

Point of Care Technologies in Plant Disease Diagnostics : An Overview

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ABSTRACT

Diagnostics is an important part of healthcare; be it for human, animal or Plant. Point-of-care (POC) testing originated in the medical field, where bioengineers created technologies for quick and simple testing of certain diseases using lateral flow devices and microchips. This advancement is significant, as it allowed individuals to access it as affordable and quick. Many nucleic acid testing based technologies like polymerase chain reaction (PCR), droplet digital PCR (ddPCR), reverse transcriptase PCR (rtPCR) and isothermal techniques like loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), rolling circle amplification (RCA) and helicase-dependent amplification (HDA) are now available for uses as POC solution. Therefore, in the agricultural sector a point-of-care solution may make a revolution in the field of biotic stress management, but it needs consideration of several key factors. First, the technology should be economically feasible, and affordable for the grassroot level people associated directly with crop production. Additionally, the device must have high sensitivity, can detect minimal presence of pests or pathogen responsible for the stresses.

Key words : Diagnostics, plant diseases, POC, PCR, LAMP, RPA, HDA

Introduction

Food security as defined by the World Food Summit (1996) is met when 'all the people, at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy lifestyle'. However, with the population rising, our country aims new target to fulfil future demand. Increasing percentage of undernourished and anemic women and children which was as high as 39 % in 2014 (<https://hdl.handle.net/10568/151385>) indicating that around half

of the Indian population is still facing hunger issues.

Two strategies can meet food sufficiency on a domestic scale; one is increasing the area under cultivation and another is to increase productivity. Compared to the, initiatives taken in increasing productivity are a realistic approach compared to the first option. Several abiotic and biotic stresses are responsible for decreasing the productivity of the crop. According to FAO (2020), pests and diseases alone cause a 20-40% reduction in crop production. Early detection is an efficient way to minimize

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crop losses due to disease incidence. Early detection could also prevent epidemics as major cultivating areas of the world follow the mono-cropping of food grains in particular seasons. This would also prevent the usage of chemicals required for managing the disease, even cultural and biological methods could bring the disease under control. Timely detection not only save the crop, but gives an eco-friendly solution to the problem. Symptoms are the primary indicator for identifying any diseases. However, symptoms sometimes recognizable at late stages of disease development, also some systemic diseases like smuts and ergots appear only after the flowering or at the crop maturity stage. This makes it difficult to save the crop and thus leads to huge financial losses. Even with virus and phytoplasma diseases, once infected, the disease becomes systemic, and thus a complete cure might not be possible. Also, in polycyclic diseases like rust managing the initial infection is crucial to prevent the disease from escalating into an

epidemic. This is why early detection should not be limited to symptoms.

In the medical field, several devices have now been developed for constant monitoring of many lifestyle diseases like diabetes, which can be done within the likes of home. This sort of on-site monitoring not only saves time and energy but also helps to keep the disease in check without the requirement of a professional. Thus, point-of-care (POC) technologies emerged in the healthcare field for rapid identification of any dysregulation in the functioning of the human body, with particular emphasis on cardiovascular health and lifestyle diseases. Pregnancy kits and lateral flow devices for detecting hormonal balance are another set of widely popular POC devices (Figure 1). The success of point-of-care devices has also led to their application in identifying infectious devices. Currently faster assays based on proteins and nucleic acids are available which could give accurate results within seconds. This prompted a similar development in plant pathology, for easy and rapid identification of a plant pathogen at an early stage.

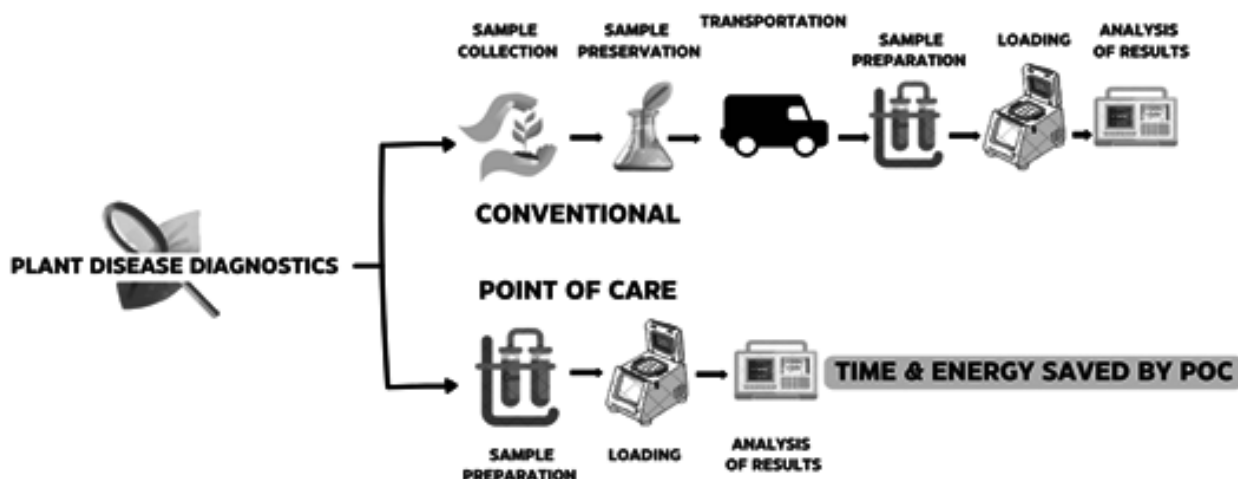


Figure 1 : Comparing conventional plant disease diagnostics to point-of-care (Adapted from Srinivasan and tung, 2015)

In plant pathology, detection and monitoring systems were primarily devised for keeping epidemics in check. Hence, several remote sensing and monitoring systems were developed that could forecast and predict the possibility of a developing disease. However, most of these models are for polycyclic diseases and do not help in point-of-care diagnostics in a farmer's field. Point-of-care detection systems are developed mainly based on protein-protein interaction or nucleic acid assays. This gives the specificity and flexibility to change the devices based on the type of infections investigated. Thus, a point-of-care-diagnostic device helps in the on-field detection of a plant pathogen rapidly with ease thus saving the crop and benefitting farmers financially. Currently, various POC devices are available in the market for the detection of plant pathogens like *Xylella fastidiosa*, *Pseudomonas syringae*, *Penicillium digitatum*, and so on. This article reviews the advanced technologies for rapid and effective identification of plant pathogens in the field.

Point of care diagnostics in plant disease management

Point-of-care diagnostics emerged in the medical field for diseases that do not require constant supervision by a physician. Development of detection kits for diseases like diabetes, infectious diseases, and cardiovascular monitoring makes the POC popular and acceptable. During the 2019 COVID pandemic, the POC testing market increased with advancements in the field of virus pathogen detection kits. The early detection of plant pathogens was based on external symptoms and signs, often confirmed

through cultural methods based on classic Koch's postulates. POC devices for plant pathogen testing can be broadly grouped into the following categories: sensors for on-field monitoring, volatile organic compounds-based detection, microfluidic-based devices, and wearable smart sensors (Buja *et al.*, 2021). Looking into its many advantages (Figure 2), researches on several technologies targeted for POC is undergoing. Let us take a look at the various technologies employed to specifically target pathogens.

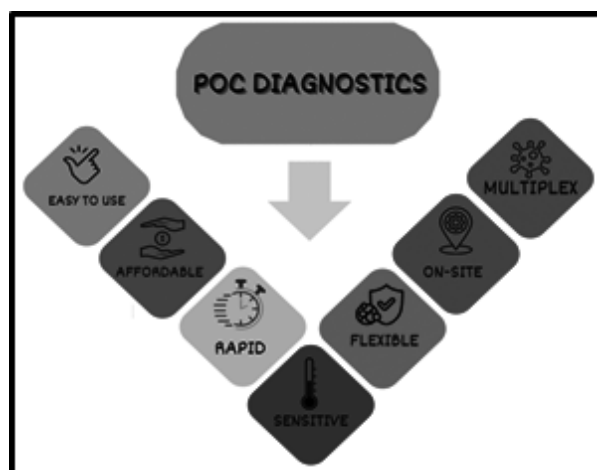


Figure 2 : Advantages of point-of-care diagnostics

Immunoassays are based on protein-protein interactions that emerged in the 1900s. ELISA technique was introduced for plant virus detection in 1977 by Clark and Adams. All immunoassays are based on the specific binding between an antigen and its corresponding antibody which is detected through electrochemical or fluorescent-based detection. These techniques have been found efficient for the large-scale on-field detection of plant viruses. Recent developments in this field led to several electrochemical-based,

optical-based antibody biosensors, and lateral flow devices that give rapid qualitative and quantitative detection of plant pathogens with much sensitivity. Nanoparticle and fluorescent-labeled immuno-biosensors with low-cost fabrication procedures have been in the market due to their ease of use and visualization (Berto *et al.*, 2019). Lateral flow assays and fluorescent-based approaches have been developed for the bacterial pathogen *Pantoea stewartii*, which could detect the pathogen with a sensitivity of 10^3 to 10^5 cfu/ml. A portable variant of immunoassay is surface plasmon-based resonance (SPR) systems, which have applications in fields ranging from clinical to environmental and agricultural. The SPR system detects the change in the refractive index after ligand and biomolecule binding and amplifies this signal as the sensor for the presence of an analyte (Khater *et al.*, 2016). Many SPR biosensors have been developed, however, a recent advancement was made in this assay for the detection of begomoviruses. This nanoparticle-based optical platform biosensor could detect the single-stranded DNA of chilli leaf curl virus with a sensitivity of 1.0 ig/ml (Das *et al.*, 2021).

However, immunoassay-based detection of plant pathogens has been less commercialized compared to nucleic acid-based techniques. This is because of expensive monoclonal antibodies. There is also the chance of false positive and negative results depending on the initial inoculum available for testing. Besides, some immunoassays like SPR require sterile conditions, and any adjuvant can hinder the test and give false results. All these demands for rapid, cheap, flexible,

and sensitive technology even in a relatively dirty atmosphere like a farmer's field.

Nucleic acid based testing emerged as a field giving highly accurate results with lower detection limits. However, techniques like Polymerase Chain Reaction (PCR) require huge machinery and skilled manpower for its operation limiting its on-field use ability. To overcome this difficulty several variants of PCR have been developed that can be adjusted for on-field testing. Droplet digital PCR (ddPCR) is a sensitive assay that can detect the presence of DNA even at a concentration of 1 copy/il. It was initially employed for the detection of human pathogens, however, this assay has been modified for plant pathogen detection as well. A ddPCR assay developed for detecting the potato blight pathogen *Phytophthora infestans* was found to be 100-fold sensitive than a conventional PCR assay (Ristanio *et al.*, 2020). While comparing this to a reverse transcriptase PCR, ddPCR shows almost the same sensitivity and has been found to surpass RT-PCR in some cases. This was observed in identifying the grapevine pathogen *Xylella fastidiosa* (Dupas *et al.*, 2020). However, PCR assays are expensive and often require massive equipment, which limits their use for on-field testing.

Isothermal techniques have been in the market since the 2000s, and they can be used in any setting without the requirement of sophisticated machinery (Figure 3). This makes them an attractive tool for the on-field detection of plant pathogens. Thermal-mediated amplification (TMA) rules the market of isothermal technologies for detection in the clinical

field followed by loop-mediated isothermal amplification (LAMP) and helicase-dependent amplification (HDA). On the other hand, when it comes to plant

pathogen detection systems, LAMP, RPA (Recombinase polymerase amplification), RCA (Rolling circle amplification) and HDA have been used respectively (Li *et al.*, 2019).

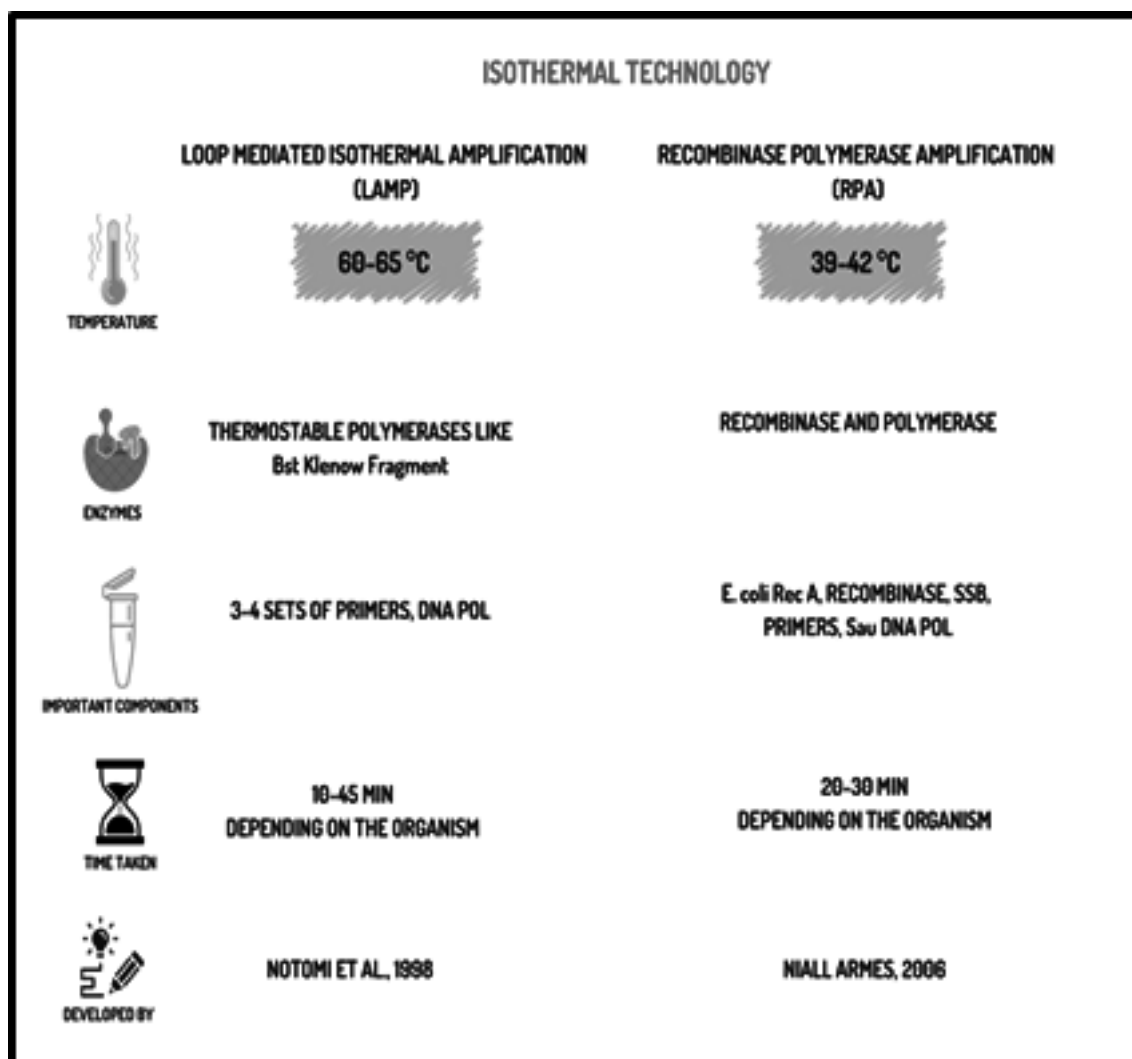


Figure 3 : Comparing isothermal technologies: LAMP vs. RPA

LAMP technology was developed by Notomi and coworkers in the 2000s. This technology became popular because the amplification using this system occurs at a constant temperature requirement of 65!, thus eliminating the need for a

thermocycler. LAMP has been optimized for several pathogens including various rice pathogens. The reaction time for the detection of *Magnaporthe oryzae* took 30 minutes while the sensitivity was found to be 10^{-6} / μ l (Li *et al.*, 2019). LAMP can also

be optimized into a microchip for point-of-care detection of plant pathogens (Prasannakumar *et al.*, 2021). A notable example of LAMP based kit developed by researchers at the University of Florida. This portable diagnostic kit includes a battery-powered device and a smartphone app, enabling on-site detection of plant pathogens such as citrus canker and tomato yellow leaf curl virus in under an hour.

Another equally appealing technology is the recombinase polymerase amplification assay (RPA). This assay is an enzymatic amplification procedure that mimics the replication in the cells of living

organisms. Replication, as we know occurs at room temperature, i.e. around 30-40°C. The amplification of this technology begins within 10 minutes and it has a lower detection limit giving higher sensitivity for the detection of pathogens. Hence, this technology can also be tailored to paper-based, electrochemical, colorimetric, or chip-based point-of-care devices. It is also a sensitive assay that is easier to use. The sensitivity of this technique was found to be 10^{-9} ng/ μ l with a reaction time of 30-45 minutes for detecting the false smut fungi *Ustilaginoidea virens* (Banerjee *et al.*, 2023). There are several examples of detecting the pathogen causing diseases through RPA based sensors (Table 1)

Table 1 : Plant pathogens detected through RPA sensors

Biosensors	Sensing plant pathogen	Detection limit	Time taken
REC POL-LF	<i>Little cherry virus 2</i>	0.1 ng	25 min
REC POL-LF	<i>Plum pox virus</i>	1 fg/ μ LRNA	30 min
REC POL	<i>Rose rosette virus</i>	1 fg/ μ LRNA	20 min
REC POL	<i>Tomato yellow leaf curl</i>	10^{-5} μ L	30 min
REC POL	<i>Xanthomonas gardneri</i>	10^6 cfu/ml	30 min
REC POL LF	<i>Phytophthora infestans</i>	500 fg	30 min

An interesting innovation using RPA assay involves a qualitative photothermal sensing strategy for the detection of the plant pathogen *Alternaria*. This method combines the RPA assay with rolling circle amplification (RCA) assay and CRISPR/Cas 12a, the gene editing tool. This can be done in any lab setting without the requirements of a sophisticated device. The sensitivity of technology was high and target DNA

could be detected even at fg/ μ l (Liu *et al.*, 2023). RCA is an enzymatic isothermal strategy that uses xDNA polymerase for rapid amplification. RCA assay alone has been useful in detecting plant pathogens like *Pectobacterium atrosepticum* and *Erwinia caratovora*.

There are several other isothermal amplification strategies, like helicase-dependent amplification (HDA), nucleic

acid sequence-based amplification (NASBA), polymerase spiral reaction (PSR), strand exchange amplification (SEA), cross-priming amplification, and CRISPR-Cas9-triggered nicking endonuclease-mediated strand displacement amplification. Although numerous methods are available, not many of the above-mentioned techniques have been exploited for detecting plant pathogens (Table 2). The major constraint associated with these isothermal strategies is the decreased sensitivity and the requirement

for optimization at each location. This limits the attractiveness of these technologies for the development of a cheap, easy-to-use, and sensitive point-of-care device. Among the isothermal strategies discussed, LAMP and RPA technology have been found the most efficient for the detection of various plant pathogens with higher sensitivity and reproducible results. This prompted the development of biosensors, microfluidic chips, and lateral flow devices based on these technologies.

Table 2 : List of isothermal amplification techniques used for plant pathogen detection

Detection method	Target nucleic acid	Advantages	Disadvantages
LAMP	DNA/RNA	Fast, isothermal, sensitivity, relatively cheap	Primer design can be difficult
RPA	DNA/RNA	Fast, isothermal, does not require denaturation	Long primers, sensitivity may vary
RCA	DNA/RNA	Isothermal, sensitivity, specificity	Expensive
SDA	DNA/RNA	Fast, isothermal	Inefficient for long transcript
HDA	DNA	Fast, isothermal, does not require denaturation	High optimization needed
NASBA	RNA	Fast, isothermal	Expensive

All the technologies that we have discussed so far target the pathogen and confirm the disease by confirming the physical presence of the pathogen through direct methods. A pathogen is the most important component of a disease,

however, it is not the only component in a disease system. A diseased condition in a plant occurs when a vulnerable host plant comes in contact with a virulent pathogen under congenial conditions for plant disease development. Hence targeting the

pathogen is an efficient strategy, even so, an equally compelling strategy is to monitor the physiology of the host plant. A stressed plant makes itself ready to combat any danger by activating the production of stress hormones and metabolites. These metabolites vary with the type of incoming stress, i.e. based on whether it is biotic or abiotic. So monitoring the plant physiology can indirectly give input about infection by any biotic, abiotic, or combined stress. These wearable sensors can be taped to plants and thus give a unified and targeted approach to reducing crop losses through smart monitoring (Oren *et al.*, 2017).

Conclusion

Point-of-care testing aims at easy, affordable, rapid, and sensitive technologies that could be applied to farmers' fields for early disease detection. A POC device is marketable only when all of these criteria are met. The POC market for plant pathogen diagnostics is not as popular as compared to the medical field. The reason might be the dynamic physiology of the plant, the combined effect of biotic and abiotic factors, poor reliability of the technologies employed, and the requirement of optimization for most of these technologies. These limitations hinder the development of the POC diagnostics market for plant pathogens. Even so, the market shares for plant pathogen testing in the POC market has been growing at 4 % and the US holds the major share in the market.

However, several researchers in the field have tried to improve the quality of diagnostics by combining different technologies for enhanced sensitivity and detection. Innovations in the field have

made it possible to produce inexpensive solutions to the constraints. Even then, the major challenge as far as India is concerned would be to introduce these technologies to the farmers and encourage them to replace their traditional testing methods with novel and efficient newer techniques so that its intended purpose would be fulfilled. Future prospects in plant pathogen detection focus on developing advanced diagnostic kits, remote laboratory services, and digital platforms that integrate IoT and AI. The success of these innovations depends on technological advancements and their proven benefits to farmers, promising a more efficient and effective approach to disease detection in agriculture.

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