A Loop-Mediated Isothermal Amplification(LAMP)-Based Kit for Rapid Visual Detection of Colletotrichum gloeosporioides

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Anthracnose disease, caused by Colletotrichum gloeosporioides, is among one of the most destructive diseases of plants worldwide. C. gloeosporioides is listed as one of the top 10 fungal pathogens. Early and accurate detection of the pathogen is of the essence and will be the key to success in control. However, current detection methods based on conventional isolation, immunology or PCR are time-consuming and laborious. Loop-mediated isothermal amplification is a newly developed nucleic acid amplification technique which does not require expensive equipment. The aim of this project was to develop a novel visual detection kit to simply and rapidly detect C. gloeosporioides in resource-poor settings. Six potential target genes were screened, and finally LAMP assay targeting at internal transcribed spacer(ITS) was developed. The reaction was optimized to be 65°C for 60 mins. All 25 C. gloeosporioides isolates collected from a range of host species yielded positive by visual detection, which was confirmed by gel electrophoresis. No cross-reactivity was observed with 32 other genetically related fungal pathogens. The detection limit of this LAMP assay was 10 fg purified genomic DNA or 5 conidia/reaction, which is 1000 times more sensitive than PCR. The detection rate of LAMP(87.5%) was higher than PCR(67.8%) in 56 samples gathered from naturally infected plants, and the pathogens were subsequently verified by conventional isolation(LAMP accuracy=100%). The simple kit for visual detection of C. gloeosporioides provides an alternative simple, rapid, sensitive, specific, and inexpensive method suitable to apply directly “on-site” in infected plants in resource-poor settings.

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