Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Leptosphaeria biglobosa* ‘brassicae’ Causing Blackleg on *Brassica juncea* var. *multisecta* in China

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Brassica juncea var. *multisecta*, a leafy mustard, is widely grown in China as a vegetable (Fahey 2016). In May 2018, blackleg symptoms, grayish lesions with black pycnidia, were found on stems and leaves of *B. juncea* var. *multisecta* during disease surveys in Wuhan, Hubei Province. Disease incidence was approximately 82% of plants in the surveyed fields (~1 ha in total). To determine the causal agent of the disease, 12 diseased petioles were surface-sterilized and then cultured on potato dextrose agar (PDA) at 20°C for 5 days. Six fungal isolates (50%) were obtained. All showed fluffy white aerial mycelia on the colony surface and produced a yellow pigment in PDA. In addition, pink conidial ooze formed on top of pycnidia after 20 days of cultivation on a V8 juice agar. Pycnidia were black-brown and globose with average size of 145 × 138 µm and ranged from 78 to 240 × 71 to 220 µm, *n* = 50. The conidia were cylindrical, hyaline, and 5.0 × 2.1 µm (4 to 7.1 × 1.4 to 2.9 µm, *n* = 100). These results indicated that the fungus was *Leptosphaeria biglobosa* rather than *L. maculans*, as only the former produces yellow pigment (Williams and Fitt 1999). For molecular confirmation of identity, genomic DNAs were extracted and tested through PCR using the species-specific primers LbigF, LmacF, and LmacR (Liu et al. 2006), of which DNA samples of *L. maculans* isolate UK-1 (kindly provided by Dr. Yongju Huang of University of Hertfordshire) and *L. biglobosa* ‘brassicae’ isolate B2003 (Cai et al. 2014) served as controls. Moreover, the sequences coding for actin, β-tubulin, and the internal transcribed spacer (ITS) region of ribosomal DNA (Vincenot et al. 2008) of

isolates HYJ-1, HYJ-2, and HYJ-3 were also cloned and sequenced. All six isolates only produced a 444-bp DNA fragment, the same as isolate B2003, indicating they belonged to *L. biglobosa* ‘brassicae,’ as *L. maculans* generates a 331-bp DNA fragment. In addition, sequences of ITS (GenBank accession nos. MN814012, MN814013, MN814014), actin (MN814292, MN814293, MN814294), and β-tubulin (MN814295, MN814296, MN814297) of isolates HYJ-1, HYJ-2, and HYJ-3 were 100% identical to the ITS (KC880981), actin (AY748949), and β-tubulin (AY748995) of *L. biglobosa* ‘brassicae’ strains in GenBank, respectively. To determine their pathogenicity, needle-wounded cotyledons (14 days) of *B. juncea* var. *multisecta* ‘K618’ were inoculated with a conidial suspension (1 × 10⁷ conidia/ml, 10 µl per site) of two isolates HYJ-1 and HYJ-3, 12 seedlings per isolate (24 cotyledons), while the control group was only treated with sterile water. All seedlings were incubated in a growth chamber (20°C, 100% relative humidity under 12 h of light/12 h of dark) for 10 days. Seedlings inoculated with conidia showed necrotic lesions, whereas the control group remained asymptomatic. Two fungal isolates showing the same culture morphology to the original isolates were reisolated from the necrotic lesions. Therefore, *L. biglobosa* ‘brassicae’ was confirmed to be the causal agent of blackleg on *B. juncea* var. *multisecta* in China. *L. biglobosa* ‘brassicae’ has been reported on many *Brassica* spp. crops in China, such as *B. napus* (Fitt et al. 2006), *B. oleracea* (Zhou et al. 2019b), *B. juncea* var. *multiceps* (Zhou et al. 2019a), and *B. juncea* var. *tumida* (Deng et al. 2020). To our knowledge, this is the first report of *L. biglobosa* ‘brassicae’ causing blackleg on *B. juncea* var. *multisecta* in China, and its occurrence might be a new threat to leafy mustard production of China.

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