Disease Notes

First Report of *Impatiens necrotic spot virus* on Begonia in Bosnia and Herzegovina

V. Trkulja, J. Mihić Salapura, B. Ćurković, I. Stanković, A. Bulajić, A. Vučurović, and B. Krstić

Affiliations

Authors and Affiliations

V. Trkulja , Department of Plant Protection, University of Banja Luka-Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, 78000 Banja Luka, Bosnia and Herzegovina

J. Mihić Salapura

B. Ćurković , Department of Plant Protection, Agricultural Institute of Republic of Srpska, Knjaza Miloša 17, 78000 Banja Luka, Bosnia and Herzegovina

I. Stanković

A. Bulajić

A. Vučurović

B. Krstić , Institute of Plant Protection, Department of Phytopathology, University of Belgrade – Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia. This research was partly supported by the grant III 43001 of the Ministry of Education, Science, and Technical Development, Republic of Serbia

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Abstract

Impatiens necrotic spot virus (INSV) and *Tomato spotted wilt virus* (TSWV) are the most serious viral pathogens in the production of ornamental plants in Europe and North America (1). During a survey for the presence of tospoviruses in July 2012, potted begonia hybrids (*Begonia* × *tuberhybrida* Voss) exhibiting foliar chlorotic rings and zonal spots accompanied by leaf necrosis and distortion, were observed in a greenhouse in the vicinity of Banja Luka (Bosnia and Herzegovina). Leaf samples collected from 12 symptomatic plants were analyzed for the presence of INSV and TSWV by commercial double-antibody sandwich (DAS)-ELISA kits (Bioreba AG,

Reinach, Switzerland). Commercial positive and negative controls and extracts from healthy begonia leaves were included in each ELISA. INSV was detected serologically in all 12 begonia samples and all tested samples were negative for TSWV. Five healthy plants of each *Petunia* × *hybrida* and *Nicotiana benthamiana* were mechanically inoculated with sap from an ELISA-positive sample (157-12) using chilled 0.01 M phosphate buffer (pH 7) containing 0.1% sodium sulphite. Local necrotic lesions [illegible] × *hybrida* and systemic chlorotic mottling on *N. benthamiana* were observed on a[illegible] inoculated plants 4 and 10 days post-inoculation, respectively. For further confirmation of INSV infection, total RNAs were extracted from all ELISA-positive begonia plants as well as mechanically inoculated *N. benthamiana* plants with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and used as template in reverse transcription (RT)-PCR. RT-PCR was performed with the OneStep RT-PCR Kit (Qiagen) using primer pair INSV-589 and TOS-R15 (3), specific to the partial INSV nucleocapsid (N) gene. Total RNA obtained from Serbian INSV isolate from a begonia (GenBank Accession No. HQ724289) and RNA extracts from healthy begonia plants were used as positive and negative controls, respectively. All naturally and mechanically infected plants as well as the positive control yielded an amplicon of the expected size (589 bp), while no amplification products were obtained from the healthy controls. The RT-PCR product derived from the isolate 157-12 was sequenced directly after purification with QIAquick PCR Purification Kit (Qiagen) and submitted to GenBank (KC494869). Pairwise comparison of the 157-12 isolate N sequence with other homologous sequences available in GenBank, conducted using MEGA5 software (2), revealed that begonia isolate from Bosnia and Herzegovina showed the highest nucleotide identity of 99.7% (100% amino acid identity) with the Chinese INSV isolate (FN400772) originating from *Oncidium* sp. To our knowledge, this is the first report of INSV on begonia in Bosnia and Herzegovina. Begonias are very popular and widely grown ornamentals in Bosnia and Herzegovina and the presence of a new and devastating pathogen could represent a serious threat for its production. Since begonia is commonly grown together with numerous ornamental plants susceptible to INSV, further investigations are needed in order to prevent spread of this potentially harmful pathogen to new hosts in Bosnia and Herzegovina.

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3340 Pilot Knob Road, St. Paul, MN 55121

USA

+1.651.454.7250

FAX +1.651.454.0766

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