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Recent Developments in Disease Diagnosis of Agricultural Crops and Food Microbes Through LAMP Assays

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Abstract: ***Loop mediated isothermal amplification (LAMP) assay is an accurate and rapid method to diagnose fungal, bacterial and viral pathogens infecting agricultural crops. It has also been proved useful for the diagnosis of gastroenteritis pathogens, Mycobacterium, Listeria, Staphylococcus, Salmonella in food samples. This technique is very useful to screen viral and bacterial strain mutations, analysis of micro - RNAs, plant pathogens and their vectors identification, single nucleotide polymorphisms and detection of genetically modified organisms.***

Keywords: Loop mediated isothermal amplification (LAMP) assay, agricultural crops, Food microbes

1. Introduction

Nucleic acid is an important biomarker of the pathogens infecting agricultural crops and food. However, the gold standard for diagnostic methods is Polymerase Chain Reaction (PCR). Loop mediated isothermal amplification (LAMP) assay is an accurate and rapid method to diagnose fungal, bacterial and viral pathogens infecting agricultural crops. It is a reliable and robust method to detect and identify microbial pathogens. Microbial contamination has become an increasing concern in the 21st century especially gastroenteritis pathogens, *Mycobacterium*, *Listeria*, *Staphylococcus*, *Salmonella* etc. in food items. LAMP assay method has also been used along with other molecular techniques like real - time detection, multiplex methods, chip based assays etc.

Polymerase Chain Reaction (PCR) is one of the standard methods in nucleic acid based molecular diagnostic applications. It amplifies specific target DNA sequences which are detected by gel electrophoresis. This technique is now being used in medicine, agriculture and food industry. LAMP assay method was developed by Notomi et al., to amplify a specific DNA region of hepatitis B virus (HBP) under isothermal conditions. This technique is very useful to screen viral and bacterial strain mutations, analysis of micro RNAs, herbal medicine identification, plant pathogen vectors identification, single nucleotide polymorphisms and detection of genetically modified organisms.

2. Principle and Method

This technique is highly specific owing to the use of 4 - 6 primers that recognize 6 and 8 independent regions containing different sequences within the target DNA. The four primers contained two inner primers (Forward Inner Primer and Backward Inner Primer and two outer primers i. e., Forward Outer Primer and Backward Outer Primer. Inner primers consisted of two different sequences that recognize a sense and an antisense sequence of the target DNA while the outer primers recognize only one external sequence of the target DNA.

This method is based on auto - cycling and high DNA strand displacement activity mediated by Bst polymerase from *Geobacillus stearothermophilus* under isothermal conditions. The reaction consists of two steps: An initial step and a combination of a cycling amplification step with an elongation/recycling step (Mori and Notomi, 2009). The initial step is performed at 65^{0}C. Primers at this temperature are able to anneal to the specific sequence. The forward inner primer hybridizes with the original reverse target sequence and the synthesis of the new forward strand starts from the 3' end flanked by the forward inner primer. The forward outer primer then hybridizes again with the same original reverse target sequence and the synthesis of this new forward strand continues until the enzyme finds the 5' end of the first strand created by the use of the forward inner primer. This separated strand creates a self hybridizing loop at one end owing to the complimentarily of the reverse sequence from the inner primer to the target sequence. In a similar way, the strand displacement of the forward strand occurs and results in dumbbell like DNA structure.

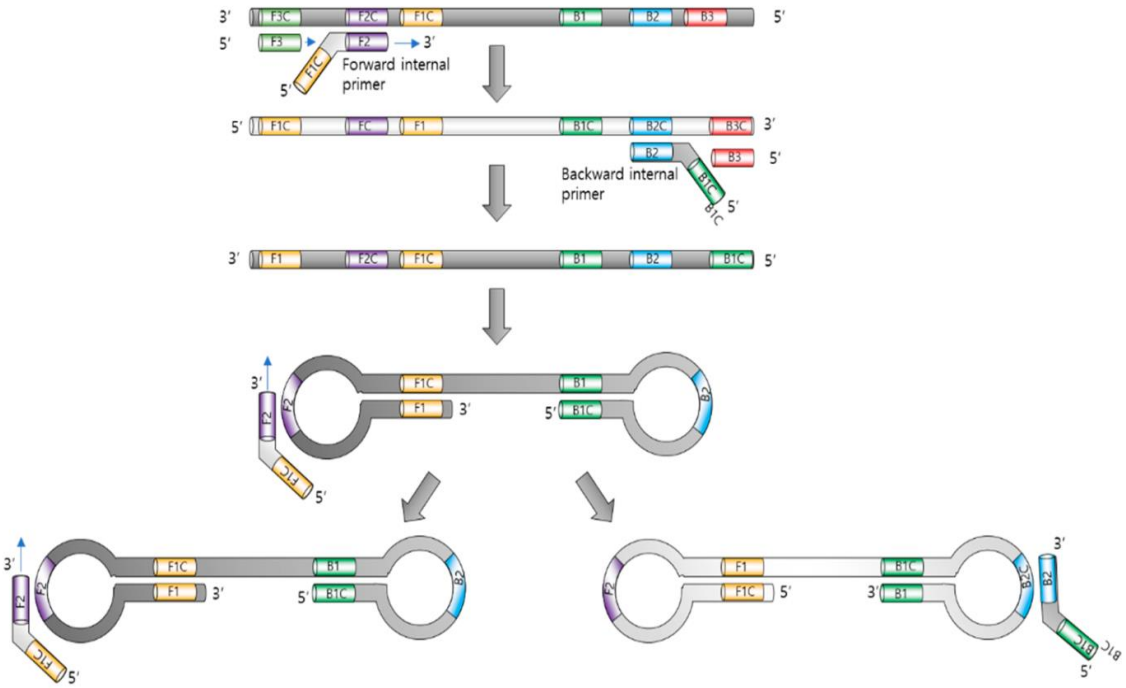

Figure 1: Basic Principle of Loop - mediated isothermal amplification (LAMP)

Reverse transcription LAMP (RT - LAMP) is a LAMP based RNA molecular detection technology which is more sensitive than RT - PCR. In this method reverse transcription reagents are added to the reaction system so that reverse transcription of RNA and LAMP amplification of cDNA can be done in the same tube.

3. Brief Review

Wheat plants are infected by *Zymoseptoria tritici* and *Parastagonospora nodorum* often occur together and form the Septoria leaf blotch complex. Accurate detection of wheat pathogens is, therefore, essential in applying the most appropriate disease management strategy. Through this method, potato virus Y can be detected. Tomato chlorotic spot virus (TCSV) is a tripartite single stranded RNA and transmitted by thrips. The disease magnitude as reported varied from 30 - 40% in South Florida (Webster et al., 2015). The Ortho - tospo has a tendency to mutate in tomato with Sw - 5 gene, therefore, there is a need to detect the virus and accordingly simple rechargeable battery operated hand warmer assisted reverse transcription - loop mediated isothermal amplification assay was developed.

LAMP and RCA (Rolling Circle Amplification) are the commonly used isothermal amplification methods (Becherer et al., 2020). Colorimetric end point detection of RT - LAMP with pH indicator (phenol red) and dual dye based end point detection is made with phenol red as well as hydroxyl - naphthol blue (200 µM) in the field and amplicons from these reactions are made in the laboratory by agarose gel electrophoresis.

The utility of LAMP for detection of pathogens in wheat was discussed by Gutierrez et al, 2022). Feng et al., (2019) reported from Japan that in lettuce pathogens like *Phytophthora pseudolactucae, Pythium irregularie, P. uncinulatum* and *P. spinosum* could be detected by LAMP in lettuce fields and thus is useful for detection of four oomycetes pathogens in lettuce. Fu et al., (2024) developed a variety of LAMP based detection methods for the identification of viruses infecting different crops. Kant et al., (2021) reported the development and application of a Loop - mediated isothermal amplification (LAMP) assay for the detection of *Pseudomonas syringae* pv. *pisi* and *syringae*. Li et al., (2022) reported wide popularity of LAMP technology in the detection of *Salmonella* in foods owing to its simplicity, rapidity and robustness.

LAMP assay could detect the virus presence from infected samples in as little as 10 - 15 min. This quick detection procedure developed for direct in - field diagnosis proved to be reliable and simple. LAMP enables nucleic acid amplification without temperature changes with simple devices besides faster amplification process in comparison to regular PCR. It is thus the most reliable technique because it amplifies nucleic acids rapidly. It requires a simple reagent consisting of Bst polymerase, $MgSO_4$ and primers.

4. Conclusion

LAMP assay technique will be useful in identification of pathogens of diverse and complex nature. There are diseases caused by pathogens viz., *Pythium, Phytophthora, Fusarium, Rhizoctonia, Verticillium* etc. which occur in association with the nematodes like *Meloidogyne* and *Pratylenchus.* This type of assay will, therefore, be an asset for diagnosis of species, pathovar and strain identification in fungi, bacteria and viruses. However, there are challenges to using LAMP for multiplex assays in a single sample and in quantization of target DNA. Further research is warranted to investigate further improvement in LAMP technology for food microbial detection.

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