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Loop-mediated isothermal amplification and its limit of detection for diagnostics of plant pathogens

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Abstract: Phytopathology deals with a branch of biology encompassing pathogens that infect plants. Pathogenic fungi, bacteria, viruses, viroids, and phytoplasmas are notorious and hard to control; preventive measures are important for managing disease as early as possible. Age-old management practices are time-consuming and labour-intensive processes. In the past, nucleic acid-based methods, such as hybridization, amplification, and sequencing, have been used extensively for the preliminary identification of plant pathogens. Recently, PCR-based methods have been widely used for the detection of plant pathogens. However, PCR methods are time-bound and require high-quality DNA extraction because of inhibitors' effects on PCR sensitivity. Several isothermal detection techniques are commonly used for the onsite detection of plant pathogens. Among them, loop-mediated isothermal amplification (LAMP) is a paradigm diagnostic tool for early plant pathogen detection. Hence, in this review, we discuss the rapid, reliable, sensitive method of the LAMP assay and the limit of detection (LOD) in different sectors of plant pathology. We also address the advantages and disadvantages of different LAMP approaches and future prospects.

Keywords: PCR; onsite detection; LAMP; sensitivity; diagnostics; LOD

According to United Nations reports, approximately 7.6 bil. people are expected to reach 8.6 bil. in 2030, 9.8 bil. in 2050 and 11.2 bil. in 2100. To meet the demands of a growing population, crop production is likely to increase by approximately 70–80% by improving the efficiency of agricultural units (Raina et al. 2022). In particular, plant diseases cause approximately 10% of global food production losses in developing and emerging countries. The major plant pathogens are viroids, viruses and bacteria, including phytoplasmas, fungi and parasitic plants (Daulagala 2021). Plant pathogenic organisms cause major yield losses in a number of highly valued crops, which has detrimental effects on society and the economy. The practices of people, such as global trade and monoculture, increase the incidence of plant diseases and the occurrence of new diseases. A marginal amount of agricultural product is lost each year due to multiple diseases, and this problem is especially prevalent in devel-

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oping countries, making crop disease management a priority in agricultural-centric economies (Agrios 2005). Proper identification of pathogens is critical for determining the most effective disease management strategy, which relies on understanding the function of the causative organism. Traditional methods of identifying plant pathogens, such as morphological and microscopic observations, require taxonomic expertise and are time-consuming. Despite their importance in pathogen diagnostics, these approaches can yield false results due to the vast array of pathogens and their manifestations, necessitating skilled professionals (Aslam et al. 2017). Alternatively, polymerase chain reaction (PCR)-based techniques have laid the foundation for developing various nucleic acid-based methods for detecting plant pathogens and have consistently yielded reliable results. Numerous improvements and adaptations to PCR have been developed to increase its efficacy, leading to the establishment of quantitative polymerase chain reaction (qPCR) as a reliable method for detecting, quantifying and typing different microbial pathogens (Lau & Botella 2017). While PCR-based techniques are widely used to detect plant pathogens, their applicability for onsite diagnostics is typically restricted because of the need for a thermal cycler and high purity of DNA (Lau & Botella 2017). Nevertheless, PCR-based methods face difficulties when it comes to detecting complex plant pathogens and implementing them in field settings. Recently developed loop-mediated isothermal amplification (LAMP) assay can amplify up to femtogram (fg) level in less than 1 h at a constant temperature of 65 °C with greater specificity. The LAMP assay involves *Bst* polymerase and specially designed 4–8 primers (2–4 primer pairs) that recognise six unique regions of the target DNA with high specificity. The number of cycles continues up to 10^9 copies of the target DNA in less than one hour (Notomi et al. 2000). Isothermal amplification platforms can be performed via chip-based lateral flow devices, making them suitable for in-field conditions (Bhat et al. 2022). Basic tools such as heating blocks can be employed in lieu of thermal cyclers to conduct the assay (Ivanov et al. 2021). The LAMP assay involves sequence-specific fluorescent probes, DNA-binding fluorescent dyes, lateral flow devices, precipitation-induced turbidity measurements, and gel electrophoresis, which can be used for the detection of amplified products (Mori et al. 2001; Craw & Balachandran 2012; Naidoo et al. 2017; Panno et al. 2020). Several isothermal amplification platforms are available, such as helicase-dependent amplification (HDA) (Schwenkbier et al. 2015), recombinase polymerase amplification (RPA) (Rojas et al. 2017) and loop-mediated isothermal amplification (LAMP) (Feng et al. 2018). In this review, we elaborately discuss the LAMP approach-based detection of plant pathogens, including plant pathogenic bacteria, fungi, viruses and viroids (Boubourakas et al. 2009; Tomlinson et al. 2010; Fukuta et al. 2013).

VISUAL DETECTION METHODS

Visual detection of plant pathogens relies entirely on symptoms and changes in the architecture of infected plant tissues. This method considers plant height, leaf colour, root system, and other factors. Pathogens are traditionally identified by characteristic disease symptoms such as lesions, blight, galls, tumours, cankers and wilt. For example, *Agrobacterium tumefaciens* infected plants stimulate tumour production; powdery mildew-infected plants exhibit whitish mycelial growth; rice blast-infected plants exhibit spindle-shaped spots, *Meloidogyne* infected roots exhibit typical root knot symptoms (Khakimov et al. 2022). Visual estimation is performed by trained personnel based on intensive research and interpretation (Mahlein 2016). At present, complex symptoms are more common and challenging for the pathological community. Gomez-Guitierrez and Goodwin (2022) reported that symptoms of wheat plants infected with *Zymoseptoria tritici*, *Parastagnospora nodorum*, *Pyrenophora tritici-repentis* and *Pyricularia oryzae* are more difficult to identify if they both exist. Hence, visual-based observations often do not yield definitive results. Moreover, these time-consuming methods require experienced personnel with sophisticated skills that add a setback for visual detection techniques. Another lacuna of visual identification of plant disease is detection only when significant damage has occurred [European Food Safety Authority (EFSA 2020)]. Hence, to increase the trustworthiness of the diagnosis, microscopy and other approaches are also needed (Mahlein 2016).

Microscope-based detection of plant pathogens employs minute objects, including plant tissues and cells, under a magnifying lens (Buttimer et al. 2017; Rizzo et al. 2021). High-precision microscopes are vital tools that are often used for precise diagnosis. Examination

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of a diseased plant sample via light microscopy is often employed for a diagnosis. Plant pathogenic fungi and nematodes associated with diseased material can often be identified immediately, eliminating the need for further steps (Putnam 1995). The initial assessment of plant disease symptoms typically involves the use of a binocular stereo-microscope. By scrutinizing the infected areas, researchers can identify various pathogenic structures, including hyphal networks, microsclerotia, conidiophores, conidia, and clusters of bacterial cells (Khakimov et al. 2022). In the detection context, microscopy has several advantages, such as high resolution, versatility and accessibility. Some setbacks are the difficulties in sample preparation, shallow depth to visualise the object, and low spatial resolution (Yang et al. 2023). Moreover, traditional approaches for diagnosing pathogens could be difficult and often associated with interpretive skills and knowledge (Rajapaksha et al. 2019).

Serological detection methods in plant pathogen diagnostics utilise the immune response to identify pathogens through antigen-antibody interactions. A number of serology-based approaches are used in the identification and diagnosis of plant diseases. Among them, enzyme-linked immunosorbent assay (ELISA), immunofluorescence and immunostrip are of paramount importance (Wang et al. 2015; Cimmino et al. 2017; Bonants 2022). The ELISA plate contains a 96-well microtiter plate where the antigen of interest binds to the desired antibody in the presence of the enzyme (Stepaniak 2004). Immunofluorescence detects and visualises particular proteins in a sample using antibodies labelled with fluorescent dyes (Yang et al. 2023). Immunofluorescence can identify pathogen-associated molecular pattern (PAMP) molecules secreted at the plant-pathogen interface (Van Vuurde et al. 1991; Jung et al. 1998; Riffaud & Morris 2002). Immunostrip is a fast diagnostic tool that uses lateral flow technology to detect particular plant pathogens within a sample rapidly. This technique uses a lateral flow apparatus to identify specific pathogens rapidly. The sample pads that absorb samples and move them into test zones, which are captured by antibodies coated on membranes, are used to identify the antigen of interest (Tian et al. 2022).

NUCLEIC ACID DETECTION PLATFORMS

PCR-based approach. The nucleic acid sequence is an excellent target tool for plant pathogenic bacteria, fungi and viruses. Techniques such as PCR and LAMP allow the detection of target DNA sequences to a particular pathogen (Venbrux et al. 2023). In PCR-based assays, high-quality purified DNA is profoundly important. To increase the reproducibility of nucleic acid-dependent detection, samples must be free of polysaccharides, phenolic, and toxic substances (Lopez et al. 2009). There are several methods for extracting nucleic acids, each of which differs in the purity of the extract, the concentration of the obtained nucleic acid, and the complexity of the extraction methods (Trippa et al. 2023). Compared with serological-based platforms, PCR is 100 times more sensitive and provides both qualitative and quantitative analysis (Ray et al. 2017). Some common PCR methods include real-time PCR, nested PCR, bio-PCR (Ballard et al. 2011), co-operative PCR (co-PCR) and multiplex PCR (Martinelli et al. 2015). Several variants of PCR have been developed to suit different applications and address specific issues. However, all PCR variants employ high heat to denature double-stranded DNA, expensive thermal cycler and high-quality DNA, limiting their use to detect pathogenic strains (Khater et al. 2017; Wong et al. 2018).

Isothermal detection methods. Isothermal amplification methods are gaining attention over conventional detection approaches (Gill & Ghaemi 2008). Isothermal amplification techniques have recently become more popular, particularly for point-of-care (PoC) diagnosis and applications (Srivastava & Prasad 2023). These techniques are used in high-throughput analysis and have excellent sensitivity and selectivity for amplification (Oliveira et al. 2021). Isothermal detection methods include nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), rolling circle amplification (RCA), helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA) and loop mediated isothermal amplification (LAMP). The most important method of plant pathogen detection is the LAMP assay, developed previously (Notomi et al. 2000). LAMP methods include RT-LAMP, multiplex-LAMP, digital LAMP, and chip-based LAMP. It is a highly effective and sensitive technique for the specific amplification of phytopathogens. LAMP uses *Bst* polymerase, and 4–8 primers uniquely recognise six distinct regions of the sequence of interest. The LAMP method can amplify a few copies of DNA to femtograms (fg) in an hour under iso-

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thermal conditions (Notomi et al. 2000). Unlike the PCR-based technique, which requires a thermal cycler, LAMP allows testing to be carried out with the help of a water bath or heat block (Tomlinson et al. 2010). The displacement activity of *Bst* polymerase and synthesis of a new DNA strand occurs at a constant isothermal temperature of 65 °C (Aliotta et al. 1996). The LAMP approach is a potential methodology for PoC applications without needing specialised staff and expensive equipment (Yigci et al. 2023). Since then, a number of developments have been made to improve LAMP assay efficiency. To transform it into a quick, field-deployable and simple sample screening method, it has been combined with a range of molecular approaches, such as multiplex, real-time, colorimetric and visual detection methods, to easily identify positive samples (Wong et al. 2018). The LAMP method has been enhanced to integrate the latest advancements in diagnostic applications, leading to the development of faster and more efficient PoC testing tools (Garg et al. 2022). Figure 1 shows a series of steps in LAMP amplification, comprising primer sets, *Bst* polymerase, nucleotides, and reaction buffer containing magnesium ions.

LAMP: Principle and working flow. The main principle of the LAMP assay is autocycling and DNA strand displacement, which are mediated by *Bst* polymerase at an isothermal temperature of 65 °C. The LAMP reaction consists of an initial step and a combination of cyclic amplification with an elongation /recycling step (Mori & Notomi 2009). Denaturation of double-stranded DNA into single strands; the FIP (forward inner primer) attaches and binds to the F2c region, and complementary strand synthesis begins in the 3' to 5' direction; the F3 primer attaches and binds to a region upstream from the FIP site; the F3 primer unlocks the newly formed strand by FIP as it synthesises a complementary strand; the backward inner primer (BIP) attaches and binds to the B2c region, and complementary strand synthesis begins in the 3' to 5' directions; the B3 primers attaches and binds to a region upstream from the BIP site; the B3 primer unlocks the newly formed strand by the FIP as it synthesises a complementary strand; As a result of strand synthesised from FIP, BIP, F3 and B3 primers contains F1 & F1c, B1 & B1c and these regions bind each other forming a dumbbell shaped structure with two loop regions; the dumbbell region forms a template for the cycling phase of further synthesis and the rounds of synthesis lead to the formation of a single-stranded structure with a sequence complementary to that of the initial dumbbell; To enhance the rate of amplification, additional primers called as loop primers which bind between F1 & F2 and B1 & B2 region. The cycle was repeated, as mentioned above, to generate large numbers of DNA strands that contained stretches of different sizes of multiple repeats of the initial DNA template, as shown in Figure 2.

LAMP primer design. Proper primer design is crucial when the LAMP platform is employed

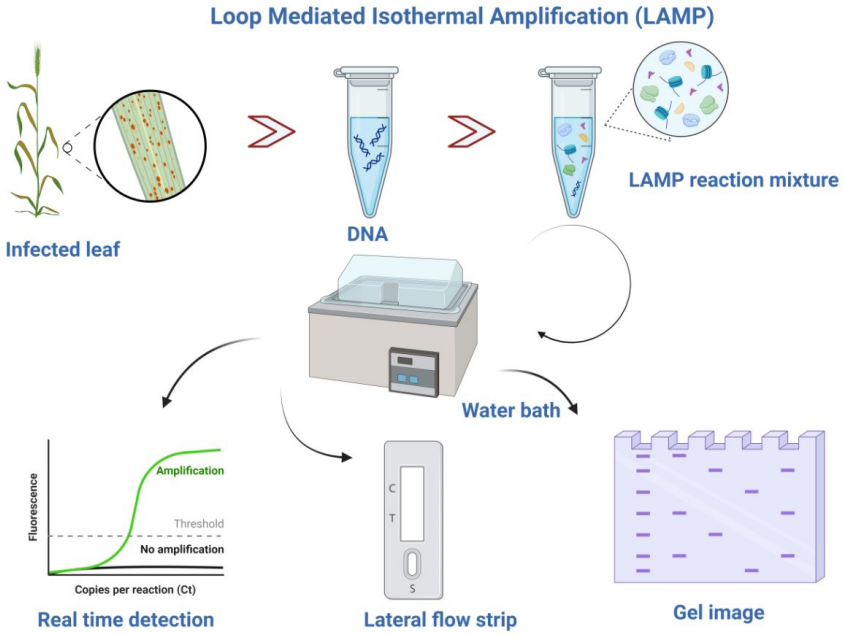

Figure 1. Steps involved in sample processing for the LAMP assay and its detection methods

The LAMP process starts with DNA extraction from an infected plant leaf, followed by amplifying the target DNA in a water bath using LAMP reagents. Detection methods include real-time fluorescence monitoring, lateral flow strips for visual detection, and gel electrophoresis for analysing amplified fragments. Image created with https://www.biorender.com/

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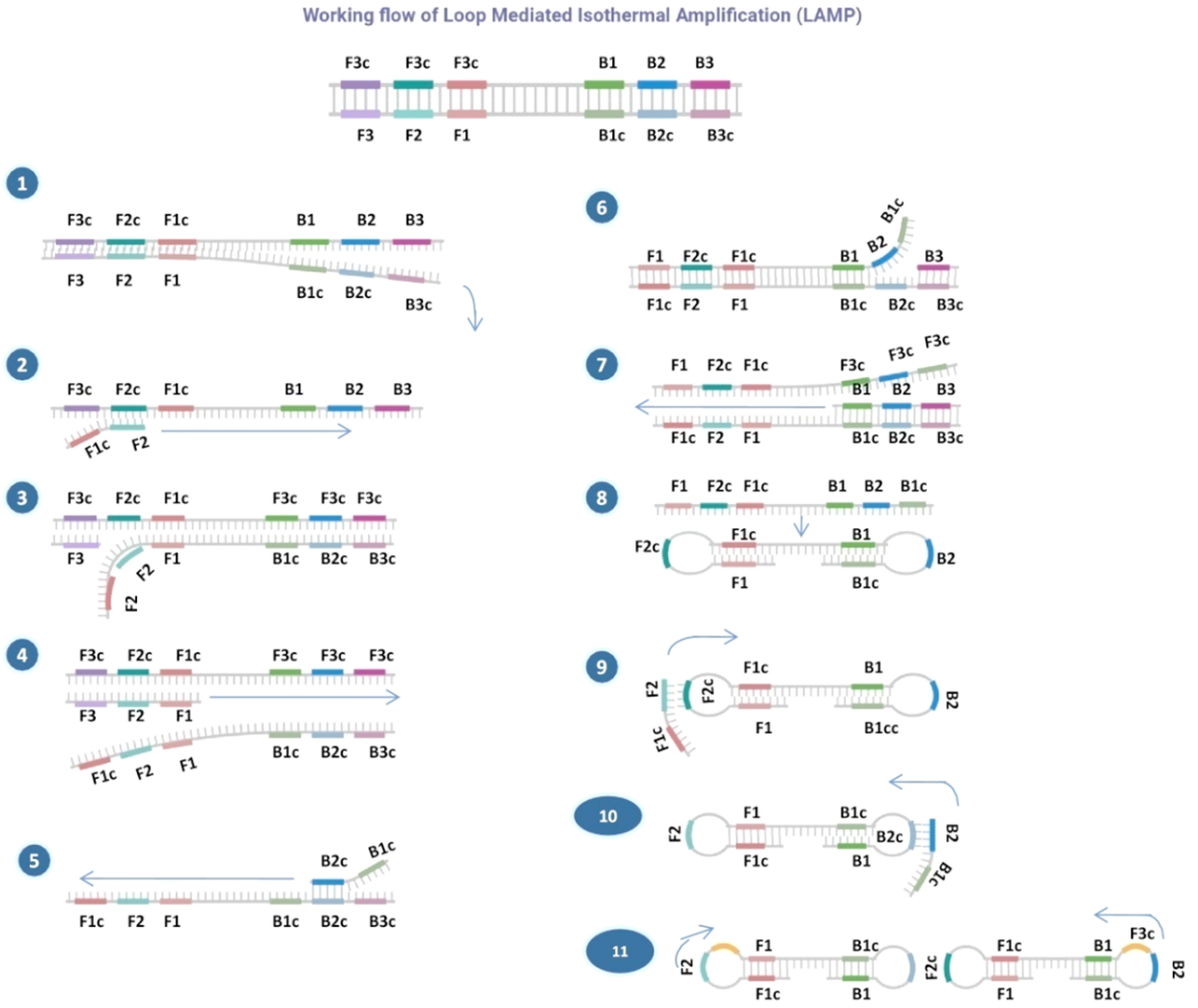

Figure 2. Workflow of loop-mediated isothermal amplification (LAMP)

LAMP primers were designed on the basis of six individual target regions of the DNA sequence. Each step represents a critical part of the LAMP process. [Forward target regions (F1, F2, F3), backward target regions (B1, B2, B3) and complementary strands of forward target regions (F1c, F2c, F3c) and backward target region (B1c, B2c, B3c)]. Image created with https://www.biorender.com/; modified according to Notomi et al. (2000)

for gene amplification. It is advisable to use primer design software only using the LAMP technique. Among the freely available computer algorithm software, PrimerExplorer (version 5) (https://primerexplorer.jp/e/) and NEB primer design (version 1.4.2) (https://www.neb.com/en/neb-primer-design-tools/neb-primer-design-tools/) are the most commonly used platforms. Some expandable open-source LAMP resources, such as electronic LAMP (eLAMP) and LAMP assay versatile analysis (LAVA), are also available (Torres et al. 2011; Salinas & Little 2012).

The following key points should be considered for the optimal primer design:

(i) The inner primer should not have AT-rich terminals on either end;

(ii) There should be a T_m value of 55–65 °C for every domain;

(iii) There should be 40–60 bp from positions 5' of F2 to 5' of F1 and from positions 5' of B2 to 5' of B1;

(iv) The stretch of the amplified DNA region (from the F2 site to the B2 site) should not be 4 200 bp;

(v) HPLC-purified FIP and BIP primers are advised because primer quality may be essential for amplification speed and repeatability (Tomita et al. 2008).

Notably, these programmes can analyse only sequences up to 2 000 nucleotides long. Further-

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more, testing these algorithms revealed that the chosen primer sets occasionally resulted in undesired secondary structures, leading to non-specific amplification. LAMP primer design is topical and challenging because of the limited number of programmes and the requirement for high specificity. LAMPrimersiQ (version 3.10) (https://github.com/Restily/LAMPrimers-iQ) is a new, freely accessible programme for designing high-quality LAMP primers. LAMPrimersiQ is an innovative algorithm that considers precise requirements when choosing primers. It can decode lengthy gene sequences and completely avoid primers that have the ability to generate homo and heterodimers. LAMPprimersiQ is designed using Python (version 3.10), the Biopython library, and the Qt framework. This program is freely available and delivered by SaaS (software as a service) (Akhmetzianova et al. 2024).

Post-LAMP detection methods. The most common methods to detect LAMP products include turbidity measurement, gel electrophoresis, colorimetric assessment with visual observation and ultraviolet (UV) light detection. Other methods include dye staining for colouration, fluorescence labelling, lateral flow assays, electrochemical detection, surface plasmon resonance, chip technology, and gold nanoparticle (GNP) aggregation resulting from DNA binding and pyrosequencing (Wong et al. 2018; Becherer et al. 2020). Real-time monitoring utilising a turbidity meter is widely adopted for detecting LAMP products. This approach involves measuring the LAMP assay every 6 s to detect white precipitation caused by the presence of magnesium pyrophosphate ($Mg_2P_2O_7$), a by-product of the LAMP reaction, at an optical density of 650 nm (Wong et al. 2018). One can visualise amplified DNA amplicons using gel electrophoresis or by adding post-amplification dyes to the mixture. Procedures require the opening of tubes, which is dangerous for contamination (Karthik et al. 2014; Fischbach et al. 2015). Various visualisation methods have been employed for closed-tube amplification reactions to address this issue. DNA-intercalating dyes such as SYBR Green, EvaGreen, Quant-iT Picogreen, ethidium bromide and propidium iodide, as well as other chemicals such as hydroxynaphthol blue (HNB), are used in closed-tube visual evaluation of LAMP products (Duan et al. 2014) and calcein (Zhou et al. 2014) or $CuSO_4$ (Tomita et al. 2008) serve as indicators (Zhang et al. 2013; Panno et al. 2020).

Detection based on turbidity. The turbidity method for detecting amplicons in LAMP products relies on the precipitation of magnesium pyrophosphate. This process involves the release of pyrophosphate ions from deoxynucleotide triphosphate, a byproduct of DNA polymerisation catalysed by DNA polymerase. The released pyrophosphate ions form a precipitate with magnesium ions present in the reaction buffer (Mori et al. 2001). Because of the large amount of DNA that LAMP produces, the pyrophosphate ion concentration is higher than necessary for precipitation. This results in the observable formation of a magnesium precipitate, which is proportional to the amplicon concentration. In contrast, PCR produces insufficient pyrophosphate concentrations to precipitate magnesium pyrophosphate (Saiki et al. 1985; Compton 1991). The merits of turbidity-based detection are that it allows real-time monitoring of the LAMP reaction without needing post-amplification processing and has increased sensitivity. The disadvantages are the instrumental dependency for turbidity measurement and the limited multiplexing of multiple targets in a single reaction, which is challenging (Mori & Notomi 2009).

Detection based on fluorescence probes. Fluorescence-based detection approaches have been extensively utilised over a period of time to observe amplification products via qPCR. These methods utilise SYBR green or TaqMan probes to produce signals (Holland et al. 1991). The primary strengths of fluorescence-based detection methods lie in their simplicity and high sensitivity. Additionally, these methods offer the flexibility of integrating fluorescence detection tools with microfluidic devices (Neethirajan et al. 2011). In accordance with Ristaino et al. (2020), SYBR green and a mobile reader (mReader) were used for onsite detection, and the detection limit of approximately 580 fg/μL was more effective than unaided visual inspection. Various visualisation methods are used in LAMP, including turbidity and colorimetric detection with dyes such as SYBR Green1 and HNB, allowing for visual detection of amplification, as clearly explained in Table 1.

VARIANTS OF LAMP

Real-time LAMP (qLAMP). After amplification of LAMP products, amplicons may lead to cross-

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Table 1. LAMP visualisation methods

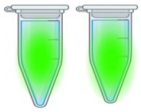
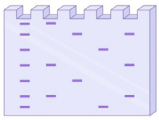
Visualisation methods	Working principle and inference	References
Turbidity 	• Pyrophosphate ions accumulate during the amplification reaction. • When these ions synergise with magnesium ions, they form magnesium pyrophosphate, which precipitates in solution. • Turbidity that results can be seen with the naked eye.	Garg et al. (2021)
SYBR Green 1 	• Fluorescence dye SYBR Green I was used to observe colour changes. • When compared to other dyes, it exhibits a notable level of efficiency. • Mainly resource-limited settings.	Lai et al. (2021); Zhang et al. (2013)
Colorimetric detection 	• The measurement of colour change by the unaided eye is usually the basis for colorimetric LAMP detection. • Which can be done by-use of various indicators such as, pH, metal binding, or DNA binding dyes.	Goto et al. (2009); Miyamoto et al. (2015); Tanner et al. (2015); Shi et al. (2021)
HNB dye 	• Colorimetric detection technique that utilise the metal indicator hydroxynaphthalene (HNB) to determine an optical signal employing an easy readout (with the naked eye). • HNB is sensitive to this pH change, and it undergoes a unique colour change from violet to sky blue in response to the drop in pH.	Reuter et al. (2020)
Gel electrophoresis 	• Agarose gel electrophoresis of amplified DNA product reaction products, displaying ladder-like amplicons as a result of concatamer reaction. • It provides additional information of specificity, non-specific amplification and size of the amplified products.	Edwards et al. (2015)
Lateral flow assay (LFA) 	• The lateral flow assay (LFA) is a paper-based platform suitable for the detection of target analytes • LFA is simple and low-cost, and the colorimetric results that are quickly obtained within 5–30 min. enable the rapid detection of target analytes. • LFA could be integrated with LAMP to meet the detection sensitivity required for practical diagnostic applications.	The milenia hybridetect platform available at: https://www.milenia-biotec.com/en/product/hybridetect/

Image created with https://www.biorender.com/

contamination, resulting in false-positive detection and non-specific amplification. To reduce post-processing errors, qLAMP shows promising and convenient detection for amplified products (Gomez-Guitierrez & Goodwin 2022). Currently, real-time LAMP methods rely entirely on the turbidity of the solution and fluorescence-emitting dyes (Panno et al. 2020). qLAMP eliminates the need for end-point analysis by real-time quantification as well as visualisation (Garg et al. 2022). Currently, fluorescent assimilating probes are standardised and are a solution for the non-specific detection of intercalating dyes (Villari et al. 2017; Gadkar et al. 2018). A number of wheat plant pathogens, including wheat dwarf virus, *Pyricularia oryzae*, *Fusarium* spp., and *Tilletia* spp., have been detected and quantified via real-time LAMP (Gomez-Guitierrez & Goodwin 2022). Moreover, qLAMP offers several benefits, such as gene expression studies, microbial quantification analysis, and high-throughput screening assays. However, the complexity of the assay, technical expertise, and limited multiplexing capability make qLAMP applications lacuna.

Reverse transcription-LAMP (RT-LAMP). RT-LAMP applications aimed at detecting the RNA of pathogenic agents responsible for diseases. It in-

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volves using reverse transcriptase enzyme to generate complementary strands from RNA, followed by amplification and detection. The process is notably straightforward and efficient, as the entire reaction occurs in a single step within a single tube, maintaining a constant temperature for the incubation of all reagents, including both enzymes (reverse transcriptase and *Bst* polymerase). Notomi et al. (2000) pioneered this approach, establishing that RT-LAMP has a promising role in the clinical diagnosis and monitoring of disease outbreaks, particularly in developing countries. Despite these advancements, quantitative RT-PCR currently remains the benchmark for diagnosing viral diseases (Garg et al. 2022).

Four primers were designed for apple chlorotic leaf spot virus (ACLSV) based on the conserved regions identified from the alignment of the coat protein-encoding gene sequences. The reaction conditions were meticulously optimised based on the temperature and reaction time. The developed RT-LAMP method could detect ACLSV DNA at concentrations as low as 0.02 μg/μL at 64 °C. RT-LAMP boasts a sensitivity 100 times greater than RT-PCR (2.29 μg/μL) (Peng et al. 2017). Moreover, RT-LAMP demonstrated great sensitivity to detect the viroid in quick, unrefined plant extracts by effectively detecting citrus exocortis viroid (CEVd) in a 1 : 1 000 dilution of total RNA (i.e., 236 pg). The assay was extremely specific to CEVd, considering there was no cross-reactivity with other citrus pathogens. This novel assay represents the first RT-LAMP technique for detecting any viroid that infects citrus. It offers a straightforward, reliable, precise, and highly sensitive approach for identifying CEVd in citrus plants within Australia (Chambers et al. 2023).

Multiplex LAMP. Multiplex LAMP, a form of multi-target diagnosis, amplifies desired sequences in a single reaction by employing multiple primers. This technique provides swift detection and requires fewer steps than qLAMP and other molecular methods such as PCR and RT-PCR. Numerous researchers have achieved a diagnosis in 20 min or less via multiplex LAMP (Sharma et al. 2022). The primer design process must be conducted precisely to ensure the components' standardisation and eliminate primer dimer formation. Using several genes, multiplex LAMP, improves the selectivity and accuracy by concurrently detecting numerous target sequences. This assay was used for the identification of *Pyricularia oryzae* and *Triticum* lineages in wheat; the mitochondrial NADH-dehydrogenase (nad5) gene served as a positive internal control for plant DNA and was simultaneously amplified with the *Pot2* and *MoT3* genes (Yasuhara-Bell et al. 2018). A multiplex LAMP-based detection system incorporating internal plant control can be utilised for efficient quarantine monitoring of *Phytophthora* pathogens (Hieno et al. 2021). The merits of this technique are its quick analysis time (less than 30 min), capacity to measure a wide dynamic range, compatibility with smartphones and other advanced technical equipment, high sensitivity (one to ten copies) and use of raw materials such as plant tissue.

Lateral flow immunoassay for onsite detection of pathogens. High-throughput microarray systems built on tiny chips are intended for amplification techniques. This method requires few samples or reagents, and several tests can be run concurrently. Extensive sample testing is needed to control the spread of a highly deadly infectious disease. This could be a laborious task with delayed results, encouraging the disease to spread even more. Inadequate sample material has also been added as a setback for onsite detection (Zhang et al. 2019). For quick sample processing, a number of miniature LAMP platforms have been developed, including microfluidic, electrochemical, paper-based and digital approaches. It offers rapid and sensitive amplification, but at the same time, it has a risk of contamination and limited quantification of samples (Garg et al. 2022).

A new microfluidic stirring device called polydimethylsiloxane (PDMS) was inserted with optical fibres to improve the optical signal for detection. Microfluidic-based plant pathogen detection has been used to detect Capsicum chlorosis virus (CaCV) on *Capsicum* (Lin et al. 2015). To detect the LAMP amplicon using lateral flow assay (LFA), a biotin or fluorescein isothiocyanate (FITC)-labelled product is diluted at the final step of the reaction. It typically consists of a test strip with three distinct lines: a positive line, a negative line, and a control line. When a sample is applied, it moves along the strip by capillary action. If the target substance is present, it binds to the labelled antibodies, producing a visible positive line. The control line, which should always appear, confirms that the test is functioning correctly, whereas the negative line indicates the absence of the target substance (Bhat et al. 2022) (Figure 3A). Com-

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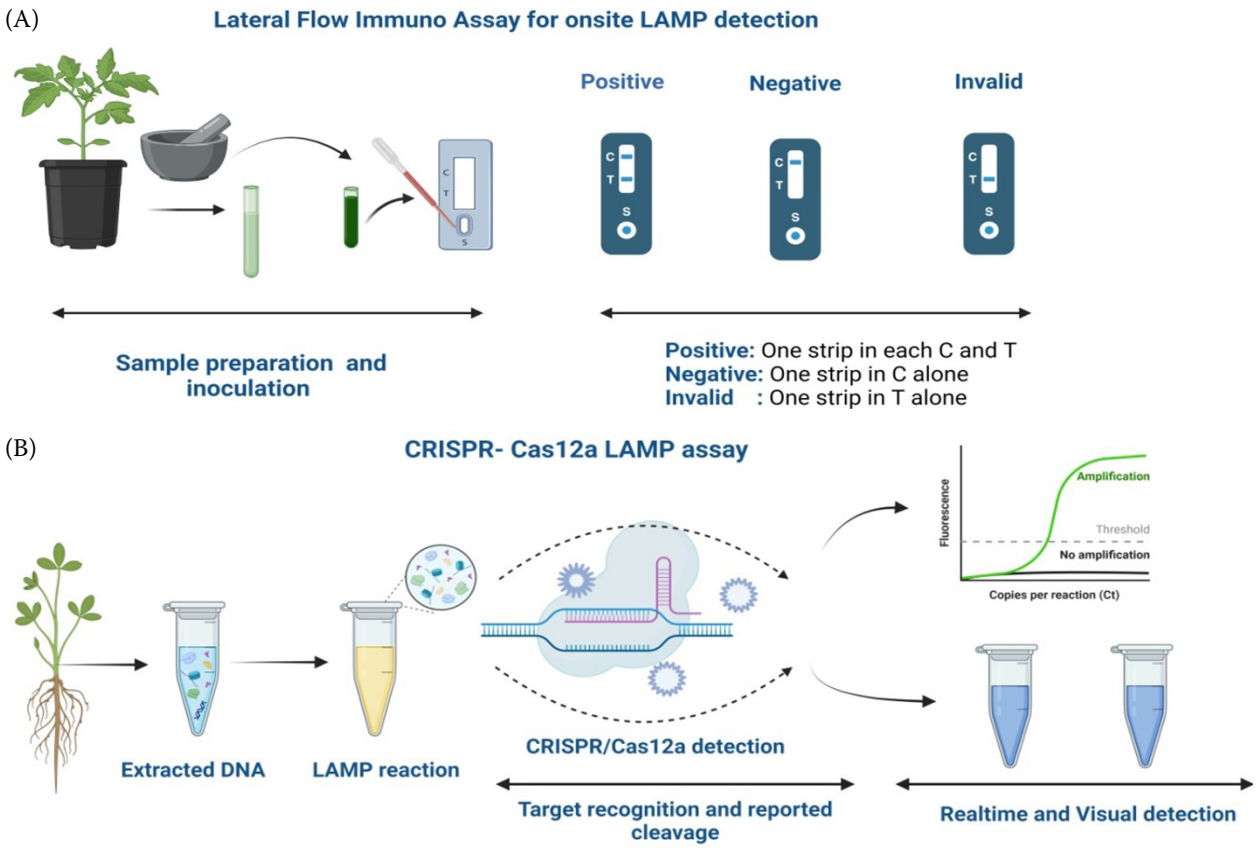

Figure 3. Illustrates two methods for onsite LAMP detection
A – the lateral flow immunoassay, which is based on results of strip patterns (positive, negative, or invalid); B – the CRISPR-Cas12a LAMP assay, which combines LAMP with CRISPR for target recognition and real-time visual detection

mercially available LFAs and LAMP detection have been successfully used to diagnose Ugandan cassava brown streak virus, cassava brown streak virus, and tomato brown rugose fruit virus (Tomlinson et al. 2013). Similarly, chromatographic LFA-LAMP successfully detected *Rhizoctonia solani* infected isolates under in-field conditions. The samples are blue in colour, and these results indicate that the LAMP assay can be easily implemented with LFAs for PoC diagnostics of *R. solani* (Patel et al. 2015).

LAMP-CRISPR-Cas detection. Genome engineering technology has become a potent paradigm tool for the precise modification of any genetic material of interest by adding or deleting the desired gene of interest. The main goal of gene editing technology is to understand the role of specific genes or regulatory elements via different approaches. It begins with DNA extraction from a plant sample, followed by a LAMP reaction to amplify the target DNA. The amplified DNA then undergoes CRISPR/Cas12a detection, where the Cas12a enzyme identifies and cleaves the target sequence. This cleavage event can be monitored in real-time or visually, indicating successful target recognition and amplification. The results are typically displayed through fluorescence, where a signal increase confirms the target DNA's presence (Figure 3B). The integration of CRISPR/Cas into loop-mediated isothermal amplification (LAMP) technology, which meets the 'ASSURED' criteria, enables the implementation of novel point-of-care diagnostic platforms (Atceken et al. 2022). CRISPR-Cas12a integrated with the LAMP assay offers high specificity and reduces non-specific amplification. CRISPR-Cas12a cr-RNA (CRISPR RNAs) reduce false positive results through conserved target sequence design (Pang et al. 2020). Hence, CRISPR-Cas12a offers potential detection of tomato bacterial wilt caused by *Ralstonia solanacearum* under PoC diagnostics (Fan et al. 2023). A LAMP assay integrated with Cas12a detected two California strains of tomato spotted wilt virus (TSWV), an RB strain carrying the C118Y mutation (CA-C118Y referred to as CA-RB) mutation, and one California WT strain with-

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out the mutation (CA-WT). The TSWV LAMP/Cas12a is deployable for in-field diagnostics using either smartphones or heat block units (Shymanovich et al. 2024).

Commercial LAMP assay kit for plant pathogen detection. As an efficient and quick alternative to conventional diagnostic techniques, LAMP has become a powerful tool for quickly and precisely identifying plant diseases. Commercial kits have been produced by a number of firms, some of which are officially recommended for routine surveillance and illness screening programs (Garg et al. 2022). A summary of commercially available LAMP assay kits for identifying different plant pathogens is provided in Table 2.

LAMP assay detection of fungal plant pathogens. The success of LAMP in detecting plant fungi relies on the selection of specific DNA sequences that are unique to the desired pathogen. These sequences can be chosen from conserved regions of the fungal genome, ensuring accurate and reliable detection. LAMP amplification of the *Ssos5* target sequence for detection of *Sclerotinia sclerotiorum*. The results obtained via the HNB method revealed a detection limit of approximately 0.1 fg/μL of genomic DNA (Duan et al. 2014). The primers in this experiment were designed to target the *MoT3* locus, which allows for pathotype differentiation, and the *PoT2* locus, which distinguishes *P. oryzae* from other fungal taxa. *P. oryzae* could be identified with a minimum of 5 pg/μL of DNA per reaction, indicating good detection sensitivity (Harmon et al. 2003). A LAMP assay was conducted to identify the target gene of *CesA4* in grapevine downy mildew caused by *Plasmopara viticola*. The LAMP protocol was validated in grapevine samples collected from different areas; of the 78 samples tested, only 62 samples were positive (79.5%). The LAMP assay is highly sensitive and can detect even ~20 fg of *P. vitocola* genomic DNA (Marimuthu et al. 2020). A LAMP assay was conducted for brown spots of rice caused by *Bipolaris oryzae* to specifically target the glycoside hydrolase gene family encoding 13 proteins using six specific primers. The detection limit is approximately 100 fg of *B. oryzae* genomic DNA (Lakshmi et al. 2022).

LAMP assay detection of bacterial plant pathogens. The success of LAMP in detecting plant bacteria relies on the selection of specific DNA sequences unique to the target pathogen. These sequences can be chosen from conserved regions of the bacterial genome, ensuring accurate and reliable detection. The LAMP test was developed using 96 strains, comprising all species of *Dickeya* and *Pectobacterium* and other closely relevant genera and five hosts; no instances of false positives or false negatives were found. For sensitivity studies, 10-fold serially diluted DNA isolated from *D. fangzhongdai* was used to determine the assay detection limit, both in the presence and absence of crude host extracts (taro, orchid, and onion). All sensitivity tests had an upper detection limit of 100 fg, and the host crude extracts did not have a negative impact (DeLude et al. 2022). Potato ring rot caused by *Clavibacter sepedonicus* infected samples was serially diluted to assess the sensitivity of the LAMP primers. This study examined six distinct dilutions of pathogen DNA ranging from 10 ng to 0.1 pg/μL. Consequently, 10 pg/μL *C. sepedonicus* DNA was the minimal detection limit required for diagnosis (Sagcan & Turgut Kara 2019). Tomato bacterial speck caused by *Pseudomonas syringae* pv. *tomato* detection was performed to identify the *hrpZ*

Table 2. Commercial LAMP assay kit for plant pathogen detection

Company	Product name	Pathogens detected	Reference
Agdia	AmplifyRP® XRT	Viruses, Bacteria, Fungi	https://www.agdia.com/
OptiGene	Genie® III & Genie II	African cassava mosaic virus; Citrus tristeza virus; *Candidatus* Phytoplasma vitis, potato virus A, X, Y	https://www.optigene.co.uk/
Eiken chemical	Loopamp® Plant DNA Detection Kit	*Phytophthora, Ralstonia*	https://www.eiken.co.jp/en
Nippon Gene Material Co., Ltd	Phytoplasma universal detection LAMP kit	*Ca.* Phytoplasma japonicum	https://nippongene.com/english/index.html
Agdia	AmplifyRP® Acceler8™	*Xylella fastidiosa*	https://www.agdia.com/
ICGENE	–	Citrus tristeza virus, plum pox virus, tomato leaf curl New Delhi virus	https://icgene.com/en/

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gene of interest. In the sensitivity test, the detection limit of the LAMP assay was 1.61 × 10 fg/μL for genomic DNA and 1.05 × 10^3 colony forming unit (CFU)/mL for bacterial suspensions without DNA extraction (Chen et al. 2020). Similarly, Zebra chip disease of potato caused by *Candidatus* Liberibacter solanacearum was identified through LAMP detection by designing a set of primers that target 16S rDNA, and the band pattern was visualised through gel images, which revealed a detection limit of 100 to 0.001 ng of pathogen DNA (Ravindran et al. 2015). Bacterial wilt of potato caused by *Ralstonia solanacearum* was serially diluted to determine the sensitivity of the LAMP assay; consequently, 10 pg/μL *R. solanacearum* was the minimal detection limit required for diagnosis (Archana et al. 2024).

The LAMP assay has emerged as a potent molecular diagnostic tool for various pathogens, including phytoplasmas. These specialised bacteria infect plants, causing diseases with significant agricultural and economic implications. The LAMP assay has gained prominence in phytoplasma detection in recent years because of its simplicity, rapidity, and high sensitivity. LAMP reaction based on the 16S rDNA gene was conducted on the Napier grass accessions using three pairs of primers, including NGS-BIP, NGS-FIP, NGS-B3, NGS-F3, NGS-FL and NGS-BL (Obura et al. 2011).

LAMP assay detection of viral pathogens. The LAMP assay has emerged as a highly effective and efficient technique for identifying viral diseases. Operating under isothermal conditions, the LAMP assay offers a rapid and sensitive means of amplifying viral nucleic acids. By targeting specific regions of the viral genome, this molecular diagnostic tool provides an accurate method for early detection, assisting in the timely management and control of viral infections. Owing to its simplicity, speed, and applicability in various settings, the LAMP assay represents a promising approach for accurate and accessible viral disease diagnosis. Wheat yellow mosaic virus (WYMV) was identified through RT-LAMP by four distinct sets of primers designed to target the virus coat protein. The reaction specificity was assessed against two wheat viruses, Chinese wheat mosaic virus (CWMV) and barley stripe mosaic virus (BSMV), while RNA from healthy wheat plants served as the negative control (Chen et al. 2021). The RT-LAMP technique demonstrated a sensitivity 100 times greater than RT-PCR's and could detect RNA diluted to 10^5 (Zhang et al. 2011). A LAMP assay was conducted for cotton leaf curl disease caused by cotton leaf curl multan betasatelite virus to specifically target the βc1 gene by using six specific primers and a detection limit as low as 22 copies of viral DNA (Rafiq et al. 2021).

LAMP assay-based detection of viroids. Viroids are small, circular RNA molecules that could incite diseases in plants. Detecting viroids is crucial for plant health management and preventing the spread of plant diseases. Traditional methods for viroid detection often involve complex procedures and are time-consuming. In recent years, molecular biology platforms such as LAMP have emerged as potent tools for quickly and accurately identifying plant pathogens, including viroids. An experiment was conducted to identify the specific target sequence of the potato spindle tuber viroid (PSTVd). Amplified DNA products were observed through either colorimetry or electrophoresis. The lower detection limit of PSTVd is approximately 100 pg dilution. Similarly, other pospiviroid species were identified by the same LAMP method protocol (Tseng et al. 2021). The comprehensive LAMP detection limits for certain key plant pathogens, including bacteria, fungi, viruses, phytoplasmas and viroids, are provided with detection thresholds ranging from bacterial pathogens detectable at 10 CFU/mL to viral pathogens at 1 fg/μL, as presented in Table 3.

ADVANTAGES AND DISADVANTAGES OF LAMP DETECTION

Advantages. LAMP can be conveniently conducted under isothermal conditions. The strand displacing activity of the *Bst* polymerase allows the generation of a new DNA strand under the isothermal condition of 65 °C (Aliotta et al. 1996). Moreover, *Bst* polymerase exhibits reduced susceptibility to inhibitors during amplification reactions and does not necessitate advanced equipment such as an expensive thermal cycler. Using two sets of primers for a concise DNA segment (approximately 200–500 bp) enhances the assay specificity, resulting in a distinctive reaction product. LAMP is 10–100 times more sensitive than the conventional PCR approach (Le et al. 2010; Li et al. 2007; Bhat et al. 2013). Compared with any other nucleic acid amplification method, LAMP

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Table 3. List of important plant pathogens and their LAMP detection limits

No.	plant diseases	Causal agent	Detection limit (dilution copies of pathogen DNA)*	References
Bacteria				
1.	Bean bacterial common blight	*Xanthomonas phaseoli* var. *phaseoli*	10 CFU/mL (cell suspension); 1 fg/mL (DNA)	de Paiva et al. (2020)
2.	Common bacterial blight	*Xa. fuscans* subsp. *fuscans*	10 CFU/mL (cell suspension); 1 fg/mL (DNA)	de Paiva et al. (2020)
3.	Soft rot and bleeding canker	*Dickeya fangzhongdai*	100 fg (18–20 genome copies)	DeLude et al. (2022)
4.	Quick decline syndrome of olive	*Xylella fastidiosa*	~0.02 pg/µL of DNA	Aglietti et al. (2019)
5.	Bacterial shot hole of peach	*Xa. arboricola* pv. pruni	1.8 ng/µL of genomic DNA	Li et al. (2019)
6.	Angular leaf spot of strawberry	*Xa. fragariae*	2×10^3 CFU/mL (pure bacteria) 300 CFU/mL (leaf & petiole)	Wang & Turechek (2016)
7.	Bacterial spot of tomato and pepper	*Xa. gardneri*	1 pg/µL of genomic DNA	Stehlikova et al. (2020)
8.	Bacterial blight of pomegranate	*Xa. axonopodis* pv. *punicae*	1 pg/µL of genomic DNA	Usharani et al. (2017)
9.	Bacterial spot of tomato and pepper	*Xa. euvesicatoria*, *Xa. vesicatoria*, *Xa. gardneri* and *Xa. perforans*	100 fg of genomic DNA and 1 000 fg in samples spiked	Larrea-Sarmiento et al. (2018)
10.	Bacterial blight of rice	*Xa. oryzae* pv. *oryzae*	10 fg to 0.01 ng genomic DNA	Lang et al. (2014)
11.	Bacterial canker of citrus	*Xa. citri* pv. *citri*	1 ng/µL and 10 fg/µL	Webster et al. (2022)
12.	Fire blight of Rosacae family	*Erwinia amylovora*	1.2×10^9 CFU/mL to 1.2×10^2 CFU/mL	Buhlmann et al. (2013)
Phytoplasma				
13.	Pear decline	*Candidatus* phytoplasma pyri	10^4 dilution	Siemonsmeier et al. (2019)
14.	Areca palm yellow leaf	*Ca.* phytoplasma	200 ag/µL	Yu et al. (2020)
15.	Napier grass stunting	*Ca.* phytoplasma	7.5 pg/µL of DNA	Wamalwa et al. (2017)
Fungi				
16.	Downy mildew of grape	*Plasmopara viticola*	33 fg of DNA	Kong et al. (2016)
17.	Fusarium head blight	*Fusarium graminearum*	0.004 ng	Gomez-Gutierrez et al. (2022); Harmon et al. (2003)
18.	Wheat blast	*Pyricularia oryzae*	5 pg and 1 pg/µL DNA	Gomez-Gutierrez et al. (2022); Harmon et al. (2003)
19.	Yellow rust of wheat	*Puccinia striiformis* f. sp. *tritici*	2 pg/µL	Gomez-Gutierrez et al. (2022); Harmon et al. (2003)
20.	Loose smut of wheat	*Ustilago nuda-tritici*	100 fg/µL	Gomez-Gutierrez et al. (2022); Harmon et al. (2003)
21.	Sunflower black stem	*Phoma macdonaldii*	100 fg/µL	Sun et al. (2022)
22.	Sheath blight of rice	*Rhizoctonia solani*	1.65 fg/µL template DNA	Choudhary et al. (2020)
23.	Late blight of potato	*Phytophthora infestans*	584 fg/µL	Ristaino et al. (2020)
24.	Phytophthora wilt of lettuce	*Phytophthora pseudolactae*	100 fg/µL	Feng et al. (2019)
25.	Damping-off of lettuce	*Pythium spinosum*	10 fg/µL	Feng et al. (2019)
26.	Damping-off of lettuce	*Pythium uncinulatum*	100 fg/µL	Feng et al. (2019)
27.	Dwarf bunt of wheat	*Tilletia controversa*	5 pg of genomic DNA	Sedaghatjoo et al. (2021)

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Table 3. to be continued...

No.	plant diseases	Causal agent	Detection limit (dilution copies of pathogen DNA)*	References
28.	Rice blast	*Magnaporthe oryzae*	100 fg	Prasannakumar et al. (2021)
29.	Fusarium wilt of chickpea	*Fusarium oxysporum* f.sp. *ciceri*	10 fg of genomic DNA	Ghosh et al. (2015)
30.	Rhizome rot of lotus	*F. commune*	10 pg/µL	Deng et al. (2022)
31.	Leaf rust of wheat	*Puccinia triticina*	100 fg dilution	Manjunatha et al. (2018)
Virus				
32.	Tomato leaf curl New Delhi disease of solanaceae	Tomato leaf curl New Delhi virus	~50 × 10^{-8} ng/µL	Caruso et al. (2023)
33.	Tomato leaf curl New Delhi disease of ridge gourd	Tomato leaf curl New Delhi virus	10^{-10}dilution of DNA	Naganur et al. (2019)
34.	Rice tungro disease	Rice tungro bacilliform virus	10^{-6} dilution of DNA	Ladja et al. (2018)
35.	Rice ragged stunt disease	Rice ragged stunt virus	10^{-1}–10^{-3} dilution of cDNA	Lai et al. (2018)
36.	Tobacco streak disease of cotton	Tobacco streak virus	10-fold dilution	Gawande et al. (2019)
37.	Piper yellow mottle disease	Piper yellow mottle virus	10^{-1} dilution	Bhat et al. (2013)
38.	Cucumber mosaic disease	Cucumber mosaic virus	10^{-4} dilution	Bhat et al. (2013)
39.	Mesta yellow vein mosaic disease	Mesta yellow vein mosaic virus	10^{-5}dilution	Meena et al. (2019)
40.	Abaca bunchy top disease	Abaca bunchy top virus	10^{-4} ng/µL	Galvez et al. (2020)
41.	Sugarcane mosaic disease	Sugarcane mosaic virus	100 ng/µL – 1 fg/µL	Keizerweerd et al. (2015)
42.	Maise streak disease	Maize streak virus	100 ng/µL – 10 fg/µL	Tembo et al. (2020)
43.	Rose rosette disease	Rose rosette virus	1 fg/µL of transcript	Babu et al. (2017)
44.	Chilli veinal mottle disease	Chilli veinal mottle virus	10 fg of RNA	Jiao et al. (2020)
Viroid				
45.	Potato spindle tuber disease (PSTD)	Potato spindle tuber viroid	100 pg	Tseng et al. (2021)
46.	Columnea latent disease	Columnea latent viroid	100 pg	Tseng et al. (2021)
47.	Tomato chlorotic dwarf disease	Tomato chlorotic dwarf viroid	100 pg	Tseng et al. (2021)
48.	Pepper chat fruit disease	Pepper chat fruit viroid	100 pg	Tseng et al. (2021)
49.	Tomato apical stunt disease	Tomato apical stunt viroid	100 pg	Tseng et al. (2021)
50.	Tomato planta macho disease	Tomato planta macho viroid	100 pg	Tseng et al. (2021)
51.	Coconut cadang-cadang disease	Coconut cadang-cadang viroid	84 fg/µL	Soliman & El-Matbouli (2006)

The table concisely overviews various plant diseases, their causal agents, and the respective LAMP assay detection limits; *CFU/mL – colony forming unit per millilitre; ng/µL – nanogram per microliter (a measure of pathogen DNA concentration); pg/µL – picogram per microliter; fg/µL – femtogram per microliter

can be completed in 30 min, as opposed to the minimum 90 min duration of PCR. Moreover, owing to the notable shift in pH from alkaline to acidic during LAMP amplification, the incorporation of pH indicator dyes enables monitoring of the LAMP reaction, with a distinct colour change. The affordability, instrument-free visualisation, and adaptability of pH-sensitive indicator dyes might enable the complete fulfilment of the potential of isothermal amplification technologies (Tanner et al. 2015).

Disadvantages. LAMP is susceptible to materials that are present in aerosols or cross-contamination. For this reason, it is advised to keep

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the rooms ventilated and test various samples independently. Since LAMP requires two reactions, one to detect the inhibitors and the other to amplify the material. Although LAMP is an excellent diagnostic tool, its byproducts are not useful for other analyses, such as cloning or sequencing (Sahoo et al. 2016). The limited suitability of the LAMP assay for exploring newly discovered genes with minimal information stems from its high specificity in detecting short target segments. Moreover, a comprehensive understanding of the structural features of the target gene is imperative. Excessive concentrations of dyes, such as calcein, HNB and ethidium bromide, hinder polymerase activity and disrupt product stability, thereby diminishing the effectiveness of LAMP (Tanner et al. 2015). To mitigate these potential risks, it is crucial to handle the process cautiously, preferably opting for colour indicators instead of electrophoresis. Moreover, the outcome of the assay can be influenced by the amplification time; an optimal duration falls within the range of 60–120 min. The duration may be shorter when loop primers are employed in LAMP. Prolonged incubation can lead to negative samples being erroneously identified as false positives. To ensure satisfactory outcomes, the mastermix preparation was completed in less than half an hour (Francois et al. 2011).

CONCLUSION & PROSPECTS

LAMP methods are emerging as a transformative force in plant pathogen diagnostics, offering various detection possibilities. The future of plant disease diagnostics is poised for significant transformation through the promising capabilities of the LAMP technique. With the evolution of technology, portable LAMP devices are expected to increase, facilitating swift and accessible diagnostics directly in the field. Furthermore, ongoing research endeavours are anticipated to result in the development of multiplex LAMP platforms, allowing the concurrent detection of several pathogens in a single reaction. This advancement greatly enhances efficiency and streamlines comprehensive plant disease testing protocols. Integrating LAMP with emerging point-of-care technologies and digital platforms will likely enhance data analysis and interpretation communication and ultimately enable real-time monitoring and reporting of results. Continuous efforts to refine primer design and optimise enzyme formulations aim to increase the sensitivity and specificity of LAMP assays, increasing their reliability for detecting low-titer infections or challenging pathogens. Additionally, rapid and precise detection may find applications in plant breeding programs, aiding in the early identification of pathogen-resistant cultivars. The establishment of global surveillance networks, facilitated by LAMP assays and networking technologies, has the potential to contribute significantly to monitoring the global spread of plant diseases. Ultimately, ongoing research initiatives, collaborative efforts, and technological advancements will be pivotal in shaping the future trajectory of LAMP in plant disease diagnostics and management, fostering its increased adoption across the agriculture industry and integration into routine plant disease monitoring programs.

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