Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Root Rot Caused by *Calonectria montana* on Sugar Beet in Heilongjiang Province, China

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Sugar beet (*Beta vulgaris* L.) is a crop with economic value in northern China, especially Heilongjiang Province. In October 2019, root rot was discovered on 'HDW09' sugar beet in Hulan (126.64° E, 46.00° N), Heilongjiang Province, China. Symptoms included lesions on root tissues, which were initially small and dark brown and gradually became irregular shapes and black. As the disease progressed, necrosis penetrated from external layers into inner tissue. Root tissues suffered severe decay, resembling several previously reported root rot diseases of sugar beet (Harveson 2006). Pieces of the transition zones (3 to 5 mm) between asymptomatic and symptomatic tissues were surface sterilized for 15 s in 1% NaClO, rinsed twice with sterilized distilled water, plated on corn meal agar (CMA) supplemented with penicillin G (50 mg/liter), and incubated at 25 ± 2°C in the dark. Isolates belonging to a *Calonectria* sp. were recovered and purified using the hyphal tipping technique. Four isolates (A1, A5, A6, and A7) were used for morphological characterization and identification by DNA sequencing. The 7-day-old colonies on malt extract agar produced buff and wooly aerial mycelia. They were sennu to umber. Chlamydospores and microsclerotia were produced abundantly throughout the medium. The isolates were also cultured on PDA at 25°C under near-ultraviolet irradiation. Macroconidiophores comprised a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle. Stipe extensions were septate, straight to flexuous, 61 to 117 µm long, 2 to 4 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 5 to 9 µm diameter. Conidiogenous apparatus was 31 to 177 µm long

and 16 to 110 µm wide ($n = 30$). Primary branches of the conidiogenous apparatus were aseptate or one-septate, 16 to 51 × 3 to 7 µm; secondary branches aseptate, 6 to 31 × 2 to 7 µm; tertiary branches aseptate, 8 to 19 × 2 to 6 µm, each terminal branch producing one to six phialides. Conidia, cylindrical, were rounded at both ends, straight, 32 to 53 × 3 to 5 µm (mean = 47 × 4 µm), one-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime ($n = 100$). Partial sequences of calmodulin (CAL) (Carbone and Kohn 1999), histone H3 (HIS), translation elongation factor 1-alpha (EF1-alpha), and beta-tubulin 2 (tub2) (Crous et al. 2004) genes of the isolates were obtained and deposited in GenBank as MW118652 to MW118667. BLAST results showed that the CAL, HIS, EF1-alpha, and tub2 sequences of A1, A5, A6, and A7 matched the sequences of *C. montana* strain CERC 8957 MF527082.1 (CAL) (99 to 100%), CERC 8930 MF527061.1 (HIS) (98 to 99%), HSP4 MN356465.1 (EF1-alpha) (100%), and HSP4 MN356460.1 (tub2) (98 to 99%), respectively. A phylogenetic tree using the maximum likelihood algorithm and sequences of the four concatenated genes was reconstructed in RAxML and revealed that the four isolates clustered in the clade of *C. montana*. The pathogen was identified as *C. montana* based on morphological and molecular traits (Liu et al. 2017; Stępniewska et al. 2020). Ten 8-week-old sugar beet plants without symptoms were selected. The roots near the ground were carefully cleaned. One mycelial plug (5 mm diameter) from a 7-day-old colony of A1 was used to inoculate each root. Ten plants inoculated with plugs of noncolonized PDA served as a control. Pathogenicity tests were repeated three times. Plants were incubated under greenhouse conditions at 25 ± 2°C and watered when the surface soil was dry. All inoculated plants showed symptoms like those in the field after 30 days; control plants remained healthy. Symptomatic tissues were plated in CMA for 7 days at 25°C. *C. montana* was reisolated from 100% of inoculated tissues and identification confirmed by molecular sequencing, validating Koch's postulates. This is the first report of *C. montana* in China causing root rot on sugar beet. The study suggests its broader host range and wider geographical distribution and lays a basis for further monitoring and managing this pathogen.

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