Diseases Caused by Oomycetes

Phytophthora cactorum as a Pathogen Associated with Root Rot on Alfalfa (*Medicago sativa*) in China

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Alfalfa (*Medicago sativa*) is the main pasture crop in China due to its economic and ecological importance. In June to August 2018, stunted plants were observed in alfalfa fields (2 years old) in Jinchang, Gansu Province. Incidence of root rot was 40 to 50%. Taproots of stunted plants showed red to dark-brown discolorations, and lateral roots were poorly developed. Shoots wilted with rotted taproots and lateral roots in severely affected plants. Twenty symptomatic plants were collected for pathogen isolation. Roots were washed under running tap water, cut into 2- to 3-mm pieces (40 per plant), sterilized in 75% ethanol for 2 min, and rinsed three times with autoclaved distilled water (ADW). Surface-dried pieces on autoclaved filter paper were put onto water agar and also a *Phytophthora* selective medium, P₅ARP(H) (Jeffers and Martin 1986). Plates were incubated at 22°C for 3 to 5 days, and then the growing hyphae were subcultured onto PDA. Thirty-two *Phytophthora*-like isolates were obtained and showed similar morphologies on PDA. Five random isolates were purified by single hyphal tip, and plugs (4 to 5 mm) from PDA cultures were incubated in Petri dishes with ADW at 22°C for 5 days. Sporangia, chlamydospores, and oospores were examined. Sporangia were usually ovoid and sometimes ellipsoid, 30.5 to 39.1 µm by 23.4 to 27.8 µm. The diameter of chlamydospores was 29.6 to 42.5 µm. Oospores had a diameter of 23.6 to 30.2 µm. Isolates were tentatively identified as *Phytophthora cactorum* based on morphological characteristics (Montealegre et al. 2016). DNA was extracted, and PCR amplifications of the rDNA ITS region and cytochrome oxidase subunit I (CoxI) (Kroon et al. 2004) were conducted. Sequences were compared with reference sequences in

GenBank using BLAST search. The 866-bp ITS sequences had identity of 99 to 100% with *P. cactorum* (e.g., EU662221, KJ128036). In addition, the 663-bp CoxI sequences showed 100% identity with three *P. cactorum* isolates (AB688156, HQ708234, and EU660851). The ITS and CoxI sequences of representative isolate Phy.c2 were deposited in GenBank as MT280033 and MT344138, respectively. Pathogenicity of the five isolates (Phy.c1 to Phy.c5) was determined on 2-week-old alfalfa seedlings (cv. Longdong) grown from seeds. Inoculum was prepared by subculturing agar plugs from edges of PDA cultures into the flask with autoclaved millet seeds, incubating at 22°C in darkness for 2 weeks, and shaking by hand every 2 days to ensure uniform colonization. Seedlings were transplanted into pots (12 × 12 cm) filled with autoclaved potting mix infested with millet-seed inoculum of each isolate at a rate of 0.5% (w/w). Control seedlings for comparison were transplanted into pots with uninfested potting mix. There were five seedlings per pot and 12 replicate pots for both inoculated and noninoculated treatments, and pots were kept under 22°C (12 h photoperiod and 65% RH) and watered every 2 days to free draining. Most (87 to 92%) inoculated plants showed stunted symptoms with poorly developed and brown-discolored roots 3 weeks after inoculation; control plants were healthy with no root disease symptoms. To fulfill Koch's postulates, reisolated cultures from discolored root tissues were confirmed as the inoculated isolates by morphological examination and ITS sequencing. The five purified isolates were submitted to the Grassland Culture Collection Center, Lanzhou University as LZU-MsR-Phy.c1 to Phy.c5. This is the first report of *P. cactorum* as a pathogen of root rot on alfalfa in China. *Phytophthora* spp. have been reported causing root rot on alfalfa in America, Australia, and Canada, on other legumes such as chickpea, and on many other crops worldwide (Musial et al. 2005; Tan and Tan 1986; Vandemark and Barker 2003), and *P. cactorum* was reported as a root rot pathogen on lavender in China (Chen et al. 2017). *P. cactorum* may be a significant pathogen associated with root rot in major alfalfa-producing areas in China that use flood irrigation during the growth season.

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