First Report of Iris yellow spot virus on Onion (Allium cepa) in Serbia

August 2008, Volume 92, Number 8 Page 1247

http://dx.doi.org/10.1094/PDIS-92-8-1247A

Disease Notes

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Iris yellow spot virus (IYSV; genus *Tospovirus*, family *Bunyaviridae*) is established in several European countries (France, Italy, The Netherlands, Poland, Slovenia, Spain, and the UK) and its distribution in the EU region has increased since 2002 (3). In July 2007, symptoms resembling those of IYSV were observed in an onion (*Allium cepa*) seed crop in the Sirig locality in Serbia. Onion plants exhibited characteristic symptoms of chlorotic or necrotic spindle and diamond-shaped lesions on the leaves and scapes. Symptomatic plants were found throughout the field and disease incidence was estimated at 80%. Leaf and scape samples were tested for the presence of IYSV and two other tospoviruses, *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV), using commercial double-antibody sandwich (DAS)-ELISA diagnostic kits (Loewe Biochemica, Sauerlach, Germany). All samples tested negative for TSWV and INSV. IYSV was detected serologically in 26 of 34 onion samples. To determine an experimental host range, samples of IYSV-infected onion plants were homogenized in chilled 0.05 M phosphate buffer pH 7 containing 1 mM Na-EDTA, 5 mM Na-DIECA, and 5 mM Na-thioglycolate (2), and host plants were inoculated with the sap. Mechanical transmission of the virus occurred rarely. All inoculated test plants were assayed by DAS-ELISA and only four species tested positive for IYSV, but not in all replications. Inoculated *Chenopodium quinoa* developed local chlorotic

lesions, *Nicotiana tabacum* cvs. Samsun and Prilep showed mild mosaic, while infected *N. benthamiana* were symptomless. For further confirmation of IYSV, conventional reverse transcription (RT)-PCR was performed on extracts made from symptomatic onion leaf material and from the ELISA-positive symptomless leaves of *N. benthamiana*. Total RNAs were extracted with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and RT-PCR was carried out with the OneStep RT PCR Kit (Qiagen) following the manufacturer's instructions. The primer pair, IYSV56U/IYSV917L, covering the entire nucleocapsid (NC) gene was used for both amplification and sequencing (1). A product of the correct predicted size (896 bp) was obtained from each of the plants assayed, and that derived from isolate 605-SRB was purified (QUIAqick PCR Purification Kit, Qiagen) and sequenced (GenBank Accession No. EU586203). BLAST analyses revealed 86 to 97% sequence identity with the NC gene from all other IYSV. The highest identity (97%) was with leek and onion isolates (GenBank Accession Nos. EF427447 and EF19888) from Spain. To our knowledge, this is the first report of IYSV infection of onion seed crop in Serbia. Thorough inspections and subsequent testing would be needed to establish the distribution and incidence of IYSV in Serbia.

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