Disease Notes

First Report of *Tomato spotted wilt virus* on Pepper in Montenegro

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Abstract

In April 2009, chlorotic and necrotic ring spots, chlorotic line patterns, and stunting were observed on greenhouse-grown pepper plants in the vicinity of Podgorica, Montenegro. Disease symptom incidence was estimated at 40%. Symptomatic leaves were tested for the presence of *Tomato spotted wilt virus* (TSWV) with a commercial double-antibody sandwich (DAS)-ELISA diagnostic kit (Bioreba AG, Reinach, Switzerland). Commercial positive and negative controls were included in each ELISA. TSWV was detected serologically in 33 of 75 pepper samples. The virus was mechanically transmitted from ELISA-positive pepper samples to *Nicotiana tabacum* cv. Samsun using chilled 0.05 M phosphate buffer (pH 7) containing 0.1% sodium sulfite (1). Inoculated test plants produced chlorotic and necrotic concentric rings and necrotic spots, consistent with symptoms caused by TSWV on *N. tabacum*. For further confirmation of TSWV infection, reverse transcription (RT)-PCR was performed with the One-Step RT-PCR Kit (Qiagen, Hilden, Germany) using three sets of primers: S70-for/S890-rev (2) and S574-for/S1433-

rev (3), both specific to the nonstructural (NSs) gene; and S1983-for/S2767-rev (2), specific to the nucleocapsid protein (N) gene. Total RNAs from naturally infected pepper and symptomatic *N. tabacum* cv. Samsun plants were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Total RNAs obtained from the Italian isolate of TSWV (GenBank Accession No. DQ398945) and healthy tobacco plants were used as positive and negative controls, respectively. The expected sizes of the RT-PCR products (820, 8[illegible] and 784 bp) were amplified from symptomatic pepper samples but not from healthy tissues. The PCR product obtained from isolate Is-344 using primers specific to N ger was purified by a QIAquick PCR Purification Kit (Qiagen), cloned into the pGEM-T Eas Vector (Promega, Madison, WI) and sequenced in both directions using the same primer pair as in RT-PCR. The sequences amplified with the two primer pairs specific to the NSs gene were obtained by direct sequencing (Bio-Fab Research Srl, Pomezia, Italy) and joined using MEGA4 software. Sequence analysis of the complete N gene (777 bp; GenBank Accession No. GU369717) revealed that the TSWV isolate originating from Montenegro shared 98.2 to 99.7% nucleotide identity (98.1 to 100% amino acid identities) with corresponding TSWV sequences deposited in GenBank. The Montenegrin isolate Is-344 was most closely related to Italian isolates from tomato (GU369725) and eggplant (GU369720). The partial (1,257 bp) nucleotide sequence of NSs gene (GU369737) showed 96 to 99.8% nucleotide identity (96.9 to 100% amino acid identity) with previously reported TSWV sequences, and in this case the highest identity was with French isolates from tomato (FR692835) and lettuce (FR692831). To our knowledge, this is the first report on the occurrence of TSWV in Montenegro. Data of this study sheds light on the importance of further survey studies and inspections of TSWV-susceptible crops cultivated in Montenegro.

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