Application of ITS-RFLP Analysis for Identifying *Colletotrichum* Species Associated with Pepper Anthracnose in Taiwan

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Anthracnose is one of the major constraints of pepper production in the hot and wet tropics and subtropics. Four causal agents responsible for the disease: *Colletotrichum acutatum* (Ca), *C. boninense* (Cb), *C. gloeosporioides* (Cg) and *C. capsici* (Cc), have been identified in Taiwan by AVRDC. Our previous studies indicated that morphotaxonomic criteria alone is not enough to effectively and accurately differentiate these *Colletotrichum* species, due to the morphological plasticity. This study is aimed to develop a rapid and accurate diagnostic tool for species identification. Sequencing of the internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) in a total of eighteen phenotypic-characterized *Colletotrichum* isolates were accomplished for analyzing nucleotide divergence among four *Colletotrichum* species. Few restriction polymorphisms were presumed to offer a simple way for species differentiation. In this study, restriction fragment length polymorphisms (RFLP) of ITS region resulting from AluI, RsaI, & BamHI digestion were employed to differentiate four *Colletotrichum* species. After PCR amplification with universal primers ITS4/ITS5, the generated products (approximately 600 bp) were respectively digested by AluI, RsaI, & BamHI, and analyzed through 2% agarose gel electrophoresis. Among these 18 isolates, three RFLP patterns were generated by AluI digestion; however, the PCR product of Ca isolates remained un-cut. Two RFLP patterns were generated by *RsaI*, and those of Cg isolates were cut into two fragments (200 & 400 bp). Two RFLP patterns were generated by *Bam*HI, and only those of Cc isolates remained un-cut. All Ca, Cb and Cc isolates had their own respectively distinguishable RFLP patterns, while Cg isolates separated into two groups. The results suggested ITS-RFLP technique is an efficient method for rapid diagnosis of *Colletotrichum* species from pepper. Recently, a total of 412 Taiwan isolates collected from pepper production areas were analyzed through ITS-RFLP fingerprinting. Among them, 245 Ca, 34 Cb, 52 Cc and 69 Cg were identified. Other *Colletotrichum* isolates (3%) were not distinguishable, which inferredto the various inter- and intra-species variations in *Colletotrichum* members. The results reflected the complex pathogen diversity of pepper anthracnose, and Ca is the predominate species in Taiwan.