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Disease Notes

First Report of *Tomato spotted wilt virus* on *Gloxinia* in Bosnia and Herzegovina

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Abstract

In June and July 2012, symptoms resembling those caused by a tospovirus infection were observed on the greenhouse-grown gloxinia (*Sinningia speciosa* Benth. and Hook.) in the Lijeve polje, in the vicinity of Banja Luka (Bosnia and Herzegovina). Infected plants exhibited chlorotic ring spots and chlorotic and necrotic patterns followed by necrosis and distortion of leaves. Disease symptom incidence was estimated at 30% out of 400 inspected plants. Symptomatic leaves were collected and tested by double-antibody sandwich (DAS)-ELISA test using commercial polyclonal antisera (Bioreba AG, Reinach, Switzerland) for two of the most important tospoviruses in the greenhouse production of ornamentals: *Tomato spotted wilt virus*

(TSWV) and *Impatiens necrotic spot virus* (INSV) (2). TSWV was detected serologically in 27 out of 30 tested gloxinia samples, and all were negative for INSV. Symptomatic leaves of five selected ELISA-positive gloxinia plants were separately ground in chilled 0.01 M phosphate buffer (pH 7) containing 0.1% w/v sodium sulphite and were mechanically inoculated on five plants of *Petunia × hybrida*. All inoculated plants produced typical symptoms of TSWV (1), necrotic spots on inoculated leaves in 2 days post-inoculation. For further confirmation of TSWV infection, total RNAs were extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) from all 27 infected gloxinia plants and tested by reverse transcription (RT)-PCR assay. A 738-bp fragment of TSWV nucleocapsid (N) gene was amplified with One-Step RT-PCR Kit (Qiagen) using primer pairs TSWV CP-f and TSWV CP-r (4). Total RNAs from Serbian tobacco TSWV isolate (GenBank Accession No. GQ373173) and RNA extract from healthy gloxinia plants were used as positive and negative controls, respectively. Amplicons of the expected size were obtained from all 27 naturally infected gloxinia plants, while no amplification products were obtained from the healthy control. After the purification with QIAquick PCR Purification Kit (Qiagen), the RT-PCR product obtained from one selected isolate 160-12 was sequenced directly in both directions and submitted to GenBank (JX468079). Sequence analysis of the partial N gene, conducted by MEGA5 software (3), from isolate 160-12 showed the highest nucleotide identity of 99.7% (100% amino acid identity) with eight pepper isolates of TSWV from Spain (FR693229, FR693231, FR693152-153, FR693078, FR693081, FR693089, and FR693092). To our knowledge, this is the first report on the occurrence of TSWV in Bosnia and Herzegovina. The presence of this harmful pathogen into a new area could have a serious threat to intensive and increasing production of ornamentals and numerous other TSWV susceptible species in Bosnia and Herzegovina. The discovery of TSWV on gloxinia should prompt more surveys, thorough inspections, and subsequent testing of other TSWV susceptible plants cultivated in Bosnia and Herzegovina.

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