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REVIEW ARTICLE

RECOMBINASE POLYMERASE AMPLIFICATION AS RAPID DIAGNOSTIC TOOL FOR PLANT DISEASES

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ABSTRACT

Different kinds of diseases are seen in plants throughout their lifecycle. Pathogens like fungi, bacteria, viruses, and nematodes are found to cause these diseases, resulting in huge yield loss to farmers. Different diagnostic methods such as ELISA, Polymerase Chain Reaction (PCR) and microscopy provide effective results but are costly, time consuming and require an established laboratory. Recombinase Polymerase Amplification (RPA) can be used as an alternative tool for these diagnostic tools. RPA is an isothermal nucleic acid amplification technique that generally works at a temperature range of 25-43°C, which is also feasible to operate in field conditions. This tool provides amplification results within 15-30 minutes, which makes it a better alternative to time consuming tools. Furthermore, it is comparatively cheaper than other commercial diagnostic tools. Amplicons can be detected using different detection platforms such as colorimetric dyes, smartphone assisted fluorescence imaging, and lateral flow dipsticks. RPA can also be integrated with other molecular tools like the CRISPR-Cas system and loop-mediated isothermal amplification (LAMP) to provide improved specificity and accuracy. RPA stands as a mobile, cost effective and accurate diagnostic tool that can play a major role in the early diagnosis of diseases resulting reduction in economic losses and an enhancement of biosecurity.

KEYWORDS

Recombinase, amplification, isothermal, fluorescence.

1. INTRODUCTION

Agricultural crops are vulnerable to pathogen attacks throughout their entire life span, from the sowing stage to harvesting. These attacks can persist even after harvesting, leading to post-harvest diseases in a variety of crops. Approximately 80% of the food consumed by humans comes from plants and plant-derived products (Home, n.d.). Different pathogens such as fungi, bacteria, viruses and nematodes interfere with metabolic and physiological functions in plants, resulting in a huge reduction in the quality and quantity of yield. (Kumar and Verma, 2018). Poor management of pest and diseases has threatened global food security where population is increasing in high rate. Historical famines such as the Irish famine (1845-54) and highlight the serious problem that can arise in future the (Bengal famine, 1943). Both famines were caused by plant pathogens where the Bengali famine was mainly caused by the fungal pathogen *Helminthosporium oryzae*, which led to Brown leaf spot disease in rice and the Irish famine was caused by *Phytophthora infestans* (Hsps.2005.35.2.341, n.d.) (Padmanabhan, 1973). It is very crucial to reduce crop losses caused by pathogens which can be done by early detection and application of effective management strategies.

Plant pathogens cause various symptoms in different parts of plants like leaves, stems, nodes, flowers, fruits and roots. Traditional method like visual inspection of these symptoms can provide accurate identification of some pathogens, but it does not play an effective role in the development of management strategies because the pathogen is already established within the plant system before producing symptoms. Microscopy can be used as a technique to detect pathogens at an early stage of symptom production by pathogens. Fungi can be distinguished microscopically by their spores, conidia types and mycelial structures. Bacteria are studied on the basis of their size, shape and colony, while nematodes are identified through study of shape, size, mouthparts, reproductive system, and tail morphology (Indarti et al., 2025). But some pathogens may share similar morphological traits and vary at the molecular level. So, microscopy cannot be applied to distinguish pathogens on the strain or race level. Different laboratory and field based molecular tools and techniques have been developed to overcome this problem and enhance pathogen diagnosis.

Nucleic acid-based detection techniques are often used for molecular identification of fungi, bacteria, and viruses. DNA or RNA is extracted from the pathogen and is amplified by polymerase chain reaction (PCR). Different primers, DNA polymerase enzymes, thermal cyclers, and dNTPs are used for amplifying specific DNA fragments, which are later detected in order to confirm the presence of the pathogen (Shen, 2019). ELISA is one of the methods based on antigen-antibody reactions that help in the detection and concentration of molecules. This method is generally used in virus detection. It can measure even in low concentrations as antibodies don't bind to molecules other than their own antigens (Aydin, 2015; Hornbeck, 1992). MALDI-TOF Mass Spectrometry is being used as a chemical analysis method for the characterization of biomolecules, proteins, and biopolymers. It helps in identification at the genus and species levels (K, 2003). Lab-based molecular techniques like PCR, ELISA, Mass Spectrometry, etc., are highly efficient in the detection and diagnosis of pathogens but consume a lot of time for processing.

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2. WORK FLOW OF RPA

In 2006, Piepenburg developed Recombinase Polymerase Amplification (RPA), an isothermal method for DNA amplification that utilizes proteins involved in DNA synthesis, recombination, and repair to amplify nucleic acids. This technique is used for detecting plant diseases. Currently, the commercialization of RPA is handled by the company Twist DX. The process relies on a complex of recombinase enzymes, which includes a strand-displacing polymerase that initiates synthesis at the 3' end of a primer at a stable temperature ranging from 25 to 43 °C, as well as single-stranded binding proteins that help stabilize the displaced strands of DNA and recombinases that interact with primers and target double-stranded DNA.

The RPA process commences when the recombinase protein uvsX from T4-like bacteriophages binds to a single-stranded DNA primer with assistance from uvsY. The presence of ATP leads to the formation of a presynaptic filament (nucleoprotein filament). To locate homologous sequences on double-stranded DNA, the recombinase-uvsY-primer complex actively hydrolyzes ATP into ADP. Upon finding homologous sequences, this complex infiltrates the DNA and forms a D-loop structure. Aligned multimers of single-stranded DNA-binding protein (gp32) provide stability to the unwound strand of DNA. Once the complex disassembles, strand-displacing DNA polymerase can access the 3' ends of both primers, initiating primer extension and resulting in exponential amplification of DNA.

Thus, the recombinase is capable of attaching to a new primer and starting multiple strand-displacement processes, which significantly accelerates DNA amplification within minutes at a consistent temperature. Amplified products can be generated within 20 to 25 minutes. In RPA amplification occurs at steady and lower temperature due to which there is no need of thermal cyclers. This makes RPA different from other isothermal detection techniques. Unlike the LAMP method, which requires four to six primers for synthesizing DNA amplicons of varying sizes (Notomi et al. 2000), RPA only needs one pair of oligonucleotide primers. Furthermore, RPA amplicons can be monitored in real time using lateral flow dipsticks (LFDs) with an oligonucleotide probe (Ghosh et al. 2018).

As laboratory-based methods require advanced facilities and skilled personnel, formulation of management strategies for disease control may not be feasible within the necessary timeframe. It is possible that established laboratory resources are not readily accessible to farmers who need to submit infected samples fortesting. If these diseases are not identified early and managed properly, it will cause serious trouble to farmers due to damage to crops. Different field based diagnostic tools have been developed such as Lateral Flow Immunoassay (LFIA) Gold Nanoparticle-based Biosensor Loop-mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA) (Piepenburg et al., 2006; Razo et al., 2018; Khater et al., 2019; Chen et al., 2020). RPA is considered a highly effective method for identifying pathogens through isothermal amplification directly in the field. It allows detection of pathogens by generating DNA amplicons very quickly at a lower cost. The amplification carried out by RPA is comparatively similar to PCR (Londoño et al., 2016). It can be integrated with other diagnostic tools to produce more accurate results effectively.

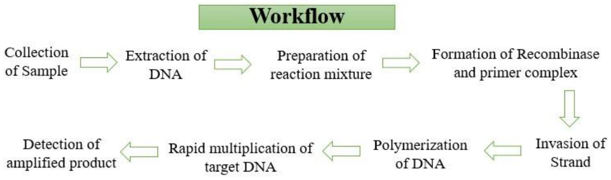

Figure 1: Workflow of Recombinase Polymerase Amplification (RPA)

3. DETECTION TECHNIQUES

Lateral Flow Assay is a technique that utilizes capillary action to facilitate the movement of a sample across a membrane. This method comprises several components, including a sample pad, conjugate pad, nitrocellulose membrane, and control line, which work together to separate the mixture's components based on their migration within the reaction membrane (Dzantiev et al., 2014; Hu et al., 2014; Singh et al., 2015). An oligonucleotide probe tagged with a gold nanoparticle effectively identifies AAC genomic DNA from *Acidovorax avenae subsp. citrulli,* serving as an on-site detection mechanism (Zhao et al., 2011). Bacterial DNA amplification can be conducted using the RPA kit (TwistDX, San Diego, USA) along with PCRD cassettes designed for Lateral Flow strips (Batra et al., 2024). The RPA-Lateral Flow dipstick presents itself as an efficient alternative to PCR due to its enhanced capability in pathogen detection (Xu et al., 2023).

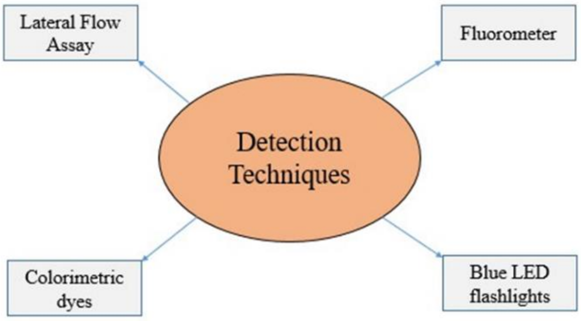

Figure 2: Different detection techniques of amplified products.

Fluorometer, blue LED flashlghts and smartphone based fluorescence readers are some examples of fluorescence based diagnosis techniques used for pathogen detection in field condition. Portable fluorometers detect the fluorescence emitted when DNA-binding dyes attach to double-stranded DNA, enabling the assessment of target DNA presence within a sample (Qubit Assays - IN, n.d.). The blue LED detection method functions by exciting fluorescent markers that produce detectable green light, which confirms the presence of DNA (Guo et al., 2023). Furthermore, a smartphone camera can facilitate the observation and documentation of this process by positioning the smartphone next to RPA-amplifiedDNA tubes in low-light conditions for image capture (Samacoits et al., 2021).

Colorimetric dyes are used for detection of amplified DNA without use of any other equipments. These dyes change their color in response to alteration in pH levels or magnesium ion concentrations. Phenol red exhibits a color change when the pH lowers due to the release of protons during the process of DNA amplification (Tanner et al., 2015). Crystal violet carries a positive charge and binds to double-stranded DNA (dsDNA) through electrostatic interactions. It appears pale violet in lower DNA concentration and deep violet at higher DNA concentration (Kong et al., 2019). Additionally, magnesium-sensitive dyes alter their color based on the amount of Mg^{2+} ions in the sample. Hydroxy Naphthol Blue (HNB) displays violet at higher Mg^{2+} concentrations and shifts to sky blue when these concentrations are reduced (Goto et al., 2009a).

4. INTEGRATION WITH OTHER DETECTION TOOL

One of the method used to amplify DNA at a constant temperature is loop mediated isothermal amplification, or LAMP. This process produces a large amount of DNA that can be detected using real-time detection techniques turbidity measurements, or the addition of other dyes as Ethidium Bromide (Goto et al., 2009b; Bekele et al., 2011). Recombinase polymerase amplification (RPA) exhibits improved sensitivity and specificity when paired with LAMP or RAMP (Lai and Lau, 2022). Because RPA functions well at lower temperatures, its DNA amplicons can be used as templates for LAMP amplification of certain targets at higher temperatures. As an integrated approach, RAMP enhances the detection threshold by up to tenfold compared to traditional LAMP and also shortens the duration needed for amplification and product formation. When the color of final products changes, it can be detected visually without the use of specialized equipment.

CRISPR-Cas system is a gene editing tool that functions as molecular scissors by cutting specific segments of target DNA. It helps to cut or eliminate unnecessary segments and addition of desired segments. A guide RNA (gRNA) targets and binds to the DNA subsequently activating the Cas enzyme if the amplified DNA or RNA contains a sequence associated with a pathogen (Chen et al., 2018). Once activated, the Cas enzyme cleaves nearby reporter molecules, generating a signal. The combination of recombinase polymerase amplification (RPA) with CRISPR technology has formed an effective hybrid model for pathogen detection. RPA amplifies target DNA, producing numerous copies of the pathogen's genetic material, which are then identified by the CRISPR-Cas system; this process results in the cleavage of a reporter probe, leading to visible signals (Chen et al., 2018; Gootenberg et al., 2017). The RPA-Cas12 assay is capable of detecting low concentrations of *Fusarium oxysporum f. sp. Cubense* tropical race 4 at levels as low as 10 femtograms, achieving sensitivity comparable to quantitative PCR while being ten times more sensitive than loop-mediated isothermal amplification (LAMP) (Aguayo et al., 2017; Matthews et al., 2025). The combination of RPA and CRISPR technology improves both the sensitivity and specificity of pathogen detection while also shortening the analysis duration. Conventional techniques typically require extended processes, but the RPA-Cas12 assay can produce results in less than an hour, making it a valuable choice for quick diagnostics in field environments.

Table 1: Examples of fungal pathogens detected using RPA and integrated diagnostic technologies.

Pathogen	Host Plant	Diagnosis method	Reference
Fusarium oxysporum f.sp. conglutinans	Thale cress	RPA-SERS	Lau et al., 2016
Phytophthora infestans	Potato	RPA	Ammour et al., 2017
Fusarium oxysporum	Strawberry	RPA–LFD	Hu et al., 2024
Alternaria triticina	Wheat	RPA-CRISPR/Cas12a	Phurbu et al., 2025
Phytophthora vignae	Cowpea	RPA-Cas12a	Lin et al., 2025

Table 2: Examples of bacterial plant pathogens detected using RPA and integrated diagnostic technologies.

Pathogen	Host Plant	Diagnosis method	Reference
Pectobacterium spp.	Tomato	RPA-LFD	Ahmed et al., 2018
Xanthomonas spp.	Tomato	RPA	Strayer-Scherer et al., 2018
Ralstonia solanacearum	Tobacco	RPA–LFD	Li et al., 2021
Xanthomonas arboricola pv. Pruni	Peach	RPA-Cas12a	Luo et al., 2021
Pseudomonas syringae pv. Actinidiae	Kiwi	RPA-LFA	Yang et al., 2023

Table 3: Examples of plant viruses detected using RPA and integrated diagnostic technologies.

Pathogen	Host Plant	Diagnosis method	Reference
Wheat dwarf virus	Wheat	RPA	Glais & Jacquot, 2015
Tomato yellow leaf curl virus	Tomato	RPA	Londono et al., 2016
Citrus tristeza virus	Citrus	RPA-LFICA	Ghosh et al., 2020
Bean common mosaic virus	Common Bean	RPA-LFD	Qin et al., 2021
Maize dwarf mosaic virus	Maize	RPA-CRISPR	Tian et al., 2024

5.CONCLUSION

Recombinase Polymerase Amplification (RPA) addresses many limitations associated with conventional diagnostic methods like PCR emerging as an efficient, rapid and reliable isothermal nucleic acid amplification technique for identifying plant diseases. Operating at a consistent and relatively low temperature range of 25–43 °C, RPA is particularly suitable for field applications and point of care testing. Unlike PCR, which requires thermal cycling and sophisticated laboratory equipment, RPA leverages the collaboration of recombinase enzymes, single-stranded DNA-binding proteins, and strand-displacing polymerases to accurately target and swiftly amplify specific DNA sequences. This method significantly reduces diagnostic time by yielding detectable amplification results within 15 to 30 minutes. Additionally, RPA streamlines assay design while maintaining high sensitivity and specificity by utilizing only a single set of primers, in contrast to the multiple primers needed for LAMP. These advantages make RPA an invaluable tool for early detection of plant pathogens, which is crucial for timely disease management and preventing substantial crop losses.

Additionally, the adaptability of RPA is enhanced by its compatibility with various detection platforms, including colorimetric dyes, smartphone-assisted fluorescence imaging, fluorescence-based detection systems, and lateral flow dipsticks. This versatility allows for both instrument-based and instrument-free visualization of amplification outcomes. Its flexibility makes it suitable for use in low-resource environments and in agricultural contexts where laboratory facilities may not be present. Moreover, integrating RPA with advanced molecular technologies such as CRISPR-Cas systems and Loop-mediated Isothermal Amplification (LAMP) has significantly improved detection sensitivity, specificity, and accuracy, enabling the identification of infections from extremely low quantities of DNA. These hybrid methodologies serve as powerful tools for modern plant disease diagnostics by merging highly specific detection capabilities with the rapid amplification offered by RPA. In summary, RPA stands out as a cost-effective, portable, and highly adaptable diagnostic tool that aids in early pathogen identification, accelerates decision-making in disease management, reduces economic losses, and enhances plant biosecurity and sustainable agricultural practices particularly in resource-limited and field settings.

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