Research Article

Development of Rapid Novel Detection Assay for *Dickeya dadantii* based on Loop-Mediated Isothermal Amplification

Sujata Singh Yadav, Yogendra Singh and Anshul Arya

Department of Plant Pathology, College of Agriculture, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India; Email: sujatasingh62@gmail.com

Abstract

Dickeya dadantii is a devastating plant pathogen that causes destructive maceration, a wide host range, and longevity in non-plant substrates and has established stalk rot in sorghum. Molecular detection of pathogen by using PCR-based methods usually requires a well-equipped laboratory and technical expertise. Effective and rapid detection methodologies are required to mitigate yield loss and time constraints associated with monitoring and management of bacterial stalk rot. In the present study, detection of *D.dadantii* has developed by using loop-mediated isothermal amplification of DNA (LAMP) based on the Indigoidine gene with hydroxynaphthol blue dye (HNB). The LAMP reaction provided more rapid and accurate results, amplifying the target pathogen at an optimal temperature of 63 C for 45 min. The sensitivity and specificity of the LAMP assay developed in the study was 10-3 ng mL^{-1}, which was more sensitive than conventional PCR. When HNB dye was added prior to amplification, samples with *D. dadantii* DNA developed a characteristic sky blue colour after the positive reaction, but in the negative reaction mixture, those without DNA or with DNA of other pathogenic bacterial do not showed sky blue colour. The results of the HNB staining method were reconfirmed when LAMP products were subjected to gel electrophoresis. The assay developed in this study can be valuable for rapid point-of-care detection, which is imperative for quarantine, eradication, disease management, and border protection, permitting early detection and prediction of disease and, reducing the risk of epidemics. The LAMP assay can be used to test crude extracts prepared directly from symptomatic lesions.

Key words: Detection, *Dickeya dadantii,* loop-mediated isothermal amplification

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