Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Rhizoctonia solani* AG-4 HGI Causing Stem Canker on *Fagopyrum tataricum* (Tartary Buckwheat) in China

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Tartary buckwheat (*Fagopyrum tataricum*) is an ideal functional food source that is gluten-free and rich in proteins, fats, vitamins, minerals, and flavonoids of various pharmaceutical uses, such as rutin, quercetin, and epicatechin (Zhou et al. 2018). *Rhizoctonia solani* AG-4 HGIII causing severe canker disease was first isolated from common buckwheat (*F. esculentum*) in Inner Mongolia of China (Zhou et al. 2015). In 2018, sunken lesion and dark brown symptoms were observed on the root and stem of 10-day-old Tartary buckwheat in Liangshan (28°21'N, 103°19'E), Sichuan Province, and Fenghuang (28°19'N, 109°48'E), Hunan Province, China. In the beginning, water-soaked spots appeared on the stem base, which gradually became rotten and necrotic, finally resulting in the damping-off and death of buckwheat seedlings. This disease had >40% incidence and led to serious losses to production in 2018. To isolate the pathogens, 10 plants with typical symptoms were collected from each location. Infected tissue was cut into 3-mm pieces from the margin between healthy and diseased tissue, surface sterilized with 1% NaOCl solution for 4 min, washed three times with sterile distilled water, dried on sterilized filter paper, and then placed on PDA with 100 mg/ml of streptomycin sulfate. After incubation at 28°C in the dark for 2 days, mycelial tips of four fungal cultures were transferred to PDA plates for purification. Colonies were initially pale white and turned brown after 2 days of incubation. The mycelium was hairy and concentrically whorled in the culture medium. Microscopic observation showed that the hyphae characteristically branched at right angles and had constriction at the base of hyphal branches. Nuclear staining showed that hyphae cells were multinuclear.

These morphological features revealed that the isolates belonged to *R. solani* (Sneh et al. 1991). Subsequently, rDNA ITS region of one isolate was amplified by PCR (White et al. 1990) and sequenced (GenBank MT078642) by Shanghai Majorbio Bio-pharm Technology Co. DNA was extracted by a Fungal Genomic DNA Extraction Kit (D3390-02, Omega). BLAST similarity analysis showed 99.96% match with *R. solani* AG-4 HGI (JQ343830) and 99.85% identity to *R. solani* AG-4 HGI isolate SX-8 (KJ170346) (Sun et al. 2015). Further, phylogenetic analysis using the neighbor-joining method (MEGA 7) clustered the isolate with the group of *R. solani* AG-4 HGI (Ireland et al. 2015). Pathogenicity was tested in the greenhouse to satisfy Koch's postulates. Tartary buckwheat plants of 7 and 50 days old were respectively inoculated near the base of the stem neck with one mycelial plug in direct contact. Ten plants in a pot were inoculated as one treatment; four pots were used for replicates. Control plants were inoculated with PDA plugs without fungi. All the plants were kept at 26°C with 14-h light, 10-h dark, and 96% humidity. After 5 days (Sun et al. 2015), >90% of the inoculated plants exhibited necrotic brown lesions on stems that was similar to symptoms observed in the field, whereas control plants remained asymptomatic. The visible characteristics and ITS sequence of the pathogen reisolated from symptomatic plants were in accordance with the original isolate (*R. solani* AG-4 HGI). Based on disease symptoms in the fields, morphological characteristics, ITS sequence analysis, and pathogenicity assay, we concluded that *R. solani* AG-4 HGI was the principal cause of Tartary buckwheat blight in Liangshan, Sichuan Province, and Fenghuang, Hunan Province, China. Previously, *R. solani* AG-4 HGI was identified as a Chinese chive pathogen (Shi et al. 2017). This is the first report of the natural occurrence of *R. solani* AG-4 HGI affecting Tartary buckwheat in China. This finding is helpful for the early diagnosis and identification of the disease, which will guide effective control methods to the devastating disease at the early stage.

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