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First Report of *Fusarium* sp. FIESC 3 on Onion Seed in Serbia

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Published Online: 29 Jun 2015 | https://doi.org/10.1094/PDIS-01-15-0082-PDN

Onion (*Allium cepa* L.) is one of the most important vegetable crops in Serbia, where it is grown on about 20,000 ha. During a routine quality control analysis on onion seed in 2014, fungal infection was observed on an average of 28% of the seed. Onion seeds placed on the blotter surface were covered by white mycelium, with reddish to purple pigmentation observed on the blotter under the seeds. Isolation of pathogen was carried out by cutting a small piece of infected seed, which was surface sterilized with 3% NaOCl for 3 min, dried and transferred onto potato dextrose agar (PDA), and then incubated for 7 days at 25°C (Burgess et al. 1994). For morphological identification, 25 isolates were single-spored and subcultured on both PDA and carnation leaf agar (CLA). Incubation lasted 7 to 10 days at 25°C, in alternating cycles of 12 h light and 12 h darkness. All of the isolates produced whitish to pale-salmon colonies, with orange pigment on the reverse surface of the PDA. On CLA, isolates formed hyaline, thin-walled, slightly curved, fusoid macroconidia with 4 to 6 septae (23 to 40 × 3.5 to 6 µm). Microconidia and chlamydospores were not observed. A pathogenicity test was conducted using Knop agar slants in controlled conditions at the laboratory (Tuite 1969). A piece of mycelium (approximately 2 to 3mm) of each isolate grown on PDA for 7 days was placed at the bottom of each test tube, and afterward a

sterilized, dried onion seed was carefully placed and slightly pressed, approximately 2 cm above the inoculum. Onion seeds placed on a solid agar without mycelia were used as negative control. Tubes were kept in the laboratory for two weeks in the vertical position at room temperature (21 to 25°C) with 12-h day/night cycle. After 14 days, fungal mycelia of 25 isolates completely covered the seedlings, causing root necrosis and seedling decay. No symptoms were observed on seedlings in the tubes which were used as negative control. Pathogen was reisolated and morphological identity was confirmed on PDA and CLA. To obtain a DNA sequence-based identification, a total DNA was extracted directly from the mycelium of the 25 isolates tested with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Following DNA extraction, the translation elongation factor 1-alpha region was amplified by PCR with the primer pair EF1 and EF2 (Geiser et al. 2004). In 25 tested isolates, an amplicon of 700 bp was amplified. Identification of one isolate was performed by sequencing the translation elongation factor (EF-1α) gene, which was deposited in the NCBI GenBank database under Accession No. KP658211. BLASTn queries of GenBank and the *Fusarium* ID-database (Geiser et al. 2004) showed 100% identity to accessions GQ505648.1 (NRRL36323) and GQ505646.1 (NRRL36318) from an unnamed phylogenetic species within the *Fusarium incarnatum-equiseti* species complex designated FIESC3 (O'Donnell et al. 2009). Based on the completion of Koch's postulates, and sequence analysis, to our knowledge, this is the first report of *Fusarium* sp. FIESC3 causing decay and rot of onion seed in Serbia. Because this fungus could reduce seed germination, the presence of this pathogen could significantly impact onion production in Serbia.

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