RECENT ADVANCES IN LATERAL FLOW ASSAY FOR DETECTION OF PLANT PATHOGENIC BACTERIA

Abstract

Plant pathogenic bacteria (PPBs) are widespread and pose a significant threat to global agriculture due to their ability to cause several diseases. Early and accurate detection of these pathogens is crucial for effective plant protection and disease management. Conventional methods for detecting plant pathogenic bacteria including serological and molecular techniques are, undoubtly reliable; however, these techniques can be time-consuming to analyse results. To cure the bacteria borne disease in plants, there is a need for ‘on site’ detection in field to enable treatment protocol precisely. To meet this aspect, generally point-of-care detection protocols are more reliable. Among several POC based assays, lateral flow assay (LFA) has emerged as a promising rapid diagnostic tool for detection of plant pathogenic bacteria. This technique has been found to be useful to detect various plant pathogenic bacteria such as, Dickeya solani, Erwinia amylovora, Ralstonia solanacearum, Xanthomonas arboricola, and Acidovorax avenae subsp. citrulli. There are also instances where LFA has been used along with molecular techniques like PCR or recombinase polymerase amplification (RPA) to enhance sensitivity and specificity for plant bacteria detection. Such combined detection approaches allow identification of bacterial pathogens more precisely. As research in this area continues, LFA is expected to play an increasingly critical role in ensuring food security and safeguarding plant health.

Keywords: plant pathogenic bacteria, molecular techniques like PCR, RPA.

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I. INTRODUCTION

Plant pathogenic bacteria (PPBs) are significant plant pathogens that are distributed worldwide (Bar-On et al., 2018). It is anticipated that approximately 150 species of the 7100 identified bacteria are responsible for various plant illnesses (Rajesh-Kannan et al., 2016). Plant pathogenic bacteria are distinguished from non-pathogenic counterparts by their ability to induce disease (physiological harm) on sensitive plants (hosts). Upon spread of infection, which typically begins with a small number of cells from the pathogen (propagules), they colonise and reproduce rapidly in living plant tissues, reaching extremely high population levels per tissue mass/area (often multiple million-fold over initial inoculum concentrations). When infected by the substantial microbial biomass in the tissue of plants they interfere directly with biochemical signalling pathways, host pathology and gene regulation. They also interfere with local and long-distance nutrient transport that ultimately results in changing in plant's developmental programme. Infection results in decreased plant development and output, lower quality of the product, following harvest degradation of crop product, loss of perennial crop plantations, and, in some situations, greater sensitivity to other biotic or abiotic causes (e.g., frosts).Plant protection is critical in the agricultural sector for food quality and quantity. Before plant diseases can be comprehended and controlled, those must be accurately diagnosed and concerned pathogens should be identified. Early pest detection techniques have the potential to halt transmission of diseases and food waste.A range of technical approaches including microscopy, physiological, biochemical, serology, and molecular tools is employed to find and detect infections caused by bacteria (Valeria Scala et al., 2018).The initial step in pest management is the observation of plant symptoms, which is done using optical techniques (Lazcka et al., 2007).Currently, a variety of methods based on various concepts are utilised as diagnostic tools, including microscopy,biochemical, serology, physiological, nutritional, molecular tools, and culture propagation(Valeria Scala 2014).There were several serological methodsbased on Enzyme linked immunosorbent assay (ELISA), and Immunofluorescence (IF) thosehave been reported to detect the pathogenic bacteria in plants. On the other hand, several molecular methods based on DNA and RNA have also been used for bacterial pathogen identification in plants. Other techniques have been used to find plant pathogenic bacteria, such as the image spectroscopy technique, electronic nose, volatile organic chemicals, and biosensors. In fact, isolation as well as culture propagation have always been the "golden" procedure and are still necessary stages for a precise diagnosis, but they are time-consuming. Plant pathogenidentification should be supplied quickly, accurately, and consistently in the early stages by utilising innovative sensor technologies in the open field. Early detection of diseased plants can help to prevent disease transmission (Valeria Scala 2014). Moreover, Asymptomatic plants can serve as a reservoir for infections, and the development of diagnostic procedures with increased specificity and sensitivity can aid in the detection of plant pathogenic bacteria even in the absence of illness symptoms or obvious indicators of the causal agent. That is when the point of care assay came. The diagnosis of pathogens should ideally be like the point of care, so that treatment can begin promptly and is not actually dependent on the availability of laboratory or highly qualified personnel. The pointofcare (POC) approach is gaining traction for its capacity to execute diagnostic tests precisely and quickly at the spot where they are required. These technologies are simple to use and produce clear outcomes with quantitative data. Lateral Flow Assay is a point of care detection method that powers low-cost, simple, fast, and portable detection instruments used in biomedicine, agricultural, food, and environmental sciences. The LFA (lateral flow assay) is a paperbased platform for detecting and quantifying

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analytes in complicated mixtures. The sample that needs to be tested is placed on a test device, and the findings are available in 5-30 minutes(Koczula et al., 2016). LFA-based assays are frequently employed in clinical laboratories, hospitals and physician's offices for the quantitative and qualitative detection of specific antibodies (Nielsen et al., 2008) and antigens (Boisen et al., 2015) as well as results of gene amplification (Rohrman et al., 2012).It was found that the lateral flow assay performed well in the diagnosis of plant pathogenic bacteria due to its conformity about 90% in field and 96% in laboratory with typical diagnostic method for detection of fire blight, its simplicity, speed and high specificity (Braun-Kiewnick et al., 2011).It provides an accurate replacement for presently accessible confirmatory tests of suspected plate isolates (e.g., serum agglutination) at least for the majority of plant samples submitted by field inspectors (Braun-Kiewnick et al., 2011). The LFIA was demonstrated to be specific to a plant bacterial disease, detecting all strains from a global collection(Pablo et al., 2017). Moreover, lateral flow assay has been combined with other assays to make the plant bacterial pathogen detection more specific and accurate. LFA has coupled with molecular assay like PCR based methods and recombinase polymerase amplification etc. for higher sensitivity (Hodgetts et al., 2014; Firas Ahmed et al., 2018; Ivanov et al., 2020). While Rapid and sensitive detection of plant bacterial pathogen has been done by lateral flow dipstick combined with loop mediated isothermal amplification(Rigano et al., 2014). In conclusion, lateral flow assay is one of the most appropriate and convenient method for on-site or in-field detection of plant pathogenic bacteria that reduces time for detection and lab expenditure.

In this chapter, we basically focus on the detection of different plant bacterial pathogenby the method of lateral flow assay, that has never been thoroughly discussed earlier. The rationale of thischapter is to bring focus for current researchers to be update on the methods including advantages and limitations of lateral flow assay for the identification of plant pathogenic bacteria.

II. BACTERIA CAUSING PLANT DISEASE

Although considered architecturally simple, bacteria are metabolically diverse and are found in large quantities practically everywhere on Earth (Sarah D. Williams et al., 2017).The taxonomy of plant bacterial pathogen is continually changing due to recent improvements in bacterial classification.The majority of plant bacterial pathogen belongs to theseensuing genera: Erwinia, Pectobacterium, Spiroplasma, Agrobacterium, Pantoea, Xanthomonas, Pseudomonas, Ralstonia, Acidovorax, Streptomyces, Clavibacter, Xylella, Burkholderia, and Phytoplasma(Sarah D. Williams et al., 2017).Plant pathogenic bacteria induce a variety of symptoms, including galls and overgrowths, wilts, soft rots, specks and blights, leaf spots, scabs and cankers.Some plant bacterial pathogens create toxins or inject specific proteins that cause death of the host cell, while others release enzymes that degrade important structural components of plant cells and their walls(Sarah D. Williams et al., 2017).Bacteria responsible for plant diseases can be dispersed in a variety of ways, including wind, rain, birds, and insects.Propagation using bacterium-infected plant material is a key method by which pathogenic bacteria are spread over long distances. However, bacterial pathogens require a wound or natural opening, such as stomata, to enter a plant host(Sarah D. Williams et al., 2017).Once inside, they use the methods outlined above to kill host cells, allowing them to expand. Within hosts, they can develop innocuously on surfaces of plants and then overwinter or withstand poor environmental circumstances or the absence of a

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vulnerable host by remaining dormant in infected tissue, infested soil or water, or in an insect vector(Sarah D. Williams et al., 2017).

III. CONVENTIONAL METHODS FOR DETECTION OF PLANT BACTERIA

There are several different conventional methods has been done to detect plant pathogenic bacteria such as serological method, molecular method, image spectroscopy technique, electronic nose, volatile organic compounds and biosensors etc. Serology-based approaches for bacterial pathogen detection are analytical instruments used for a variety of targets and provide supplementary proof for causative linkages within the disease and the pathogenic agent. Serology is useful, rapid, and cost-effective for large-scale examinations of symptomatic materials(Valeria Scala et al., 2018).The ELISA is a test that combines antibody specificity with colour change to identify a target (E Ward et al., 2004). The antigens associated with plant pathogen may be recognized by antibodies through this process but, often coupling of the antibody to an enzyme that ultimately generate colour as a sign of positive test when substrate is added (E ward et al., 2004). Indirect ELISA is used in several commercially available kits for pre-screening plant samples for identification of bacteria. Indirect immunofluorescence (IF) is another serological method other than ELISA which is a fluorescence microscopy-based optical technique that has been used to detect bacterial pathogen in plant tissue (E Ward et al., 2004). The molecular testing might be quite specific and rely on hybridization or amplification procedures. The majority of bacterial pathogen assays detect DNA, that's simpler to manufacture and more robust than highly specific RNA(Valeria Scala et al., 2018).PCR has been used in molecular diagnostic assays since the past few years because it is more practical, simple, and fast than hybridization procedures. This approach was created to detect plant disease bacteria such as Erwinia amylovora (M Rosello et al., 2002) or P. syringae pv. actinidiae (E Biondi et al., 2013). Because it is quite sensitive, it is useful for very rare templates; however, the chance of false positive findings is substantial.Some writers recommended employing the Droplet Digital polymerase chain reaction (ddPCR) in plant pathology diagnostics(Gutiérrez-Aguirre et al., 2015).Aside from Xanthomonas citri subsp. citri, ddPCR has recently been recommended as a method for detecting Xylella fastidiosa among phytopathogenic bacteria (T Dreo et al., 2014).Then there is a technique image spectroscopy has application in agriculture offers the possibility of an automated non-destructive method for detection of plant diseases(L Belasque et al., 2008).Fluorescence, multispectral or hyperspectralimaging, fluorescence spectroscopy, infrared spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and visible/multiband spectroscopy are some of the approaches utilised for plant disease identification (S Sankarana et al., 2010).A non-optical indirect approach for detection of plant diseases is based on characterisation of the volatile chemical signature of affected plants.

IV. REQUIREMENT FOR QUICK DETECTION OF PATHOGENS IN PLANTS

To control bacterial infections in plants, there is a need for precise identification of these pathogens. Many successful techniques and protocols have been developed over past decades by different investigators as described in the section of conventional methods for plant bacterial detection. However, there is a less reliability on these tests due to inherent lack of specificity and sensitivity.

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Immunological basis of detection of plant pathogenic bacteria is considered more accurate in terms of specificity and sensitivity. On this basis, pathogens are typically detected using well optimized techniques such asenzyme linked immunosorbent assay (ELISA), immunofluorescence, immunoblot, affinity chromatography, radioimmunoassay (RIA), and agglutination method to detect antigen-antibody complexes. Such methods are used for direct identification of organisms or, antigenic components of microbes or any soluble products of the microorganisms present in host tissue. Among these methods, few are designed for rapid detection of pathogens to enable quick diagnosis of diseases that enables timely and on-site treatment options for plant. It is pertinent to note that, several immunological approaches are laboratory based, sensitive and specific; however, are time consuming and needs skilled technician to conduct the task. Most importantly, in-field detection in case of plants without availability of laboratory or highly trained staff is only possible with point-of-care assay where the treatment should start immediately. Plant disease point-of-care (POC) testing might be utilised to improve management of plant diseases in resource-limited situations. The point-of-care (POC) approach is gaining popularity for its ability to execute diagnostic tests precisely and quickly at the location of need.These technologies are simple to use and produce clear outcomes with quantitative data, and mainly it prevents the transmission of pathogen. Lateral flow assay is one of the most precise POC assay which is basically a paperbased platform for detecting and quantifying analytes in complicated mixtures. The sample is deposited on a test device, and the results of the same showed in 5-30 minutes (Koczula et al., 2016). This assay has been used to detect many bacterial pathogens in plants.

V. GENERAL PRINCIPLE OF LATERAL FLOW ASSAY

Lateral flow immunoassay (LFIA) is a paperbased assay for detecting target probes in the matrix using simple, cost-effective, and economical equipment (Sher et al. 2017).In 1976, the first LFIA was introduced to detect the presence of human chorionic gonadotropin (hCG) in sample of urine (Gnoth and Johnson 2014).The immunodiagnostic technology was presented in two variations in 1980: lateral flow test and a flow-through test. Now both these techniques are broadly used in various fields including in the detection of plant pathogenic bacteria. Lateral flow assay is called by different names in different places like lateral flow device (LFD), dipstick test,LFIA, quick test,express test, rapid testalso called aspen side test etc.

The principle underlying LFA is simple: the fluid sample (extract) encompassing the analyte of interest flows across several zones of the polymeric strips, on which molecules (antigens and antibodies) come into contact with the analyte are attached, with no intervention of external pressures (capillary action) (Kuczula et al., 2016). The architecture of LFIA is somewhat like a strip constituting overlapping membranes that has been placed on a backing card for greater stability and handling (Kuczula et al., 2016). The four constituents of this strip are sample pad, conjugation pad, nitrocellulose membrane, and absorption pad (Sajid et al. 2015).The sample is mounted on the surface of the absorbent sample pad at the edge of one end of the strip, which has been soaked with buffer salts and surfactants to render the sample suitable for communication with the detection system. (Kuczula et al., 2016).From the sample pad the treated sample moves towards the conjugate pad next to it on the strip where the labelled antibodies have dispensed. The sample, along with the conjugated antibody linked to the target substance, travels across the strip into the area of detection zone. (Kuczula et al., 2016).The detection zone is simply a membrane that's porous comprised of

nitrocellulose that contains certain particular biological components. Those components are mostly antibodies or sometimes antigens which are immobilized in lines.If the analyte of interest is present in the sample, it will be bound by the conjugate levels and will migrate with the assay to the membrane of reaction. Biological constituents (antibodies or antigens) are constrained on the reaction membrane to the test line, whereas the reagents used for control are restrained to the control line (Gupta et al., 2021).A reaction on the test line shows that the sample component has been recognised, whilst a response displayed on the control line shows that the liquid flow across the strip is appropriate (Kuczula et al., 2016).The read-out, displayed through the lines with varying intensities, can be examined visually or via a dedicated reader.The liquid moves along the device due to the capillary force of the strip material, and an absorbent pad is placed at the end of the strip to maintain the movement (Kuczula et al., 2016). Absorbent pad, as the name suggest it absorbs the excess material from the sample and prevents the backflow of the liquid.

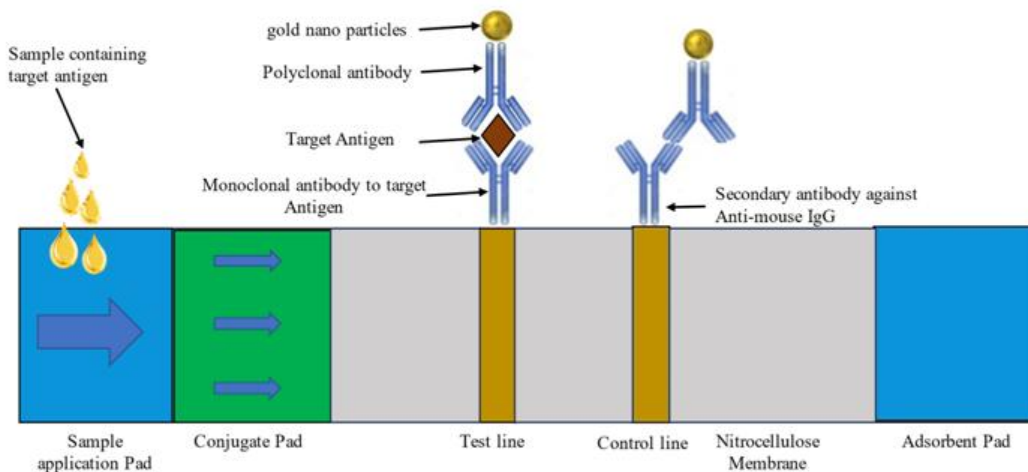

Figure 1: Lateral Flow Assay Showing Positive Result of Detection of Pathogen

VI. LATERAL FLOW ASSAY IN DETECTION OF PLANT PATHOGENIC BACTERIA

Many studies have been performed previously that shows lateral flow assay being one of the precise and quick detection method for the detection of plant bacterial pathogen. We discussed this aspect as follows. One of the studies aimed to develop a lateral flow immunoassay (LFIA) for the quick detection of a major potato disease, potato blackleg, caused by Dickeya solani and Dickeya dianthicola (Safenkova 2016).This studyis based on the creation of a test system that will detect the plant bacterial pathogen Dickeya. After immunizing rabbits with bacteria from D. dianthicola and D. solani, polyclonal antibodies that were specific to various Dickeya strains were produced. The LFIA has been developed using the discovered gold nanoparticles and antibodies. The LFIA approach allowed for the examination of potato extracts in 10 minutes under ideal conditions, with an optical limit of detection of 4x 105 CFU/ml for tubers and 1x 105 CFU/ml for leaves. The assay was run on extracts of potato stem and tuber, and real-time polymerase chain reaction was used in 92.1% of samples to corroborate the LFIA results (Safenkova 2016).One objective of this study was to distinguish between Dickeya and Pectobacterium in potatoes with blackleg symptoms. For

Dickeya strains, the acquired antisera had high titers, whereas for Pectobacterium strains, they had low titers. Although there were more false-negatives with the LIFA, the LFIA's results almost perfectly correlated (92.1% overlapping for the examined potato stems and tubers) with those of the realtime polymerase chain reaction (PCR) approach (Safenkova 2016). The current analytical procedures for the detection of D. solani and D. dianthicola are time-consuming and technically challenging (Palacio-Bielsa A et al., 2007; Czajkowski R et al., 2015). The newly created LFIA offers a low-cost, quick, and sensitive way of detecting potato blackleg brought on by D. dianthicola and D. solani(Safenkova 2016).Erwinia amylovora is another plant bacterial pathogen that induces fire blight, a quarantine disease that damages almost all of Rosaceae plants (Razo et al., 2021). Razo et al., developed a lateral flow immunoassay (LFIA) to detect E. amylovora as well as compare different plant parts for the testing of LFIA optimisation in their study. The specificity of the produced LFIA was estimated against 11 strains of E. amylovora and related species. LFIA demonstrated a high degree of specificity and produce no positive results with unrelated species(Razo et al., 2021).The conclusiveness of LFIA was confirmed by examining artificially infected sample of leaves from the plants like black raspberry, pear and apple. The result obtained very quickly through the LFIA in around 10minutes for all testing strains (Razo et al., 2021). Another deadly and financially essential bacterial pathogen of potatoes and other agricultural crops is Ralstonia solanacearum. Hence, the rapid and sensitive method was developed by Panferov et al., 2016 with low limit of detection (LOD) which is based on Lateral flow immunoassay (LFIA). Silver enhancement was used to reduce the LOD of LFIA.The LFIA with silver enhancement was shown to be ten times more sensitive (LOD 2102 CFU/mL; 20 min) than the standard analysis (LOD 2103 CFU/mL; 10 min) (Panferov et al., 2016). The LFIA was able to identify all strains of R.solanacearumwhere there were no non-specific reactions were observed (Panferov et al., 2016).The LFIA has also been successful in detecting the bacterial pathogen Xanthomonas arboricolawhich is the causative agent of bacterial spot disease of almond and stone fruits, also a major imminence to Prunus species (Pablo et al., 2017).Polyclonal antibodies were used for developing this assay, which was then mixed with carbon nanoparticles and placed on nitrocellulose strips. According to study of Pablo et al., LFIA was very specific towards detecting the different strains of Xanthomonas arboricola(2017) though there were very less cross reactivity was also observed. On the other hand, self-paired monoclonal antibody lateral flow immunoassay strip developed for rapid detection of Acidovorax avenae subsp. citrulli (Zeng et al., 2016).The highly specific monoclonal antibody 6D against Acidovorax avenae subsp. citrulli (Aac) was tested in this work. The Aac bound with Aac test McAb at the test line which was recognized by gold labelled antibodies bound with Aac (Zeng et al., 2016). Fire blight is acontagious disease occur due to another pathogenic bacteria Erwinia amylovora that poses a global danger to pome fruit cultivation. Braun-Kiewnick et al. developed an E. amylovora-specific lateral-flow immunoassay with a limit of detection log 5.7 CFU/ml, which corresponds to pathogen levels in symptomatic plant material(2011).On-site validation in ring testing proved efficient and reliable detection as compared to other detection methods like subsequent plating and PCR analysis (Braun-Kiewnick et al., 2011).The ease of use, inspector embracing oneself, and quicker diagnosis (15 minutes with the immunoassay where 2 days for laboratory provided samples) make the immunoassay a powerful tool for enhancing phytosanitary oversight of fire blight (Braun-Kiewnick et al., 2011).

Lateral flow assay being very rapid assay and a suitable detection method for in-field detection of plant pathogenic bacteria but, according to some researches when it coupled with

other molecular assays LFA shows more sensitivity. One of those researches shows the combination of the lateral flow assay with a genome-informed recombinase polymerase amplification assay for identifying Dickeyaspecies in infected plant tissues with no necessity of DNA isolation (Boluk et al., 2020). Dickeya species cause blackleg and soft rot diseases in potatoes as well as other plant species over the world, causing significant economic losses and this is why the need of rapid detection raised. With 34 sample strains from every Dickeya species and 25 strains from different genera and species, the assay specificity was confirmed; no false positives or negatives were observed (Boluk et al., 2020).The research revealed only Dickeya strains had 100% identity, which was expected given that the targeted genome region was unique to Dickeya species.A lateral flow assay (LFA) paired with isothermal DNA amplification was developed for the quick, specific, and sensitive identification of D. solanicaused potato blackleg disease (Ivanov et al., 2020).Ivanov et al. developed this method to identify DNA amplicons using lateral flow test strips. This same assay was performed for detection of Pectobacterium species which causes severe bacterial soft rot disease in fruits and vegetables including potato and tomato (Ahmed et al., 2018). They obtained the same result as Boluk et al., with no false positives or negatives found in 26 Pectobacterium sp. strains and 12 non-Pectobacterium species. To improve the reliability and precision of the established test, RPA probe and primersfor host control have been developed (Ahmed et al., 2018).In this experiment, the limit of detection was 10 fg with spiking sensitivitywhereas the assay developed by Boluk et al., had the limit of 1fg. Crown gall disease is another hazardous plant disease caused by the soil-inhabiting bacterium, Agrobacterium. Early and quick detection of this menacing bacterium is the key to manage the crown gall disease. This is when Fuller et al. designed primers made from oligonucleotides and probes that target virD2 for use in a molecular diagnostic tool that utilises isothermal amplification and lateral flow detection(2017). The incorporation of lateral flow detection into the utilisation of these oligonucleotide primers in isothermal amplification lowered the onerousness of the procedure and eliminated the need for specialised tools required for molecular diagnostics (Fuller et al., 2017).The technique represents a step forward in the fast molecular identification of pathogenic Agrobacterium spp. Again, Xanthomonas campestris is the causative agent of banana Xanthomonas wilt, which is a severe danger to banana production and most importantly it can be spread by wide range of mechanism. The need for early and rapid detection made Hodgetts et al., to develop a lateral flow device for on-site detection of Xanthomonas campestris(2014A polyclonal antibody (pAb) was generated in this study and used in a lateral flow device (LFD) configuration to detect Xcm in the field quickly. Both naturally and artificially infected banana plants were successfully detected, and the limit of detection in this device was 105 cells/ml (Hodgetts et al., 2014).Xav has never been detected in banana, despite the fact that the pAb is not completely specific for Xcm.As a result, the LFD can be utilised as a first-line screening tool in the field for identifying Xcm (Hodgetts et al., 2014).LAMP (Loop Mediated Isothermal Amplification), a novel DNA amplification approach, was modified for the detection of Candidatus Liberibacter asiaticus, which is linked to a variety of plant diseases(Rigano et al., 2014).The aforementioned methodology was paired with a Lateral Flow Dipstick (LFD) instrument to identify the amplicons visually, removing the requirement for gel electrophoresis. The assay proved extremely specific for the microorganism under study. There was no cross-reaction with any of the other phytopathogenic bacteria or fungi DNA tested(Rigano et al., 2014).

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VII. CONCLUSION

Lateral flow based approaches for detection and identification of pathogenic microorganisms is a popular practice as quick detection strategy. However, this particular technique has been commonly used for identification of various pathogens in humans enabling quick diagnosis of several diseases. Use of lateral flow based approach for plant bacteria detection has gained demand recently as this technique offers a unique opportunity for diagnosis of bacteria borne plant disease precisely. We summarized this concept citing several examples in this book chapter. Further, when combined with molecular assays like PCR or LAMP, LFA shows enhanced sensitivity and specificity, making it a reliable tool for early and accurate detection. Overall, LFA has the potential to revolutionize plant pathogen diagnostics, offering a practical and efficient method for detecting PPBs in the field. Its simplicity, speed, and accuracy make it an invaluable tool for plant protection, enabling timely interventions to minimize crop losses and enhance agricultural productivity. As research in this area continues, LFA is likely to become an integral part of plant disease management strategies, contributing to the sustainable growth of the agricultural sector.We believe that this book chapter will throw light on readers to educate themselves for lateral flow based approaches in precise detection of plant bacteria. We further believe that, this technique is expected to play an increasingly critical role in ensuring food security and safeguarding plant health.

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