for BRNV 81 to 85% nt identity with other isolates from Finland, Scotland and USA while the sequence of RLMV shared highest nucleotide identity of 88% with RLMV isolates from Scotland. To our knowledge, this is the first report of BRNV and RLMV infecting raspberry in BiH and as an important component of raspberry mosaic disease, it poses a new concern to the raspberry industry in BiH.

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