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Applications of LAMP Based Diagnostic Kits in Crop Management

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Abstract

Pathogens such as bacteria, fungus and viruses are interminable factors responsible for food loss and plant infection. Advanced disease detection methods play a pivotal role in the prevention of damage to crop yield and ensuring minimum deprivation of plants during growth, harvest and storage. In this study, we have discussed the characteristic features of traditional methods of detection and identification of pathogens, namely, polymerase chain reaction (PCR), fluorescence in-situ hybridization (FISH), enzyme-linked immunosorbent assay (ELISA), immunofluorescence (IF), flow cytometry, colony forming unit, DNA-RNA based methods, enzyme and antibody based detection methods etc. All these methods require high-tech infrastructure and laboratory facility. In the present article, we have attempted to review the various applications of loop-mediated isothermal amplification (LAMP) technique and it's commercial benefits in the field testing of pathogens and plant diseases.

Keywords: LAMP method; Plant pathogens; Isothermal amplification technique; Traditional methods of detection of pathogens; Field testing

Introduction

Human population is increasing at exponential growth in all parts of the world. Based on the recent report on food security released in 2010 [1]. Requirement of food will increase due to continuous increase in the human population leading to 70% more food requirement by 2050 [1]. Vegetarian population of the world is estimated to be 1.5 billion in 2010 [2]. Apart from the humans all the feedstock animals also rely on food produced by the plants. The demand of food is higher than the supply because of continuous decrease in the agricultural land. Under such circumstance, effective crop management is essential. The crop losses due to pathogenic infection was around 20-40% [3]. Rice, wheat, barley, maize and soybean are majorly consumed crop globally which was reduced by 12% due to plant diseases. Whereas the crop yield of groundnuts and potatoes were affected around 24% and of wheat and cotton around 50% and 80% [4]. Economic losses due to pathogens in US only was estimated to be 40 billion dollars so worldwide loses can be imagined [5,6]. With the evolution of science, researchers have found numerous techniques to identify pathogens but all of them require high initial cost, hi-tech infrastructure facility and experienced researchers. Therefore, these technologies are confined to the laboratories or research institutes located in cities. There is an urgent need to develop and popularize technologies that are suitable to the rural areas for speedy testing of pathogens to avoid epidemics and for launching crop protection measures to manage pathogens [7-29].

Discussion

Laboratory based technologies for detection and identification of plant pathogens

Identification of the pathogens plays a pivotal role in controling of plant diseases and in taking relevant crop protection measures Fang et al. [11], have divided the methods for pathogen detection in two categories: direct methods and indirect methods. Direct methods includes detection of pathogens through the molecules produced by them or by using their DNA/RNA etc. A comparison of direct pathogen detection methods is complied in the Table 1.

Whereas indirect detection methods works on the plant stress profiling and plant volatile profiling etc. All the methods mentioned in the Table 1 requires high end laboratory and skilled professionals [12-45].

Table 1: Comparison of methods for detecting plant disease causing bacteria.

Method	Principle	Advantages	Limitations	References
PCR	Detection of nucleic acid of pathogen	Vastly used technique for humans, highly reliable and very sensitive method	It requires DNA extraction, PCR amplification.To perform the, PCR machine, reagents and high end laboratory is required	[7]
FISH	Hybridization of DNA probes and target gene	High sensitivity.	False positive results, low sensitivity	[8]
ELISA	Detects diseases based on antibodies and color change in the assay	Low cost, visual color change can be used for detection.	Low sensitivity for bacteria, not suitable for early detection	[9]
IF	Fluorescence microscopy-based optical technique	High sensitivity, used for detecting onion crop infection by a fungus *Botrytis cinerea*	False negative results, low sensitivity	[10,11]
FCM	Laser-based optical technique	Fast detection in 30 minutes, proven for soil borne bacteria such as *Bacillus subtilis* in mushroom	Costly, require high end instrument	[12]
Thermography	imaging the differences in surface temperature of plant leaves and canopies	Works by measuring surface temperature, Rapid and high sensitivity	Unable to distinguish between diseases that produce similar thermographic patterns.	[13]
Fluorescence imaging	chlorophyll fluorescence is measured on the leaves	precise detection of leaf rust and powdery mildew infections	Technique in a field setting is not feasible	[14]
Hyper spectral techniques	Detects changes in reflectance resulting from the biophysical and biochemical characteristic changes upon infection	robust and it provides a rapid analysis of the imaging data, Used for *Magnaporthe grisea* infection of rice, *Phytophthora infestans infection of tomato and Venturia inaequalis* infection	Cannot identify specific pathogen	[15-17]
GC	Profiling of the volatile chemical signature of the infected plants	high specificity, stage-wise disease detection	requires sampling of pre-collected VOC for a longer time before analysis	[10]

Abbreviations: PCR- polymerase chain reaction;IF- immunofluorescence;GC- Gas chromatography; FISH- fluorescence in-situ hybridization; ELISA- enzyme-linked immunosorbent assay; FCM- flow cytometry; CFU- colony forming unit.

Principle of LAMP technology

LAMP (Loop-mediated isothermal Amplification) method was reported by Notomy [46]. It is highly specific and sensitive technique to discriminate variations at the single nucleotides polymorphism. It is also a simple, cost effective, rapid and accurate method to be used in the field testing of pathogens. LAMP method does not require specific laboratory set up, it requires only stable heat providing instrument which can be operated through batteries also. The various applications of LAMP technique in the detection of plant pathogenic bacteria, viral and fungal diseases are compiled in the Table 2.

Table 2: LAMP based method used for detection of various bacterial, viral and fungal diseases.

Disease	Disease Type	Detection of Pathogen	Reference
Edwardsiellosis, fish disease	Bacterial	*Edwardsiella tarda*	[47]
Periodontal disease	Bacterial	*Porphyromonas gingivalis*	[48]
Periodontal disease	Bacterial	*Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola*	[49]
Fire blight of pear and apple	Bacterial	*Erwinia amylovora*	[50]
Mumps disease	Viral	*Mumps virus*	[51]
Newcastle disease	Viral	*Newcastle disease virus*	[52]
human influenza A	Viral	*Human influenza A virus*	[53]
Wheat stripe rust	Fungal	*Puccinia striiformis f.sp. tritici (Pst)*	[54]
chromoblastomycosis	Fungal	*5.8S rDNA* gene of 39 fungal species	[55]

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Plant root disease	Fungal	*Arbuscular mycorrhizal fungi*	[56]
soybean Phytophthora root rot	Fungal	*Phytophthora sojae*	[57]

The technique involves the amplification of nucleic acids of plant microbes under isothermal conditions. Primers designed for the assay will amplify the stem-loop DNA structures of microbes with several inverted repeats of the target fragments, so as to detect and differentiate polymorphism among bacteria and fungal pathogens. Technology has been commercially developed by number of diagnostic companies, wherein the concentrations of white precipitates of magnesium pyrophosphate was detected using fluroscent dye like syber green. Fluorescence of syber green dye is the indication of amplification of target nucleic acid fragments of microbes present in human or animal or plant samples [47-56].

Organism specific genes or rRNA region is selected for designing LAMP specific primers. Instead of single primer pairs, LAMP uses 3 or more than three primer pairs, which makes it highly specific for target identification. Several tools and software are available for LAMP specific primer designing but validation is required for all the primer sets. Protocol of LAMP includes crude or pure DNA isolation and isothermal amplification of the DNA using LAMP specific primers followed by visual or spectrophotometeric analysis for detection of change in color as a result of amplification. Few methods uses fluoresce in isothiocyanate (FITC)/biotin for labeling the primers followed by detection based on specially treated lateral flow test strips (Milenia strips, Milenia Biotec Gieben, Germany) [57].

Commercial development of LAMP based devices

Currently, most researchers are using LAMP method for detecting targeted pathogen by SYBR green or any other fluorescent dye based detection protocol. In this protocol, DNA or cDNA is amplified using target specific LAMP mediated primers. As the amplified products contains loop structures in the positive samples they retains the fluorescent dye and changes their color. This traditional method uses water bath, dry bath or thermal cycler for providing temperature to the reaction. Singleton et al. [58] have developed a device named as"Non-instrumented nucleic acid amplification (NINA) platform" (Figure 1) which is an electricity-free platform capable of isothermal amplification and detection of a variety of pathogens. This is a kind of point of care device as it neither requires any specific laboratory/ infrastructure nor electricity. It uses MgFe fuel pouch to activate the heater. MgFe is added to the bottom of the heater and mixed with 5ml of saline which is commercially available as a blow-fill-seal cartridge. Within 12 minutes, temperature increases up to 61.5 ˚C +/21.5 ˚C which is desired temperature for performing the assay. They have validated the detection of HIV-1 with a ß-actin positive internal amplification control from processed sample to result within 80 minutes time with optimum sensitivity and reproducibility. The heater was used to amplify specific LAMP amplicons in which loop primers used were labeled with hapten to enable capture using antibody binding of FITC followed by visual detection of captured amplicons via streptavidin colloidal gold. LAMP positive reactions were analyzed using Milenia lateral flow test strips (Milenia Biotec Gieben, Germany) (Figure 2). Limit of detection for HIV viral assay performed by this instrument was reported to be 75 copies/reaction or 8,333 viral copies/mL of extracted plasma [58].

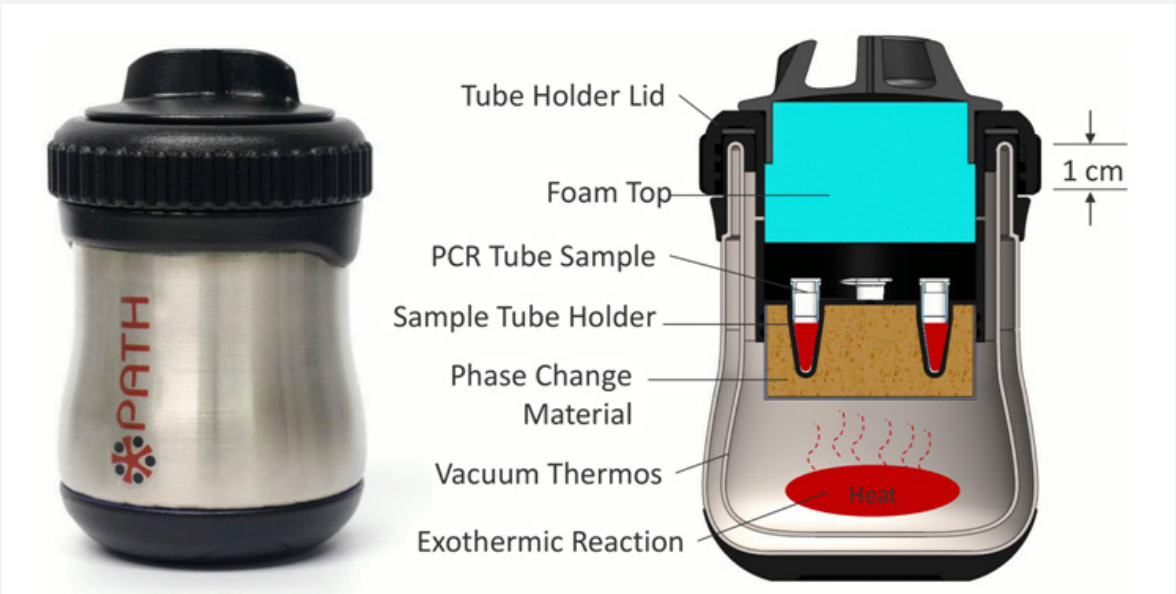

Figure 1: PATH NINA heater. (Right) Cross-section showing the internal components of the heater. Approximate dimensions of the heater are 80 mm diameter by 120 mm height. (Figure 1 is reproduced without modification from Singleton et al. [58]).

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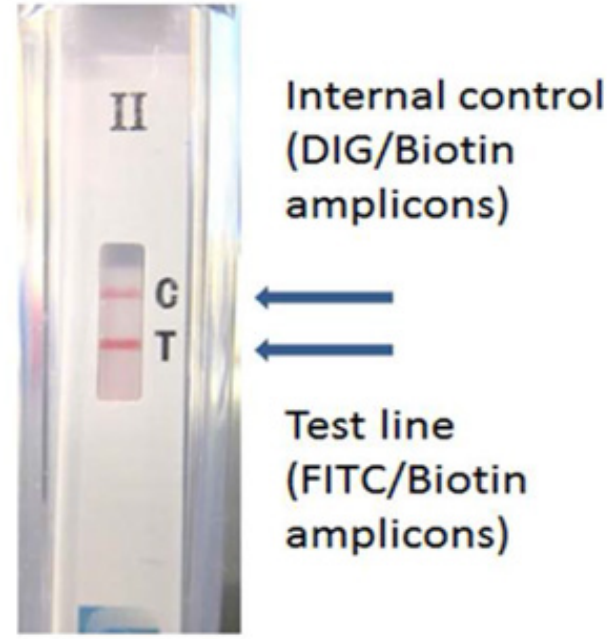

Figure 2: HIV LAMP amplicon detection via Milenia test strips: Test showing positive results for DIG/Biotin and FITC/Biotin labeled amplicons (Image source: Singleton et al. [58]).

Future application

Maize is agriculturally and industrially very important crop for India and world. Its demand is estimated to be doubled by the year of 2050. However, crop productivity is reduced by biotic and abiotic stresses. Both fungal and bacterial pathogens play significant role in the reduction of maize crop yield in India. Early diagnosis of pathogens will decrease the considerable economic loss to Farmers. Xcelris labs is working on developing a LAMP based diagnostic test for detection of *Fusarium sps, Pseudomonas sps,* and *Sclerophthora sps* affecting maize crops. After successful development and validation of kit in field, it will help in early stage diagnosis of pathogens in Sorghum, Rice, Wheat, Oats and other Poaceae family members.

Conclusion

LAMP technology is very well studied as a research and investigative project, which has significant impact in the crop management in rural areas of India. Development of LAMP based devices can contribute significantly to crop improvement. Large amount of crop damage in rural India due to delay in the identification and detection of pathogen causing the disease. Therefore, field testing of plant pathogens will help farmers to take crop protection measures, namely, spraying insecticides or pesticides, release of biological predators, fertigation in green house or poly house, early harvesting of crop produce or fruits, proper storage of food grains and so on. LAMP based devices can be made available at Taluka level or village level by bringing awareness in ''farmers training centers''.

Conflict of interest and Disclaimer

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