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DISEASE NOTES

# First Report of *Wheat spindle streak mosaic virus* on Wheat in Croatia

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*Wheat spindle streak mosaic* (WSSM; genus *Bymovirus*, family *Potyviridae*), transmitted by plasmodiophorid *Polymyxa graminis* Led., is one of the most important wheat viruses that cause significant yield losses (Deb and Anderson 2008). In March 2013, irregularly distributed, light green to yellow, circular patches in a wheat crop characteristic of soilborne viruses were observed in one local winter wheat (*Triticum aestivum* L.) cultivar Super Žitarka in the Karanac locality (Baranja Country, Croatia). Initial symptoms included light green to yellow dashes, mottling, and chlorotic, spindle-shaped streaks with green center on the leaves, which became yellow and eventually necrotic. Affected plants were stunted, with dark brown, slightly swollen, and enlarged roots. The long period of wet and cool spring weather most likely favored disease development and severe symptoms were visible by the end of vegetation with estimated incidence of 40%. The roots of infected plants were stained in lactophenol cotton blue and cystosori of *P. graminis* were observed in all assayed wheat roots by light microscope. Because of that, soil samples were collected from this locality and used in bait plant test. Wheat bait plants showed chlorosis and mild mosaic 6 weeks after sowing, and cystosori were detected in their roots. Using double antibody sandwich (DAS)-ELISA test


(Loewe Biochemica, Sauerlach, Germany), WSSM was detected serologically in all 15 collected wheat samples as well as in five wheat bait plants. For further confirmation, total RNA was extracted from leaves of all naturally infected and wheat bait plants using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and served as a template in reverse transcription (RT)-PCR. RT-PCR was carried out with One-Step RT-PCR Kit (Qiagen) using degenerate primers, WMVCPF and WMVCPR (Clover and Henry 1999), yielding an 879- to 882-bp fragment corresponding to the coat protein (CP) gene of both WSSMV and *Wheat yellow mosaic virus* (WYMV). Total RNAs extracted from healthy wheat leaves as well as RNase-free water were included as negative controls in RT-PCR analysis. Amplicons of the expected size were obtained from all 15 naturally infected and five bait wheat plants, while no amplification products were observed in the healthy controls. After the purification with QIAquick PCR Purification Kit (Qiagen), the RT-PCR product obtained from one selected isolate 361-13 was sequenced directly in both directions using the same primer pair as in RT-PCR (GenBank Accession No. KP257576). Pairwise comparison of the 361-13 isolate CP sequence with other homologous sequences available in GenBank, conducted using MEGA5 software (Tamura et al. 2011), revealed that wheat isolate from Croatia showed the highest nucleotide identity of 99.2% (100% amino acid identity) with the WSSMV isolate (AJ237926) originating from the United States. To our knowledge, this is the first report of WSSMV occurrence on wheat in Croatia. Wheat is the most important field crop in Croatia and the presence of this harmful virus could represent a major threat to its production. Further investigation toward establishing distribution of WSSMV and *P. graminis* as its vector will be conducted, followed by testing the resistance of local cultivars.

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