Title Evaluation of phenotypic and molecular criteria for the identification of *Collectotrichum* species causing pepper anthracnose in Taiwan

Author Zong-ming Sheu and Tien-cheng Wang

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Abstract

Pepper anthracnose is one of the major constraints for pepper production in the hot and wet tropics and subtropics. Survey and morphological identification results of AVRDC suggested that *Colletotrichum acutatum* (Ca), *C. gloeosporioides* (Cg) and *C. capsici* (Cc) are the causal agents of the disease in Taiwan. Understanding the pathogen profile is a prerequisite for disease control including breeding for resistance. Therefore, the objective of this study is to evaluate several phenotypic traits and specific primers for differentiating *Colletotrichum* spp. associated with pepper anthracnose in Taiwan for establishing the identification criteria. Several species-specific PCR primers derived from the sequence of the internal transcribed spacer (ITS) region the rDNA gene have been reported for identifying *Colletotrichum* species. Specificity to *Colletotrichum* species of several reported or own designed primers was evaluated in this study. Total of 25 isolates, formally identified as Ca, Cg and Cc at AVRDC, were studied. All isolates were single conidial cultures and maintained on silica gel at 4°C as described by Perkins (1962). Each isolate was transferred from stock cultures to potato dextrose agar (PDA) for 5 days as an initial working culture, which were subsequently transferred to PDA, casein hydrolysis medium (CHM) or potato dextrose broth (PDB) for the characterization of their conidial morphology, colony morphology and growth rate on PDA, protease activity on CHM and PCR reactions with species-specific primers. Primer CalNT2 (5'-GGGGAAGCCTCTCGCGG-3') (Sreenivasaprased et al. 1996) and CgINT (5'-GGCCTCCCGCCTCCGGGCGG-3') (mills et al. 1992) were tested for their specificity to Ca and Cg isolates, respectively. Because no specific primer of Cc was published, four ITS sequences of Taiwan Cc isolates (Coll 318, 322, 388, and 433) were analyzed through multiple sequence alignment with 8 each published Ca & Cg sequence from NCBI website. A specific primer for Cc was designed from ITSI (CcINT; 5'-CTCCCCGTCCGCGGGTGG-3'). To determine their specificity, the three primers were paired with a reverse primer ITS4 (5'-TCCTCCGCTTATGATAGC-3'). Among the evaluated criteria, growth rate, and specific-PCR were considered reliable for assisting species identification. Overall, ten isolates were identified as Ca, which had slow growth rate (usually less than 8 mm/day), salmon-red or olive gray color, mostly with strong protease activity, and both typical and atypical fusiform conidia. Eight isolates were identified as Cg. Most of them grew fast on PDA (more than 10 mm/day) and mostly with gray to dark gray color, occasionally with white or pale orange color. Except on isolate, they showed low or no protease activity. The conidial shape is usually cylindrical with obtuse end. Seven isolates were identified as Cc.

They could be easy distinguished based on their special falcate spore. All of them showed low or no protease activity. Results of specific-PCR showed good specificity of both CaINT2/ITS4 and CcINT/TS4 against tested Ca or Cc isolates, respectively. The expected single specific fragment (490 bp or 460 bp) was amplified from all tested Ca or Cc isolates respectively, but not from the other species. However, poor specificity of primer pairs CgINT/ITS4 was observed. The expected product 450 bp was amplified from 6 out of the tested Cg isolates, as well as from few Ca and Cc isolates. This reflects the amplexity of Cg species in Taiwan, and a specific sequence in ITS for all Cg isolates might not exist. Recently, a total of 228 Taiwan isolates collected from pepper were characterized by using the above-mentioned phenotypic and molecular criteria. Among them, 144 isolates of Ca, 42 isolates of Cg and 42 isolates of Cc were successfully identified. The results indicated that *C. acutatum* is the predominant species caused pepper anthracnose in Taiwan.