Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Maize Stalk Rot Caused by *Fusarium kyushuense* in China

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In 2017 to 2019, a field survey for maize stalk rot was conducted in 21 counties (districts) across Guangxi province, China. It caused yield losses of 20% to 30%. Maize plants with stalk rot were collected during the late milk stage, and pieces of diseased pith tissue were cultured as previously described (Shan et al. 2017). Fungal colonies and mycelia with morphological characteristics of *Fusarium* species were subcultured onto fresh potato dextrose agar (PDA) and carnation leaf agar (CLA) plates. Based on morphological characteristics and molecular detection by amplification of *Fusarium* genus-specific primers (Duan et al. 2016), 39 *Fusarium* isolates were identified. Five isolates from Du'an, Pingguo, Debao, and Daxin had abundant, pale orange to yellow aerial mycelia with deep red pigments when grown on PDA. The average growth rate was 8.0 to 12.0 mm/day at 25°C in the dark. The fungi produced two types of spores on CLA. Microconidia were ovoid to clavate, generally zero- to three-septate, and 4.6 to 9.4 µm in length ($n = 30$). Macroconidia were slightly curved with an acute apical cell, mostly three- to four-septate, and 19.4 to 38.2 µm in length ($n = 30$). No chlamydospores were observed. The five isolates were initially identified as *Fusarium kyushuense* based on morphology. PCR was performed to amplify three phylogenetic genes (*TEF1-α*, *RPB1*, and *RPB2*) (O'Donnell et al. 1998), and species-specific primers kyuR1F/kyuR1R (5'-TTTCTCCTACCAAGGAGCAGATCATG-3'/5'-TCCAATGGACTGGGACGCCAAAACACC-3'), kyuR2F/kyuR2R (5'-CA GATATACATTTGCCTCGACAC-3'/5'-TACTTGAGCACGGAGCTTG-3')

were used to confirm species identity. The obtained sequences were deposited in GenBank as MT997084, MT997080, MT997081 (*TEF1-α*); MT550012, MT997085, MT997086 (*RPB1*); and MT550009, MT997089, MT997090 (*RPB2*), respectively. Using BLAST, sequences of *TEF1-α*, *RPB1*, and *RPB2* were 99.33% (MH582297.1) to 100% (MG282364.1) similar to those of *F. kyushuense* strains. Based on phylogenetic analysis with maximum likelihood methods using tools of the website of CIPRES (<http://www.phylo.org>), isolates GX27, GX167, and GX204 clustered with *F. kyushuense* with 100% bootstrap support. Pathogenicity of the three isolates was tested using seedlings and adult plants as previously described with modification (Ye et al. 2013; Zhang et al. 2016). The primary roots of three-leaf seedlings were inoculated by immersing the roots into a 1×10^6 macroconidia solution, incubating for 6 h at 25°C, and transferring to normal growth conditions (26°C, 16 h light/22°C, 8 h dark). The second or third internode above the soil surface of flowering-stage plants grown in a greenhouse was bored with a Bosch electric drill to make a hole (~8-mm diameter), inoculated with 0.5 ml of mycelia plug, and sealed with petrolatum. The inoculum was created by homogenizing five plates of flourish hyphal mats (~125 ml) with a kitchen blender and adjusting to a final volume of 200 ml with sterilized ddH₂O. No symptoms were observed in the seedlings or adult plants that were mock-inoculated with PDA plugs. Three days postinoculation (dpi), roots of the infected seedlings turned dark-brown and shrunk, and the leaves wilted. Typical stalk rot symptoms observed in the inoculated plants were premature wilting of the entire plant and hollow and weak stalks, leading to lodging; the longitudinal section of the internodes exhibited obvious dark brown necrosis and reddish discoloration at 14 and 30 dpi, respectively. *F. kyushuense* was reisolated from the inoculated stalk lesions but not from the control. This is the first record of stalk rot caused by *F. kyushuense* on maize plants in China. However, *F. kyushuense* is known to cause maize ear rot in China (Wang et al. 2014) and can produce type A and type B trichothecene mycotoxins in kernels (Aoki and O'Donnell 1998). The occurrence of maize stalk rot and ear rot caused by *F. kyushuense* should be monitored in China due to the potential risk for crop loss and mycotoxin contamination.

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