Development of a Rapid Detection Method for *Peronospora destructor* using Loop-Mediated Isothermal Amplification(LAMP)

In-Seong Lee[1], Seung-Hee Nam[2], and Kwang-Yeol Yang[1*]
[1]Department of Plant Biotechnology, College of Agriculture and Life Science, Chonnam National University, Gwangju 61186, South Korea; [2]Institute of Agricultural Science and Technology, Chonnam National University, Gwangju 61186, South Korea

Abstract

Downy mildew caused by *Peronospora destructor* is one of the most destructive diseases of onion. Early and rapid detection of *Peronospora destructor* is important for the disease forecasting and management. In this study, we developed a loop-mediated isothermal amplification (LAMP) assay using DNA extracted from leaf blades of diseased onion for visual detection. A set of four primers was designed from the internal transcribed spacer (ITS) region of *Peronospora destructor*. The LAMP reaction was optimal at 64°C for 45 min. When hydroxynaphthol (HNB) was added, samples with *Peronospora destructor* DNA developed a sky blue color but those negative control or with the DNA of other plant pathogenic fungi did not. Results of LAMP reaction were reconfirmed by gel electrophoresis. This detection limit of the LAMP assay for *Peronospora destructor* was 7.25fg/㎕ of genomic DNA per reaction, which was 10 times more sensitive than conventional PCR. These results suggest that LAMP assay is suitable for the early and rapid detection of *Peronospora destructor* in infected onion, and contributes to the effective control of onion downy mildew in the field.

Introduction

What is onion downy mildew?

- Onion downy mildew caused by *Peronospora destructor* Berk, is an economically important disease causing losses both in the yield and quality of onion (*Allium cepa* L.).
- Infection in onion causes early defoliation, reduced size and poor storage ability of bulbs. The pathogen attacks the plants at all stages of growth and all parts of the plant may be invaded.

What is Loop-mediated isothermal amplification (LAMP)?

- LAMP is a novel molecular biological detection method that specifically detects genomic DNA by using a set of 6 specific oligonucleotide primers (Notomi et al., 2000).
- In the LAMP reaction, samples are amplified at a fixed temperature through a repetition of two types of elongation reactions occurring at the loop regions: self-elongation of templates from the stem loop structure formed at the 3'-terminal and the binding and elongation of new primers to the loop region (Notomi et al., 2000).
- LAMP is widely applied in many fields for on-site detection because of its cost, rapidness, simplicity of operation, high specificity, and efficiency, such as disease diagnosis (Notomi et al., 2000).
- LAMP can detect target without gel-electrophoresis by using visual-detection reagent like calcein, HNB (Tomita et al., 2008, Goto et al., 2009).

Results

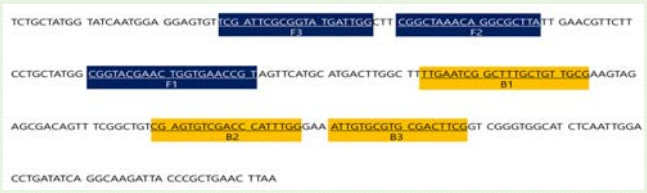

Fig. 1. Schematic representation ITS region of *P. destructor* and LAMP primers used in this study.

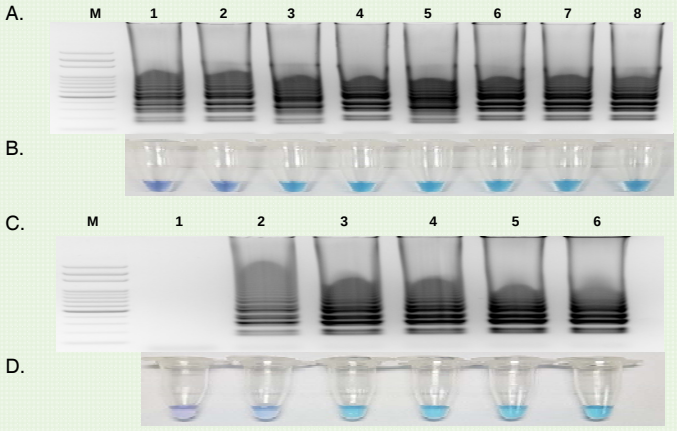

Fig. 2. Optimization of LAMP reaction temperature (A, B) and reaction time (C, D). Assessment was based on HNB visualization of color change in (B)and (D) and on gel electrophoresis in (A) and (C). In (A) and (B), 1 = 55, 2 = 56, 3 = 57, 4 = 59, 5 = 61, 6 = 63, 7 = 64, 8 = 65°C. In (C) and (D), 1= 15 min, 2 = 30 min, 3 = 45 min, 4 = 60 min, 5 = 75 min, and 6 = 90 min. M indicates a 100-bp ladder

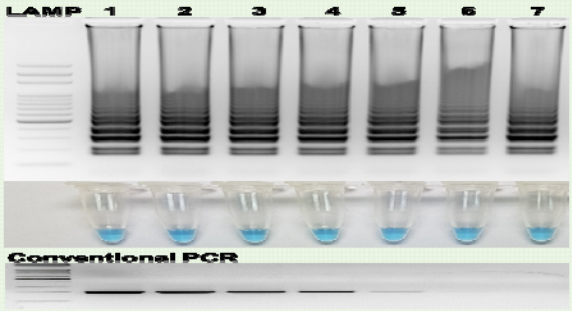

Fig. 3. Comparison of Sensitivity of LAMP assay versus conventional PCR for detection of *P.destructor* genomic DNA. Concentrations of template DNA per reactions LAMP and conversional PCR were; 1 = 7.25 ng/㎕ DNA, 2 = 10^{-1}(725 pg/㎕), 3 = 10^{-2}(72.5 pg/㎕), 4 = 10^{-3}(7.25 pg/㎕), 5 = 10^{-4}(725 fg/㎕), 6 = 10^{-5}(72.5 fg/㎕), 7 = 10^{-6}(7.25 fg/㎕).

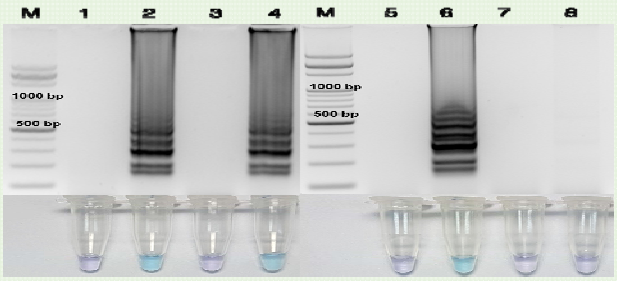

Fig. 4. Specificity of LAMP assay of *P. destructor*. Assessment was based on HNB visualization of color change and gel electrophoresis analysis of the LAMP products. 1, sterile water; 2, *P. destructor*; 3, Normal onion blades; 4, Infected onion blades; 5, sterile water; 6, *P. destructor;* 7, *Sclerotium cepivorum*; 8, *Venturia nashicola*. M indicates a 100-bp ladder

Conclusions

- We have recently developed a LAMP assay for detection of *Peronospora destructor* and demonstrated that the assay is specific and efficient.
- The new LAMP assay will contribute to the effective control of onion downy mildew in the field.

References

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