Loop-mediated isothermal amplification assay: A novel disease diagnostics tool in plant virology

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Abstract

Loop-mediated isothermal amplification (LAMP) is an innovative molecular technique that has revolutionized the field of plant virology diagnostics. This assay offers a rapid, specific, and cost-effective method for detecting plant pathogens, including viruses, under isothermal conditions. Unlike traditional PCR methods, LAMP does not require thermal cycling, making it highly suitable for point-of-care and field applications. The simplicity of the LAMP assay, combined with its high sensitivity and specificity, allows for the early detection and management of plant viral diseases. This article summarizes the principles of LAMP, its application in plant virology and the advantages it offers over conventional diagnostic methods. The potential of LAMP to enhance plant disease management and improve crop health is discussed, high lighting its significance as a novel diagnostic tool in plant virology.

Keywords: polymerase chain reaction (PCR), Loop mediated isothermal amplification (LAMP), rapid, specific, cost effective

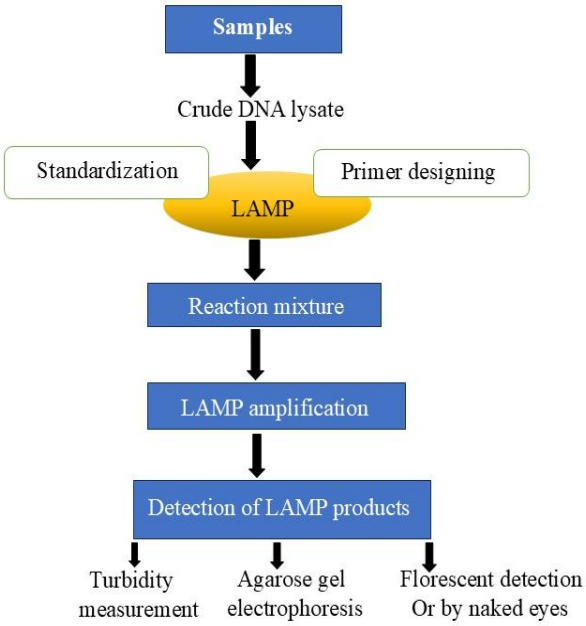
Introduction

Plant viral diseases pose significant threats to agricultural productivity and food security worldwide. Traditional diagnostic methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) (Hutchings and Ferris, 2006) have been widely used for detecting plant viruses, but they often require sophisticated equipment and extensive technical expertise. The development of the LAMP assay represents a breakthrough in the field of plant virology diagnostics. LAMP is a rapid, highly specific, and cost-effective molecular technique that amplifies DNA under isothermal conditions, eliminating the need for thermal cycling. Its simplicity and robustness make it ideal for point-of-care and field applications, enabling early and accurate detection of plant viral pathogens. As a novel tool in plant virology, LAMP offers promising solutions for addressing the challenges associated with plant viral disease diagnostics.

Principle of LAMP methods

- Isothermal amplification: - Unlike PCR, which requires thermal cycling, LAMP operates at a constant temp. (typically60-65^{o}C).
- Strand displacement DNA synthesis: - The LAMP method utilizes a DNA polymerase with strand displacement activity. This enzyme can synthesize new DNA strands while simultaneously displacing the existing strands, creating single-stranded DNA loops.
- Use of multiple primers: - LAMP employs 4-6 primers that recognize 6-8 distinct regions of the target DNA. These primers include: Forward outer primer (F_3), Backward outer primer (B_3), Forward inner primer (FIP), Backward inner primer (BIP) and Loop primers (LF & LB) to accelerate the reaction. The use of multiple primers enhances the specificity of the amplification.
- Formation of loop structures: - The inner primers (FIP & BIP) initiate DNA synthesis by binding to specific regions of the target DNA. As the DNA polymerase extends these primers, it displaces the complementary strands, forming single-stranded loops. These loop structures serve as templates for subsequent rounds of amplification, leading to rapid and exponential amplification of the target DNA.
- High sensitivity & specificity: - The combination of multiple primers and isothermal amplification results in high sensitivity and specificity. LAMP can detect very low amounts of target DNA making it suitable for early diagnosis.
- Ease of detection: - The amplification products can be detected visually through turbidity, color change, interpretation of results without the need for sophisticated equipment.

- Steps in the LAMP reaction: - The primers bind to their complementary sequences on the target DNA. The DNA polymerase extends the primers, displacing the existing strands and creating single-stranded loops. The loop structures enable continuous and exponential amplification of the target DNA. The amplification products are detected using methods such as turbidity, colorimetric dyes or fluorescence.
- Applications of LAMP: - Rapid detection of infectious diseases caused by plant pathogens and pests. Detection of food borne pathogens. Detection of contaminants in water and soil.



Essential components for LAMP method

1. Oligonucleotide primers- LAMP requires a set of specifically designed primers to target multiple regions of the DNA. These primers include:

Forward outer primer(F_3), Backward outer primer(B_3), Initiate DNA amplification by targeting external regions of the target sequence. Forward inner primer (FIP) and backward inner primer (BIP). Designed with two distinct sequences connected by a short linker, targeting internal regions of the target DNA. Loop forward primer (LF) and loop backward primer (LB). accelerate the amplification process by binding to loop regions.

2. Enzymes for LAMP- Bst DNA polymerase derived from Bacillus Stearothermophilus. Exhibits strands displacement activity and operates at a constant temperature of 60-65◦c.
3. Newer DNA polymerases in LAMP- Bst 2.0 DNA polymerase is a more efficient variant of Bst polymerase, offering faster reaction times. Improved amplification efficiency, particularly for difficult templates. Bst 3.0 DNA polymerase enhanced performance for multiplex LAMP assays. Optimized to work with loop primers for faster and more sensitive detection. Phi29 DNA polymerase (limited use) known for high fidelity and strand displacement activity, specialized applications.

Optimization and detection of amplification

- Optimization – LAMP requires 4-6 primers targeting specific regions of the DNA (Notomi et al., 2000). Proper design is crucial for specificity and efficiency. Maintaining a constant temperature (usually 60-65◦c) is essential for the isothermal nature of LAMP. Using a high-fidelity DNA polymerase with strand displacement activity ensures robust amplification. Adjusting mg^{2+} levels can significantly impact the reactions efficiency.
- Detection methods – Adding dyes like SYBR Green or Hydroxy Naphthol Blue allows visual confirmation of amplification. Real-time monitoring using fluorescent probes provides quantitative data. Detecting changes in precipitation is a simple and cost-effective methods (Safavieh et. al) Emerging technologies are making LAMP detection more portable and user-friendly.

LAMP in quantitative analysis

LAMP can be adapted for quantitative analysis by monitoring the amplification process in real-time using fluorescence or turbidity measurements. The time to reach a detectable signal (threshold time) can be correlated with the initial concentration of the target DNA or RNA. Colorimetric LAMP assays use dyes like Eriochrome Black T, which change color during the reaction. By measuring the absorbance at specific wavelengths, the initial DNA concentration can be quantified. Handheld devices have been developed for real-time colorimetric LAMP, combining simplicity with quantitative accuracy.

These devices are particularly useful for point-of-care diagnostic. Quantitative LAMP is used in various fields, including infectious disease diagnostic, food safety testing, and environmental monitoring.

Conclusions

Loop-mediated isothermal amplification (LAMP) has emerged as a transformative tool in plant virology diagnostics. Its simplicity, rapidity, and cost-effectiveness make it highly suitable for detecting plant viruses, even in resource limited settings. By enabling nucleic acid amplification under isothermal conditions, LAMP eliminates the need for complex thermal cycling equipment, making it ideal for point-of-care applications. The techniques high specificity, achieved through carefully designed primers, ensures accurate identification of plant pathogens. Furthermore, advancements in visualization methods, such as colorimetric and fluorescence-based-detection, have enhanced its usability and reliability. LAMP's adaptability to real-time monitoring and quantitative analysis further solidifies its role in modern plant virology.

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