Diagnosis of tar spot of corn caused by Phyllachora maydis using Loop-Mediated Isothermal Amplification (LAMP) and conventional PCR.

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The diagnosis of tar spot of corn currently relies on visual assessment. PCR is used for plant pathogen detection but requires specialized equipment and is not suitable for field applications. The loop- mediated isothermal amplification (LAMP) assay is a new method that is emerging as a simple and sensitive diagnostic tool. Here conventional PCR and LAMP tests for diagnosis of Phyllachora maydis in diseased corn leaves are described. The calmodulin gene of P. maydis was used as a target to design PCR and LAMP primers in the Open Reading Frame (ORF) and in a region extended 1 kb on both ends of the ORF to identify a unique region of the gene. Six primer pairs for PCR and five primer sets for LAMP were designed and synthesized. We tested the PCR primers on 13 P. maydis samples from the United States, five P. maydis samples from Ecuador, and 43 isolates from other corn pathogens. One PCR primer pair was specific for all P. maydis isolates. The LAMP assay is performed at 65 °C for 40 min and the amplification is observed by the addition of SYBR Green to the reaction. A green color reaction, that is visible to the naked eye, indicates amplification in the presence of P. maydis DNA, otherwise the reaction turns orange. The LAMP assay is a promising technique for the rapid detection of P. maydis in diseased corn leaves in contrast with conventional PCR which requires a well-equipped laboratory.