RFLP identification of *Colletotrichum* species isolated from chilli in Thailand

R. Pongpisutta, W. Winyarat, C. Rattanakreetakul

Acta Horticulturae 973: 181-186: 2013.

Abstract

Fifty eight isolates of *Colletotrichum* species were obtained from anthracnose diseased chilli fruits from different plantations at Chiang Rai, Nakhon Si Thammarat, and Sukhothai. The morphological features studied were colony morphology, conidial sizes, and shapes. All isolates were classified into three species as *C. acutatum* (*Ca*), *C. capsici* (*Cc*), and *C. gloesporioides* (*Cg*). Based on colony characteristics, each species was grouped into 5, 8, and 7 phenotypes, respectively. However, the characteristics of *Colletotrichum* species showed large variation in morphology, so a molecular marker technique was required to distinguish between them. PCR analysis of the internal transcribed spacer region of the ribosomal DNA (ITS1-5.8S-ITS2) was used for identification with universal primers ITS4/ITS5. PCR products of approximately 600 bp were obtained. Three restriction enzymes, *Alu*I, *Bam*HI, and *Rsa*I were used to study polymorphisms in the ITS regions of the isolates. The digested ITS banding pattern of *Ca* showed one band at 500 bp when digested with *Bam*HI, while *Alu*I and *Rsa*I failed to digest the amplified products. *Bam*HI and *Rsa*I did not cut the DNA fragments of *Cc*, whereas *Alu*I digested into two bands of 200 and 400 bp. The amplified product of *Cg*, when digested with *Alu*I, resulted in two bands of 200 and 380 bp, when digested with *Bam*HI resulted in one band of 500 bp, and when digested with *Rsa*I, generated two bands of 200 and 380 bp. Consequently, *Alu*I and *Rsa*I were considered to be useful to distinguish between species of *Ca* and *Cg*. Nucleotide sequencing of ITS products was examined and identified the same three species as the morphological identification. This method is proposed for specific identification of the chilli anthracnose causal agents.