Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Cercospora nicotianae* Causing Frog Eye Spot in Cigar Tobacco in Hainan, China

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Cigar tobacco (*Nicotiana tabacum* L.), sun air-cured tobacco, originally from South America, has a main use of rolling cigar wrapper that is different from flue-cured tobacco. In April 2018, diseased leaves were observed in cigar tobacco in some fields in Danzhou city (109.58°E, 19.53°N) and Wuzhishan city (109.52°E, 18.78°N), Hainan. Twenty to 40% of plants were infected (total 8 ha), thereby affecting local leaf production. The symptoms appeared as small, circular or irregular, sunken, brown patches developing into white centers and obvious dark brown margins with necrotic spots of 0.2 to 0.8 cm in most middle and lower leaves at mature stage. To determine the causal agent, 10 leaves from five cigar tobacco plants (cv. Nuowei 2) collected from Danzhou were used for pathogen isolation. Sections of infected leaf tissues were surface sterilized by 5% NaClO for 3 min, 70% ethanol for 40 s, rinsed in sterilized distilled water (SDW), placed onto potato dextrose agar (PDA) plates under aseptic conditions, and incubated at 28°C. After 7 days, predominant and consistent colonies that were nearly circular, smooth edges, generally hard, leathery and wrinkled surface, dense aerial hyphae, and producing red pigment were obtained and purified by picking hyphal tips to PDA at 28°C. One culture, HN4-1-7 from Hainan, was deposited in the Chinese General Microbial

Cultural Center (CGMCC 3.19604). Frog-eye lesions can be covered by tiny black dots on two sides, and the fruiting bodies were amphigenous. Conidiophores were bluish yellow brown and gradually lightened at tips, zero to four knee points, apical or subapical section, zero to 14 septa, measured 45.1 to 506.4 × 2.3 to 11.7 µm. Conidia were needle-shaped to clavate, colorless, erect or curved, measured 37.2 to 169.6 × 1.9 to 5.5 µm. Further comparisons were completed with CGMCC 3.19604 by PCR and BLAST sequence analyses of the partial ITS rDNA region (GenBank no. MK752900), TEF gene (GenBank no. MK881748), ACT gene (GenBank no. MK881749), CAL gene (GenBank no. MT127561), and HIS gene (GenBank no. MT185579) as described by Groenewald et al. (2012). The results showed high identity of all the five sequences to the *Cercospora nicotianae* isolates DQ835073, DQ835099, DQ835119, DQ835146, and DQ835173. Based on the microscopic observation and molecular characteristics, isolate CGMCC 3.19604 was identified as *C. nicotianae*. The pathogenicity of CGMCC 3.19604 was evaluated in greenhouse experiments. Twenty 60-day-old cigar tobacco leaves (cv. H211) and flue cured tobacco leaves (Honghuadajinyuan) were sprayed with hyphae suspensions of CGMCC 3.19604 until runoff, respectively, and the experiment was repeated once. For controls, leaves of two cultivars were similarly wounded and inoculated with SDW. All plants were incubated under 90% humidity and 28°C with a 12-h photoperiod/day. After 9 days, the same disease symptom was observed on inoculated leaves of cigar tobacco as the naturally infected leaves, but not on control leaves. *C. nicotianae* was reisolated from lesions and confirmed by micromorphological characteristics, fulfilling Koch's postulates. All tests were repeated once. To the best of our knowledge, this is the first report of *C. nicotianae* causing frog eye spot in cigar tobacco in Hainan, China. Appearance integrity and uniformity play important roles in high-quality cigar wrappers, but the incidence of frog eye spot can seriously affect appearance quality of cigar wrappers and lead to increased direct losses to local cigar tobacco production. In addition, the symptom of frog eye spot is similar to brown spot disease caused by *Alternaria alternata*, often causing their symptoms to be confused and then delaying prevention at the right time. Because cigar tobacco is a major industry in Hainan, better understanding of its diseases is relevant in order to establish disease control strategies.

Reference:

Groenewald, J. Z., et al. 2012. Stud. Mycol. 75:115.

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