Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum chlorophyti* Causing Peanut Anthracnose in China

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Peanut (*Arachis hypogaea* L.) is an important oil crop in China, mainly in Shandong and Henan Provinces. In 2018 to 2019, a black spot disease on the leaves and stems was found in two fields (a total of 10 ha) in Niulingguanzhuang village of Yishui county in Linyi, Shandong Province, China, and 20 to 40% of plants were infected, thereby reducing the amount of marketable product. Natural symptoms were circular, dark brown-to-black lesions (2 to 6 mm in diameter), with coalescent necrosis on leaves and black necrosis in stems. Six symptomatic leaves and stems collected from six plants of two fields were used for isolation. Infected tissues were surfaced sterilized with 0.5% NaClO for 2 min followed by 70% alcohol for 30 s and washed twice with sterile water. The tissues were placed on PDA at 28°C for 5 days. Felt- or fleece-forming colonies about 4 cm in diameter that were circular, flat, and with a dark center and white narrow margins were formed. Three isolates (LSJF-4, HSGF, and HSJF) were purified, and one culture (LSJF-4) was deposited in the China General Microbiological Culture Collection Center (CGMCC 3.19617). The chlamydospores were dark brown, verruculose, organized in chains and clusters, yellow-brown to black-brown, and mostly spherical, 10.3 to 18.4 × 8.3 to 11.4 μm ($n = 50$, avg. $12.6 \pm 2.1 \times 10.1 \pm 1.1$ μm). The conidia were colorless, crescent or sickle-shaped, with one acute end and one blunt end. They were 16.7 to 24.3 × 3.6 to 5.5 μm ($n = 50$, avg. $21.2 \pm 1.7 \times 4.5 \pm 0.36$ μm), with dark brown, straight, septate

setae, 40.3 to 86.9 × 3.6 to 5.5 μm ($n = 50$, avg. $70.0 \pm 19.1 \times 4.5 \pm 0.7$ μm). Setae were straight, dark brown. These morphological characteristics of our isolates were identical to *Colletotrichum chlorophyti* (Damm et al. 2009). The internal transcribed spacer (ITS, ITS1/ITS4) rDNA of three isolates (LSJF-4, HSGF, and HSJF) was amplified using PCR and sequenced, as described by Damm et al. (2009). The sequencing results (MK796409, MN756650, and MN756651) were submitted to GenBank, and BLAST analysis showed 99.0% identity with those of *C. chlorophyti* (GU227894). Actin (ACT, ACT-512F/ACT-783R), beta-tubulin (Tub2, T1/T2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GDF1/GDR1), chitin synthase (CHS1, CHS-79f/CHS-345R), and histone (H3, Cy1H3F/Cy1H3R) of LSJF-4 (MN688800, MN688797, MN097811, MN688799, and MN688798, respectively) (Damm et al. 2009) were also sequenced and analyzed with BLAST. Results showed high identity of all five sequences to CGMCC 3.19617 (GU228286/GU228287 in GAPDH = 99.6%, GU228384/GU22385 in CHS1 = 96.8%, GU228090/GU228091 in H3 = 99.0%, GU227992/GU227993 in ACT = 98.7%, and GU228188/GU228189 in Tub2 = 98.8%). Therefore, CGMCC 3.19617 was identified as *C. chlorophyti* based on morphological and molecular characteristics. To determine pathogenicity, a conidial suspension containing 10⁶ conidia/ml was sprayed on leaves and stems of six 40-day-old peanut seedlings. Three control plants were sprayed with sterile water. All treated plants were kept moist (>85% RH) at 30°C for 48 h in darkness and then kept at 60% RH and 28°C. Two weeks postinoculation, small, dark, near elliptical lesions appeared on inoculated leaves and stems, similar to naturally infected plants. Controls remained symptomless. *C. chlorophyti* was reisolated from infected tissues and identified based on the previous methods, fulfilling Koch's postulates. The test was repeated twice. *C. chlorophyti* has been reported as a pathogen associated with soybean anthracnose isolated from the Netherlands (Damm et al. 2009). *C. chlorophyti* was first detected on soybean plants imported from Uruguay (Li et al. 2017) in China. To our knowledge, this is the first report of *C. chlorophyti* causing peanut anthracnose in China. *C. chlorophyti* may be a new threat to leguminous plant species, especially peanuts. We proposed paying close attention to taking necessary control measures.

References:

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