Loop Mediated Isothermal Amplification

Subjects: Agricultural Sciences & Agronomy | Plant Sciences | Virology

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Definition

The recent progress of molecular diagnostics has allowed the generation of different sophisticated tools, like loop-mediated isothermal amplification (LAMP). This technique has become a well-established in different fields, including medicine, agriculture, and food industry, due to its high specificity, analytical sensitivity, technical simplicity, short analysis time, and low cost. LAMP involves isothermal amplification of target DNA and is highly accordant with point-of-care analysis. It has great potential to improve plant protection diagnostics, especially for in field analyses, detection of plant quarantine pathogens or virus pathogens in early infection stages. In this review, the authors provide detailed overview of the LAMP, describing in particular evolution of the technique, design and main features of the primer set, different visualization methods of LAMP results, its evolution and use in different fields, reporting in detail LAMP application in plant virology, and the main advantages of this technique.

1. Loop Mediated Isothermal Amplification (LAMP)

LAMP is a nucleic acid amplification method developed by Notomi and coworkers [1] to amplify a specific DNA region of hepatitis B virus (HBV) under isothermal conditions. The procedure enabled a fast, sensitive, and specific detection of a selected target opening new possibilities in the diagnostic field. Since this first report, the application of LAMP has been increasingly used as an alternative method to those based on PCR. LAMP, in fact, is continuously being implemented in the medicine, agriculture, and food industry, with approaches that include the screening of pathogen mutations, analysis of fungicide resistant mutations, analysis of micro RNAs, herbal medicine identification, plant pathogen vectors identification, single nucleotide polymorphisms analysis, and detection of genetically modified organisms [2][3][4][5][6]. The reasons for the development of the LAMP methodology were founded on the attempt to overcome some drawbacks of the conventional PCR, a method that requires the acquisition of a high-cost ‘thermal cycler’ equipment. The necessity of a high precision in heating/cooling ramps and temperatures produces occasional reduced specificity for the identification of the selected targets [2].

In addition, the polymerase enzyme is quite sensitive to inhibitors usually present in nucleic acid extracts, especially isolated from plant matrices [7]. Conversely, the isothermal amplification by LAMP does not require any specific equipment, while it provides high specificity owing to the use of four to six primers that recognize between six and eight independent regions, all of them addressing a specific target region. In addition, the robustness of the enzyme used in the LAMP methodology reduces the inhibitors problems [1],[8][9][10]. The benefit of LAMP to be easily adapted for point-of-care analysis makes the technique a valid method for surveys or quarantine programs where rapid, reliable, and specific analysis is required.

2. Principles of LAMP

The LAMP technique 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 [8]. The isothermal amplification is carried out at 60–65 ◦C, the optimum temperature for *Bst* polymerase activity [11]. In the pioneering study of Notomi and coworkers [1], the reaction of LAMP employed a set of four primers that were able to recognize six different sequences within the target HBV DNA. The four primers contained two inner primers (forward inner primer (FIP) and backward inner primer (BIP)) and two outer primers (forward outer primer (F3) and backward outer primer (B3)). Inner primers

consisted of two different sequences that recognized a sense and an antisense sequence of the target DNA, while the outer primers recognized only one external sequence of the target DNA [1].

3. LAMP Application in Plant Virology

Many plants, such as vegetable and herbaceous crops, fruit trees, and weeds, are affected by viral and viroidal diseases, which cause considerable economical losses worldwide. Therefore, it is extremely important to develop rapid and accurate detection methods, in order to accelerate the diagnosis process and apply appropriate intervention measures, especially in agro-ecological contexts, such as intensive cultivations. During the last years, detection of these agents became challenging. The wide use of LAMP technology has significantly facilitated this task. Currently, LAMP has an important influence in plant virology diagnostics. The first LAMP protocols in phytopathology were reported in 2003 by Fukuta and coworkers for the detection of two plant viruses on horticultural crops, tomato yellow leaf curl virus (circ-ssDNA+/−) [12] and japanese yam mosaic virus (ssRNA+) [13]. Subsequently, the LAMP protocols were developed for different plant viruses and viroids including economical and agronomical important agents. for the detection of around 100 viruses, belonging to 23 different families and 47 genera. Furthermore, RT-LAMP protocols were developed for eight viroid species, belonging to two families and four genera. Recently, the development of LAMP portable devices has allowed a ‘real-time’ detection pathogen directly on-field [14][15], facilitating their diagnosis during the routine surveys or in sanitary selection or eradication programs.

4. Advantages and Drawbacks of LAMP Assay

Among all the techniques based on the nucleic acid amplification, the LAMP assay, for its characteristics, high robustness, simplicity, and applicability in a resource-limited context, is widely used as an excellent diagnostic method with possible application in developing countries where many plant diseases are endemic [8].

Other advantages of this technique include the following:

- Compared with other RNA/DNA amplification methods (i.e., PCR, RT-PCR, and RT-qPCR), LAMP shows a comparable sensitivity and specificity [16].

- A typical LAMP test is rapid, and it is completed in about one hour; if loop primers are used, it requires no more than 30 min [10].

- LAMP works at a constant temperature, thanks to the high strand displacement activity of *Bst* polymerase [1].

- LAMP only needs a water bath or a block heater that can be used under field condition [1],[17][18]. Furthermore, it does not require specialized personnel.

- When combined with reverse transcription, amplification of RNA virus sequences can be carried out in a LAMP assay with high efficiency.

- The robustness of the LAMP allows to also analyze unprocessed samples, which can be used as a template, as the activity of the *Bst* polymerase is not influenced by the presence of inhibiting substances. For example, in plant virus diagnosis, it could be possible to use direct crude plant extracts in order to avoid total RNA or DNA extraction, shortening the processing time, allowing the simultaneous analysis of multiple samples, and drastically reducing the total cost for single analysis [19].

- The result visualization can be performed with naked-eye or real-time methods, through the use of SYBR Green I, EtBr, HNB, or Calcein [20]. There is no need for post-amplification processing, reducing the time and the risk of contamination.

- The detection limit of LAMP assay is comparable to an end point PCR, while it is higher compared with other isothermal amplification techniques such as NASBA, 3SR, and SDA, all of which having a limit of detection of less than 10 copies [1].

The major constraint of the LAMP assay is the proper designing of the primer. For this reason, multiplexing approaches for LAMP are less developed than for PCR. Furthermore, as the products of LAMP are a mixture of stem–loop DNAs and a cauliflower-like structure, the technique is not suitable for cloning purposes. The excellent sensitivity of the LAMP method makes it vulnerable to contamination, in fact, one of the principals limiting factors of the LAMP is represented by the possible contaminations that occur when electrophoresis is carried out for the visualization of the results. In fact, the LAMP product is so robust that it does not easily degrade, and this implicates the possibility of carrying over the contamination [21]. This problem can be easily overcome by pre-incubating the LAMP mix with Uracil-N-glycosylase [22]. As described above, another very easy method to prevent carry-over contamination is to use DNA binding dyes or metal ion indicators in the LAMP mixture. With this procedure, it is not necessary to open the tube when the amplification reaction has been completed and the detection is performed as a single “closed-tube” technology [23]. Finally, to avoid contaminations, master mix preparation should be made on ice in less than 30 min.

In plant pathology, it is necessary to avoid the spread of different pathogens and the introduction of new pathogens or pests in a new area [24]. Up to now, many LAMP reports for detecting different plant pathogens causing important plant viral, bacterial, phytoplasma, and fungal diseases are available.

In summary, the LAMP technique represents an important methodology to avoid the spread of endemic diseases or the introduction of dangerous pathogens into new geographic areas.

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Keywords

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