

First Report of Beet Yellows Virus Causing Virus Yellows in Sugar Beet in Serbia

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Several viruses have been described to infect sugar beet (*Beta vulgaris* var. *saccharifera* L.), but virus yellows disease is one of the most important diseases in many sugar beet growing areas. It is caused by four viruses in either single or mixed infection, including the poleroviruses beet western yellows virus (BWYV), beet mild yellowing virus (BMYV), and beet chlorosis virus (BChV) and a closterovirus, beet yellows virus (BYV) (Hossain et al. 2021; Stevens et al. 2005). In August 2019, five samples of sugar beet plants showing yellowing on interveinal leaf tissue were collected in a sugar beet crop in the Novi Sad locality (Vojvodina Province, Serbia). Double-antibody sandwich (DAS) ELISA commercial antisera (DSMZ, Braunschweig, Germany) were used to test the collected samples for the presence of the most common sugar beet viruses: beet necrotic yellow vein virus (BNYVV), BWYV, BMYV, BChV, and BYV. Commercial positive and negative controls were included in each ELISA test. BYV was serologically detected in all sugar beet samples, but no other viruses tested were found. The presence of BYV in sugar beet plants was further confirmed by conventional reverse-transcription (RT) PCR. Total RNAs were extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and used as a template in the RT-PCR. Total RNAs extracted from healthy sugar beet leaves and molecular-grade water

were included as negative controls in the RT-PCR analysis. RT-PCR confirmed the presence of BYV in all naturally infected plants using four sets of specific primers (Kundu and Ryšánek 2004), whereas no amplification products were obtained in the negative controls. The RT-PCR products derived from the isolate 209-19 were purified and directly sequenced in both directions using the same primer pairs as in RT-PCR (accession nos. OQ686792 to OQ686794). Multiple sequence alignment of the L-Pro and N-terminal part of the MET genes showed that the Serbian BYV isolate had the highest nucleotide identity (99.01 and 100%, respectively) with several BYV isolates in GenBank originating from different parts of the world. Sequence analysis of the HSP70 gene showed the highest similarity (99.79%) with the BYV-Cro-L isolate found in Croatia. In a semipersistent type of transmission test, aphids (*Myzus persicae* Sulzer) were allowed to feed on BYV-infected leaves of an ELISA-positive sample (209-19) for 48 h, and then the aphids were transferred to five plants each of *Spinacia oleracea* cv. Matador and *B. vulgaris* ssp. *vulgaris* cv. Eduarda for a 3-day inoculation access period. All test plants were successfully infected and exhibited symptoms in the form of interveinal yellowing up to 3 weeks postinoculation. RT-PCR confirmed the presence of BYV in all inoculated plants. A study by Nikolić (1951) suggested a possible presence of BYV based on symptoms observed on sugar beet plants in fields, but to our knowledge, this is the first report of BYV in sugar beet in Serbia. As sugar beet is one of the most important industrial crops in Serbia, the presence of BYV could lead to significant losses, considering that aphid vectors are widespread under Serbian environmental conditions. The discovery of BYV on sugar beet should prompt a more detailed survey and subsequent testing of susceptible hosts to determine the distribution and incidence of BYV in Serbia.

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