Title Detection of latent infection of *Colletotrichum gloeosporioides*, causal agent of anthracnose disease in raw mango fruits by polymerase chain reaction

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Abstract

Anthracnose disease is a major problem for mangoes meant fro exporting in many countries due to the invasion of *Colletotrichum gloeosporioides* in all mango tissues. The initial infection occurs during flower bloom and then exists as a latent infection in mature green-mangoes. The disease symptom thereafter appears when the fruits are ripening. A rapid method to detect the latent *C. gloeosporioides* infection in green mangoes is becoming necessary for proper assessment of the decay and loss of mangoes when they arrive in the terminal market, and also to provide suitable methods for disease control. Polymerase chain reaction (PCR) is often used to detect and identify plant pathogens using highly specific oligonucleotide primers. In this experiment, CgInt-ITS4 primers amplifying he conserve regions of the 25S-28S rRNA gene specific only on genomic DNA of *Colletotrichum* sp. but not on *Phomopsis* sp., *Botryodiplodia theobromea*, and *Aspergillus niger*. The ITS1-4 (ITS1-ITS2 and 5.8 rRNA gene), ITS4-5 (the region between small-large nuclear rDNA and 5.8 rDNA) and CAP20 (appressorium frming gene) primers showed the specific amplification on those genes of *Colletotrichum* sp., giving amplified fragment sizes of 590, 590 and 610 bp, respectively but also amplified unknown DNA regions from other fungi. In contrast, CgmPG2 primer (designed from polygalacturonase gene) was not specific for *Colletotrichum*. The sensitivity of the PCR, the minimum amounts of fungal genomic DNA needed, was evaluated. The results revealed that the lowest amount of fungal DNA which could be amplified was 1 fg by the ITS1-4 and ITS4-5 primers and 10 pg by Cglnt-ITS1 primer. Latent infection of *C. gloeosporioides* is raw mangoes without disease symptom and ripen mangoes with disease symptom was detected. The results showed that Cglnt-lts4 and ITS1-ITS4 primers could use to detect the latent infection from both samples. On the other hand, amplification by ITS4-ITS5 primers surprisingly showed on extra DNA band from the raw mango samples.