

Waranun Winyarat 2011: Characterization and Aggressiveness of *Colletotrichum* Species Causing Chilli Anthracnose. Master of Science (Plant Pathology), Department of Plant Pathology. Thesis
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Fifty eight isolates of *Colletotrichum* causing chilli anthracnose collected from Chiang Rai, Nakhon Si Thammarat and Sukhothai, using morphological and physiological characteristics were classified into 3 species as *C. acutatum* (*Ca*) *C. capsici* (*Cc*) and *C. gloeosporioides* (*Cg*). Based on colony characteristics, each species was grouped into 5, 8 and 7 phenotypes, respectively. However, *Colletotrichum* species showed highly variation in morphology, so molecular marker technique was required. PCR analysis of the internal transcribed spacer region of the ribosomal DNA (ITS1-5.8S-ITS2), were used to identify with universal primers ITS4/ITS5. PCR products were generated approximately 600 bp. Three restriction enzymes, *AluI*, *BamHI* and *RsaI* were used to study polymorphisms in the ITS regions of the isolates. The restriction fragments were generated by electrophoresis in 2% agarose gels. *AluI* and *RsaI* did not cut the DNA fragments of *Ca* while *BamHI* and *RsaI* failed to digest amplified product of *Cc*. *AluI* was useful to distinguish between species of *Ca* and *Cg*. Nucleotide sequencing of ITS products was examined and identified to 3 species as morphological marker identification. Moreover, *Cg* was clustered into 2 groups from this study.

Protease activity was tested on Casein hydrolysis medium (CHM) and casein from bovine milk medium (CBM). Each isolate produced an enzyme digesting both substrates, a clear zone was developed surrounding the fungal colony. The clear zone was assessed on CHM, *Ca* displayed the greatest width of 10.10 mm while *Cg* and *Cc* showed 2.75 and 1.85 mm, respectively. Also, clear zone was evaluated on CBM, *Ca* and *Cg* produced clear zones of 4.62 and 0.93 mm, respectively whereas *Cc* showed no clear zone. Protease activity was detected in 1%CBM. The result indicated that *Cg* produced the highest protease activity of 6.67 unit/mg while *Cc* and *Ca* illustrated the activity of 4.03 and 3.63 unit/mg, respectively.

Inoculation test was studied on two varieties of chilli fruits as Bangchang and Jinda with mycelial disc (MD) and conidial suspension (CS) and found that MD inoculation of *Ca* and *Cg* on Bangchang fruits showed absolutely infection of 100% disease incidence whereas *Ca* illustrated 96.67% of disease incidence. Whilst the conidial suspension inoculation of three species on jinda fruits was the effective method showing disease incidence of 100%. Aggressiveness evaluation of *Colletotrichum* species on Jinda variety showed greater lesion size than on Bangchang variety. On the other hand, there was correlation of inoculum inoculations illustrating severity on Bangchang variety, disease area of MD inoculation of *Ca*, *Cc* and *Cg* were 16.55, 16.06 and 3.85 %, respectively and of CS inoculation were 7.43, 6.27 and 3.76%, respectively. There was no relationship between clear zone production and protease activity to disease severity. Fungal enzyme may not support disease severity but may be a minor factor to support fungal infection to host plant.

Student's signature

Thesis Advisor's signature