Diseases Caused by Fungi and Fungus-Like Organisms

Identification of *Colletotrichum liriopes* as the Causative Agent of Anthracnose in Buckwheat (*Fagopyrum esculentum*) in China

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Buckwheat (*Fagopyrum esculentum*), belonging to the Polygonaceae family, is one of the most important “functional food” crops in China. In fall 2020, buckwheat plants grown in the field exhibiting stem canker symptoms were found in Tongxin County, Ningxia Province, China. Symptoms included stem canker, dieback, and extensive vascular discoloration. Cankers were bleached, silvery-white to dark gray, slightly sunken, oval to linear with slightly tapered tips, and pycnidia formation was also observed within the cankers. Disease incidence was ~30% and moderate to high severity across the field. Symptomatic tissues were cut into 1- to 2-cm pieces, surface sterilized (75% ethanol for 30 s and 0.1% NaClO for 2 min), washed four times with sterile distilled water, dried in sterile filter paper three times, and placed on potato dextrose agar (PDA) at 25°C. Fluffy mycelium was visible for all isolates after 48 h of incubation. Twenty-five single isolates were hyphal-tip purified on PDA. Six representative isolates were used for further study. The fungal colonies on PDA were flat with an entire margin, gray aerial hyphae, light brown pigmentation, appressed slimy mycelium, within which numerous brown-black perithecia formed. Colonies on oatmeal agar (OA) were flat, with flocculent mycelium, conidiomata, and conidia, and the reverse side was black to smoke-gray. Sparse brown-black perithecia were observed within the mycelium. Conidia were hyaline, one-celled, smooth-walled, rarely finely verruculose, aseptate, slightly curved, both sides gradually tapering toward the round to slightly acute apex and truncate base, measured 15.7 to 23.7 µm

long × 2.8 to 5.7 µm wide (avg. 20.2 × 4.2 µm, $n = 100$). Genomic DNA was extracted from the same six isolates, the internal transcribed spacer (ITS) region, and the genes encoding beta-tubulin (*TUB*), chitin synthase (*CHS*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and histone H3 (*HIS3*) were independently amplified with primers *V9G/ITS4*, *T1/Bt-2b*, *CHS-354R/CHS-79F*, *GDF1/GDR1*, and *CYLH3F/CYLH3R*, respectively (Damm et al. 2019). Sequences for all six isolates were identical. The sequences of the representative isolate 9J1 were deposited in GenBank (accession nos. MW819604, MW836580, MW836577, MW836578, and MW836579). The results of BLASTn showed that the ITS, *TUB*, *CHS*, *GAPDH*, and *HIS3* sequences of 9J1 were greater than 98% (555/557, 477/486, 258/259, 242/248, and 339/345 bp, respectively) identical to those of *Colletotrichum liriopes* (MT645674 [ITS], GU228098 [*TUB*], MT663546 [*CHS*], MH291255 [*GAPDH*], and MH292811 [*HIS3*]). MrBayes phylogenetic analysis using concatenated sequences of ITS, *TUB*, *CHS*, *GAPDH*, and *HIS3* showed that the isolate clustered monophyletically with strains of *C. liriopes*. Based on morphological and molecular characteristics, the isolate was identified as *C. liriopes*. To fulfill Koch's postulates, spores of isolate 9J1 grown on OA for 14 days were harvested in 0.01% Tween 20, and the suspension was adjusted to 10⁷ spores/ml. Six 1-month-old potted buckwheat plants were inoculated by spraying the spore suspension until runoff. Plants were kept in the greenhouse inside a plastic bag for 3 days to maintain high relative humidity. Six control plants were sprayed with sterile deionized water and kept under the same conditions. Two weeks after inoculation, all inoculated plants showed stem canker symptoms as described above, whereas control plants remained healthy. The pathogen was successfully reisolated from leaf and stem symptomatic tissue and identified as *C. liriopes* based on morphological features and DNA sequence analysis, fulfilling Koch's postulates. *C. liriopes* has been reported causing anthracnose on *Eria coronaria* (Yang et al. 2011), *Liriope spicata* (Chen et al. 2019) in China, *L. muscari* in Mexico (Damm et al. 2009), *Rohdea japonica* in Korea (Kwon and Kim 2013), and in the United States (Trigiano et al. 2018). To our knowledge, this is the first report of *C. liriopes* causing anthracnose on buckwheat worldwide. Occurrence of this disease may represent a significant impact for buckwheat production because this crop is the major agricultural commodity in some parts of China. More studies are needed to understand the epidemiology of this disease and foster disease management programs in China.

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