Diseases Caused by Oomycetes

First Report of *Pythium aristosporum* Causing Corn Stalk Rot in China

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Corn (*Zea mays* L.) stalk rot, caused by various pathogens, is one of the most prevalent corn diseases worldwide. In October 2019, a survey was carried out to determine pathogenic fungi causing corn stalk rot in three fields (~120 ha) in Harbin city (44.04°N, 125.42°E), Heilongjiang Province, China. In each field, 100 plants at five sampling points were assessed at the milk stage (R3) of development. Disease incidence was 12%. Symptomatic plants showed rapid death of the upper leaves, drooping ears, and stalks were soft, hollow, and water-soaked with white hyphae present on the outside of the stalk. Pieces of tissue (0.25 cm²) from 15 individual diseased stalks (five plants per field) were surface disinfested in 0.5% NaOCl for 5 min, rinsed three times in sterile distilled water, and cultured on potato dextrose agar containing streptomycin (50 µg/ml). After 3 days of incubation, a total of 12 fungal cultures with uniform characteristics were isolated and subcultured by transferring hyphal tips onto V8 selective medium. Colonies on V8 selective medium were creamy white and floccus, with a growth rate of 20 mm/day at 26°C in darkness. Oospores were mostly plerotic, and oogonia walls were 1.3 to 2.7 µm thick ($n = 50$); globose oogonia, 23.9 to 30.5 µm in diameter ($n = 50$), and one to eight antheridia. Based on these

characteristics, the isolates were identified as *Pythium* sp. (van der Plaats-Niterink 1981). Genomic DNA was extracted from single conidial cultures of representative isolates (MZYJF1, MZYJF3, and MZYJF7), and the internal transcribed spacer (*ITS*) region and cytochrome oxidase subunit II (*CoxII*) gene were amplified and sequenced using the primers ITS1/ITS4 (Yin et al. 2012) and COX2f/COX2r (Hudspeth et al. 2000), respectively. Partial nucleotide sequences of 796 and 573 bp for the *ITS* and *CoxII* amplicons, respectively, were obtained and deposited in GenBank (accession nos. MW447501 for *ITS* and MW471006 for *CoxII*). MegaBLAST analysis of the *ITS* and *CoxII* sequences of MZYJF1 isolate showed 100% similarity with sequences from *Pythium aristosporum* strain ATCC 11101. The isolates were identified as *P. aristosporum* based on the fact that *P. aristosporum* has aplerotic oospores and less antheridia per oogonium than *P. arrhenomanes* (Van der Plaats-Niterink 1981). A pathogenicity test was performed on corn cultivar Xianyu 335 at tasseling stage (VT) in the field. An oospore suspension, obtained from isolate MZYJF1 grown on V8 agar medium for 4 weeks (Green and Jensen 2000) and diluted to 1×10^4 oospores/ml using the blood cell counting method, was injected into the base of the maize stems of six healthy plants (1.5 ml/plant) using a syringe. Control plants were injected with sterile distilled water. All inoculated plants showed symptoms 25 days after inoculation that were similar to those observed in the field. The oomycete of *P. aristosporum* was reisolated from symptomatic plants on V8 agar medium and identified according to morphological and molecular characteristics. No symptoms were observed on the control plants. *P. aristosporum* has previously been reported causing damping-off of pea in the Columbia basin of Central Washington (Alcala et al. 2016) and on soybean in North Dakota (Zitnick-Anderson and Nelson 2015). To our knowledge, this is the first report of *P. aristosporum* causing corn stalk rot in China. Corn stalk rot caused by *P. aristosporum* poses a threat to significantly reduce the quality of corn. Thus, its distribution needs to be investigated and effective disease management strategies developed.

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