First Report of Soybean Dwarf Virus Infecting White Clover (*Trifolium repens*) in Finland

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Soybean dwarf virus (SbDV, genus *Luteovirus*) is a single-stranded positive-sense RNA virus able to infect several legume species. SbDV was first reported in Japan, where it was associated with significant yield losses in soybean (Tamada 1969). Since then, the virus has been detected worldwide. In Europe, the virus has only been reported from Germany (Abraham et al. 2007; Gaafar et al. 2020). In July 2018, several white clover plants (*Trifolium repens* L.) with leaf discoloration were observed in different locations in Oulu region in northern Finland. Individual plants were collected and analyzed for the presence of viruses using small-RNA (sRNA) sequencing (Kreuze et al. 2009) and reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted using an E.Z.N.A. micro RNA kit (Omega Bio-Tek, U.S.A.). For sRNA analysis, sequencing libraries were constructed using the TruSeq small RNA library prep kit (Illumina, U.S.A.) and sequenced on an Illumina MiSeq platform. On average, 1.3 million single-end reads were obtained per sample, of which 27% were 18 to 25 nt long and used for the subsequent analysis. Contig assembly and virus identification with VirusDetect software (Zheng et al. 2010) detected SbDV in five out of six white clover samples analyzed. Depending on the sample, 26 to 39 contigs (with lengths up to 301 to 469 nt) aligned to the complete genome of a SbDV isolate previously described from white clover in United States (accession no. JN674402). The cumulative alignment coverage ranged from 35.5 to 65.3%, with nucleotide identities between 94.4 and 97.3%. Additionally, two samples seemed to contain an unidentified closterovirus and one contained

white clover cryptic virus 2. No additional viruses were detected from two of the samples. To confirm the presence of SbDV, the samples were tested by RT-PCR using primers MDF, MYF, and MUR in multiplex (Schneider et al. 2011) together with the SuperScript III One-Step RT-PCR System with the Platinum Taq DNA polymerase kit (Thermo Fisher Scientific, U.S.A.), essentially as instructed by the manufacturer. A RT-PCR product of approximately 400 bp was produced from each of the five samples that previously tested SbDV positive by sRNA analysis. No products were produced from the sample that was SbDV negative in the sRNA analysis. Direct sequencing of two of the PCR products produced 347- and 361-bp sequences (GenBank MZ355392 and MW929169) that were 95.7 and 95.2% identical, respectively, to a SbDV isolate (accession no. AB038148) that causes yellowing on soybean and is transmitted by *Acyrtosiphon pisum* (Terauchi et al. 2003). To our knowledge, this is the first report of SbDV in Finland. SbDV is transmitted only by aphids (neither mechanical nor seed transmission occurs). In siRNA analysis all the isolates from Finland formed contigs that aligned almost perfectly (100% coverage with ≥99% nucleotide identity) to a coat protein (accession no. EF466131) of an SbDV isolate transmittable from white clover to faba bean by *A. pisum* (Abraham et al. 2007), an aphid common in Finland. Although significant yield losses by SbDV have only been reported on soybean (Tamada 1969), the virus also causes symptoms in other legume crops, such as growth reduction on pea (Tian et al. 2017) and faba bean (Abraham et al. 2007), both of which are cultivated in Finland.

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