Disease Notes

First Report of *Iris yellow spot virus* Infecting Onion in Bosnia and Herzegovina

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Abstract

In July 2012, a survey was conducted to determine the presence of tospoviruses in Bosnia and Herzegovina, symptoms resembling those caused by *Iris yellow spot virus* (IYSV; genus *Tospovirus*, family *Bunyaviridae*) were observed in an onion (*Allium cepa*) seed crop in the Gornji Karajzovci locality (Region of Banja Luka). Symptoms included chlorotic to necrotic, straw-colored, spindle- and diamond-shaped lesions, variable in size and randomly distributed on the leaves and particularly on the scapes. Later the lesions enlarged and coalesced, causing scape breakage. Affected plants occurred

throughout the field and disease incidence was estimated at 20%. Symptomatic plants were sampled and assayed by double-antibody sandwich (DAS)-ELISA test using commercial polyclonal antisera (Bioreba AG, Reinach, Switzerland) against IYSV and two other tospoviruses, *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). Commercial positive and negative controls were included in eac test. IYSV was detected serologically in 19 of 20 screened samples and none of th samples tested positive for TSWV or INSV. The virus was mechanically transmitte from an ELISA-positive sample (302-12) to five of each *Petunia* × *hybrida* and *Nicotiana benthamiana* using chilled 0.01 M phosphate buffer (pH 7) containing 0.1% sodium sulfite (1). All inoculated *P.* × *hybrida* showed local necrotic spots, while *N. benthamiana* developed mild mosaic 4 and 10 days post-inoculation, respectively. However, difficulties were encountered in reproducing the disease symptoms on mechanically inoculated onion plants corroborating a previous study (2). Serological findings were verified with reverse transcription (RT)-PCR. Total RNAs from all naturally infected onion plants as well as mechanically infected *N. benthamiana* plants were extracted with the RNease Plant Mini Kit (Qiagen, Hilden, Germany). RT-PCR was performed with One-Step RT-PCR Kit (Qiagen) using IYSV-specific primers IYSV56U/IYSV917L (3), designed to amplify an 896-bp fragment of the S RNA which includes whole nucleocapsid (N) gene. Total RNAs from Serbian IYSV isolate from onion (GenBank Accession No. EU586203) and from healthy onion plants were used as positive and negative controls, respectively. An amplicon of the expected size was obtained from each of the plants assayed as well as from positive control, but not from the negative control. The amplified products derived from onion isolate 302-12 was purified (QIAquick PCR Purification Kit, Qiagen), sequenced directly (JX861126), and compared with known IYSV isolates. Sequence analysis of the complete N gene, conducted with MEGA5 software (4), revealed the highest nucleotide identity of 99.5% (100% amino acid identity) with IYSV onion isolate (DQ658242) from Texas. To our knowledge, this is the first report of IYSV in Bosnia and Herzegovina. Onion is an important and traditionally grown vegetable crop in Bosnia and Herzegovina and the presence of IYSV could represent an important constraint to onion and other susceptible host production. The discovery of IYSV on onion should prompt more detailed surveys, thorough inspections and subsequent testing to establish the distribution and incidence of IYSV in Bosnia and Herzegovina.

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