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EXPLORING CUTTING-EDGE TECHNOLOGICAL ADVANCEMENTS IN PLANT DISEASE DIAGNOSIS AND MANAGEMENT

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ABSTRACT
Advancement in agriculture for diagnosing pathogens and applying management strategies are utmost necessary for improving food security. Modern methods of detection of pathogens play a crucial role in directly helping sustainable disease management. Modern methods are used over traditional ways of disease diagnosis because of their preference regarding time-consuming processes and as well as effectiveness regarding the correct and authentic result. Among all the methods of Polymerase Chain reaction (PCR), real-time time-PCR and Quantitative PCR an important at the molecular level of the detection of pathogens as they really show the correct and definite results leading to low fake outcomes. A comparison made between PCR methods and Loop-mediated isothermal amplification (LAMP) was found to be a feasible one for detecting plant pathogens as no long procedure was required to make it time-consuming. But Biosensor is regarded as an overall accurate means of pathogen detection at modern molecular methods. When it comes to modern management strategies, there has been a lot of research done on Integrated Disease Management (IDM), which has a sustainable crop production principle. As IDM works to reduce the use of pesticides and fungicides by employing alternative methods of controlling plant pathogens, the priority level rises. Aside from IDM, plant immunity is important for managing plant pathogens through defense genes and maintaining disease resistance in plants by regulating a variety of gene expression mechanisms in host plants. Precision agriculture, such as remote sensors, is responsible for disease management strategies that are indirectly linked to regular crop monitoring and stress protection in plants.

KEY WORDS
Agriculture, detection, fungi, molecular, pathogens, precision.

The proliferation of plant pathogens poses a significant challenge to the economic and ecological stability of various regions worldwide. Plant pathogens encompass a range of detrimental organisms such as viruses, bacteria, fungi, nematodes, and parasitic plants. These pathogens lead to the development of diseases affecting various parts of plants, including leaves, stems, roots, vascular systems, and fruits. Plant pathogens employ specific mechanisms to invade and harm plants, exacerbating the spread of diseases and causing visible symptoms to manifest (Al-Ani & Furtado, 2020). Dating back to ancient times, lacked scientific understanding of the causal factors behind disease development. Instead, people attributed the occurrence of plant diseases to supernatural causes such as the malevolent influence of the "evil eye" on community plants. Between the years 1600 and 1800, significant advancements in microscopy occurred, enabling the identification of various microorganisms such as protozoa, fungi, bacteria, and viruses. In 1728, a French scientist named Duhamel de Monceau made a notable contribution to plant pathology by describing the saffron disease affecting crocus plants. Through his research, he definitively demonstrated that the disease was caused by a contagious fungus called Rhizoctonia (Yuen et al., 2020). The severity of a potential disease is influenced by multiple factors that

contribute to its development. Additionally, the visibility and manifestation of disease symptoms depend on various factors, including the specific stage of disease development. Different diseases have unique stages at which they exhibit distinct symptoms, some of which may be visible at early stages, while others become apparent later in the disease progression. Detecting diseases can be challenging for forecasters due to various environmental conditions, such as temperature and relative humidity. Fluctuations in these factors can complicate the identification and prediction of diseases (Adikaram et al., 1986, Agrios, 2004). Different crops in Nepal face specific diseases that have become significant concerns for pathologists. In 2022, the occurrence of Citrus greening in districts like Palpa raised serious alarm among experts, necessitating urgent efforts to address the issue. Rust problems in wheat, club root in crucifers, late blight in potatoes, and early blight in tomatoes are currently major concerns in Nepal. Extensive research is being conducted by senior pathologists at the Plant Pathology Division of the Nepal Agricultural Research Centre to combat these diseases effectively (Pokhrel et al., 2021) . Accurate identification and diagnosis of plant diseases are crucial in today's era of climate change and globalization. These diseases result in significant yield losses and substantial economic impacts. Ensuring food security and preventing the spread of invasive pests and pathogens rely on precise identification. Furthermore, efficient and cost-effective management of plant diseases requires accurate, sensitive, and specific diagnostic methods (Balodi et al., 2017). New means and technologies have been developed and implemented in recent years to enhance the promptness and reliability of plant disease diagnostics and overcome the limitations associated with traditional methods (Khakimov et al., 2022).

METHODS OF RESEARCH

A large number of papers were carefully examined, with sources including a wide range of academic journals, reputable newspapers, authoritative books, and governmental websites. A well-rounded and multifaceted perspective on the topic ensured by this extensive approach to information sourcing, which included insights and findings from various domains and viewpoints.

RESULTS AND DISCUSSION

Initially, conventional PCR techniques were utilized, but they were subsequently supplanted by more advanced PCR methods like Real-time PCR, quantitative PCR, and multiplex PCR. This transition occurred because conventional PCR was deemed less accurate and more labor-intensive (Schena et al., 2013). Therefore, there is a need to develop rapid, sensitive, and precise methods for pathogen detection. Quantitative real-time PCR techniques play a crucial role in achieving accurate detection with minimal false positives. Moreover, these methods are valuable for distinguishing between closely related organisms, making them a dependable and reliable approach. Real-time PCR relies on detecting the increasing fluorescence emitted by a reporter molecule during the reaction. This reporter can be a dye that binds to double-stranded DNA or sequence-specific probes (Carlos Garrido et al., 2012). A study done by Garrido et al. (2009) showed that the newly developed assays (Real time PCR) exhibited significantly higher sensitivity, ranging from 10 to 100 times more sensitive than conventional PCR techniques previously utilized for diagnosing strawberry anthracnose. In comparison to ELISA methods, real-time PCR showed improved sensitivity in identifying the disease, as it yielded positive results for samples of strawberry plant material that had tested negative using ELISA.

Wheat plants can face infections from different pathogen species, some of which may produce similar symptoms. An example of this is the Septoria leaf blotch complex, caused by *Zymoseptoria tritici* and *Parastagonospora nodorum*, which often co-occur. Precise identification of wheat pathogens is crucial to determine the most suitable disease management approach. Loop-mediated isothermal amplification (LAMP) is a modern molecular technique that has quickly gained popularity for detecting plant pathogens

(Gomez-Gutierrez & Goodwin, 2022). In this experiment, researchers employed the Loop-Mediated Isothermal Amplification (LAMP) technique to detect *Fusarium graminearum*, the causal agent of Fusarium head blight (FHB). They tested DNA from 177 strains encompassing 21 genera of filamentous fungi and two genera of yeast. The LAMP primers were designed based on a 2042 bp fragment of the gaoA gene from *F. austroamericanum* isolate NRRL 2903. Direct application of LAMP to barley grains and wheat seeds allowed for efficient detection. The choice of the gaoA gene as the target was justified as *F. graminearum* is the only species exhibiting galactose oxidase activity in its culture supernatants (Niessen & Vogel, 2010). Recently, researchers have developed a foldable microdevice platform based on fuchsin colorimetric detection using LAMP to detect P. oryzae and *Sarocladium oryzae* in rice seeds. However, to apply this approach to other pathogen species, standardization will be necessary (Prasannakumar et al., 2021). Notably, other portable devices have been created for detecting LAMP products, such as the ESE-Quant tube scanner from Qiagen, Netherlands, and the Bio-Rad CFX96 Real-Time PCR system, both of which were used for portable real-time LAMP and fluorescence measurement to detect Ustilago maydis (Cao et al., 2017). Additionally, a POCKET platform (Point-Of-Care Kit For the Entire Test) has been developed, which can be coupled with isothermal amplification techniques(Xu et al., 2020). RT-qPCR demonstrated the highest sensitivity in comparison of the LAMP and PCR assays, followed by nested PCR, the LAMP assay, and traditional PCR. It was discovered that the LAMP assay could be used for field detection, potentially doing away with the need for costly thermal cyclers, gel electrophoresis, and time-consuming DNA extraction techniques. The LAMP specificity test revealed no amplification in healthy plant tissues, species that are closely related to it, or other fungi, proving that LAMP is a feasible field assay(Khan et al., 2018). The loop-mediated isothermal amplification (LAMP) technique amplification process was shown below figure in Figure 1.

Observing field conditions without physical contact in a field can be considered a form of remote sensing. Typically, remote sensing can be categorized into two main types: active remote sensing and passive remote sensing. In the field, passive remote sensing is commonly employed, which involves sensing the electromagnetic energy that is reflected from plants. The primary source of energy for passive systems is usually the sun. These sensors, which operate without actively transmitting signals, can be installed on satellites, manned or unmanned aircraft, or directly on farm equipment (Nowatzki, 2017). Coming to History of remote sensing in Agriculture, a committee dedicated to remote sensing was formed to address the issue of crop loss resulting from pests and diseases in 1960. As part of the progress made by the Purdue Research Committee, an aerial survey was planned. In 1970, the Corn Blight Watch marked a significant milestone in agricultural remote sensing by utilizing an airborne Multispectral Scanner (MSS). This pioneering effort represented the initial widespread implementation of remote sensing techniques in agriculture (Macdonald, 1984). Aerial photography, a form of remote sensing, was extensively employed in the detection of the devastating cotton root rot pathogen in Texas, where cotton is widely cultivated. Photographs taken from airplanes were used to identify and monitor the presence of cotton root rot, caused by the fungal pathogen *Phymatotrichopsis omnivora.* This aerial approach provided valuable insights into the distribution and severity of the disease in cotton fields (Wang et al., 2020). In a research study on citrus greening, hyperspectral image analysis was conducted using various methods such as image-derived spectral library, mixture tuned matched filtering (MTMF), spectral angle mapping (SAM), and linear spectral unmixing. The study concluded that the MTMF method exhibited higher accuracy compared to the other methods. Moreover, SAM analysis using multispectral images showed comparable accuracy (87%) to the MTMF results and outperformed SAM analysis on hyperspectral images in terms of accuracy (Lee, 2012).

Satellite imagery systems and non-image approaches like infrared (IR) photography are not widely prevalent in many countries. Currently, the primary method for disease detection involves visual observation, followed by the time-consuming process of Polymerase Chain Reaction (PCR) based on DNA sequences if uncertainty remains. There is a growing need to develop a rapid, cost-effective, and reliable sensor system for health

monitoring in agriculture. This calls for exploring existing technologies that can be adapted to develop a ground-based sensor system capable of monitoring plant health and diseases under field conditions (Sankaran et al., 2010). The utilization of remote sensing technologies has the potential to significantly enhance the spatial mapping of diagnostic results, leading to increased sustainability and safety in agriculture. This, in turn, can help reduce the need for costly pesticide applications in crop protection (Gogoi et al., 2018).

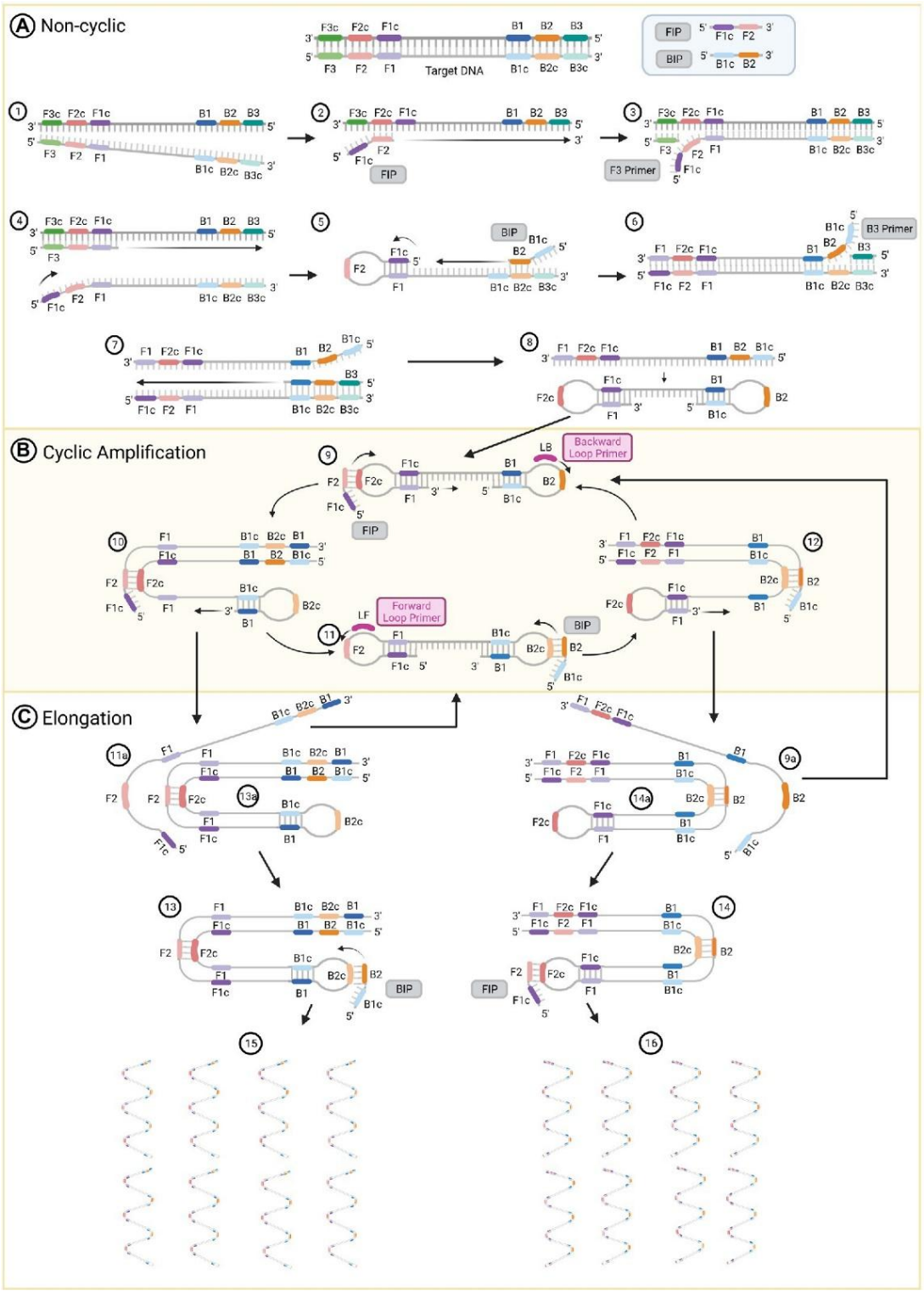

Figure 1 – The loop-mediated isothermal amplification (LAMP) technique amplification process: (A) Non-cyclic steps that produce a DNA strand with two loops at their 5’ and 3’ ends; (B) Cyclic amplification steps and (C) elongation.

The detection of pathogens at a minute level often requires a range of molecular methods, but some of these techniques suffer from inconsistency and time-consuming processes. As a result, optical and electrochemical biosensors are employed to achieve rapid and accurate pathogen detection, significantly expediting the results (Dyussembayev et al., 2021). A highly potent device, the biosensor combines a biological sensing element and physiochemical transducer. When pathogens come into contact with this device in a solution, it triggers the generation of an electronic signal (Elmer & White, 2018). A microfluidic electrochemical immunosensor was developed for the rapid detection of Xanthomonas arabicola in walnut species. The biosensor utilized covalently immobilized monoclonal anti-XA antibodies as its sensing element. Compared to the ELISA test kit, the biosensor demonstrated a significant reduction in detection time, providing results within just 30 minutes, whereas the ELISA test took 90 minutes for the same detection(Regiart et al., 2017). The detection of *Pseudomonas syringae* using the electrochemical sensor proved to be more crucial compared to conventional PCR, especially considering its role in identifying latent infections in plants (Lau et al., 2017). A rapid-response surface plasmon resonance sensor was developed for early detection of Asian rust in soybean leaf extracts. It covalently immobilizes anti-Phakopsora pachyrhizi antibody on a gold substrate using cysteamine-coupling chemistry with a self-assembled monolayer (SAM) of thiols. The immunosensor shows a linear response range of 3.5 to 28.0 g mL^{-1} for the target antigen (r2 = 0.996). Two factors, antibody amount for immobilization and surface blocking, were optimized to enhance specificity and sensitivity in detecting the pathogen in soybean leaf extracts (Mendes et al., 2009). A novel multi-targeting protocol was developed for the detection of three highly significant bacterial phytopathogens using an acoustic biosensor known as the Quartz Crystal Microbalance (QCM) for DNA detection. Unlike conventional methods that rely on mass-based discrimination, this innovative approach monitors and distinguishes DNA amplicons based on their respective lengths. The selection of these phytopathogens was based on their scientific and economic importance (Papadakis et al., 2015).

Integrated pest management (IPM) is where the IDM terms were derived. A movement to create more eco-friendly crop protection techniques started in the late 1960s. Although crop scouting was primarily motivated by economics to determine spray schedules, it was the first concrete step toward an IPM strategy. IDM recommends using pesticides sparingly and only when absolutely necessary (Pandey et al., 2016). A well verse of diseases is managed when a proper concentration of nutrients is loaded in plants. Nutrients such as N, K, P, Mn, Zn, B, Cl and Si plays a great role for disease resistance in plants that automatically reduce the infestation of pathogens leading to the sustainable agriculture(Dordas, 2008). For example: A high nitrate concentration in soil will the reduce the infestation of various types of disease such as *Fusarium oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani* and *Pythium sp*. Right application of Potassium can reduce the infestation of helminthosporium leaf blight and increase grain yields in wheat spp. According to Graham (1983), Ca confers resistance to Pythium, Sclerotinia, Botrytis, and Fusarium. Ca can be mobilized in *Colletotrichum trifolli*-induced lesions on alfalfa and aid the pathogen's growth by promoting the pectolytic enzyme polygalacturonic acid transeliminase's macerating activity (Kiraly, 1976). Mycorrhizal fungi have a great deal of potential for use as biocontrol agents for diseases that are transmitted through the soil and roots. Some species have been reported as being effective pest controllers for leaf spot diseases as well as phytoparasitic nematodes. It is necessary to assess the mycorrhizal symbionts in the natural system under field conditions for effective and persistent disease management. When compared to using a single species, the use of mixed inocula of mycorrhizal symbionts can be more productive and produce better results(Mukerji & CIANCIO, 2007). *Paxillus involutus*-mycorrhized *Pinus resinosa* roots had an inhibitory effect on *Fusarium oxysporum f. sp. Pini* (Duchesne et al., 1987). The main goal of integrated Disease management is to maintain disease magnitude below a economic threshold level that crop yield and quality reductions that have a negative impact on the producers(Zadoks, 1985). In order to accomplish one or more objectives, disease management requires the integration of tactics. To lessen disease intensity, these objectives include: i) eliminating or reducing the initial inoculum; ii) lowering the infection rate; and/or iii)

cutting down on the amount of time that pathogen populations and host populations interact (Nutter Jr & Guan, 2001).

Infection with the pathogen that causes the disease, cultivar non-pathogenic races of the pathogen, other pathogens, pathogens from other species, and, less frequently, products of infectious agents have all been used to immunize plants against diseases for at least a century (Chester, 1933). The research on systemic acquired resistance (SAR) and systemic induced resistance (ISR) over the past ten years has raised a remarkable awareness of the critical role that certain microbes, natural products, and chemicals play in promoting the expression of defense genes and disease resistance in plants. As an example, taken from Fusarium crown rot in tomato, it is found that various disease control mechanism such as disinfestation, cultural practices, and allelopathy are unable to treat soilborne pathogens. Consequently, the cytology of infection of root tissues from susceptible plant cultivars has advanced significantly over the past ten years (Brammall & Higgins, 1988) . It has been demonstrated that in tomato plants that were susceptible, the pathogen quickly spread through a large portion of the root tissues, severely damaging the host cells. A study, Benhamou et al., (1998) found that combining P. fluorescens and chitosan increased tomato plants' reactivity to *F. oxysporum f. sp. radicis-lycopersici*(FORL) as measured by the development of enlarged wall appositions and significant phenolic compound deposition. This initial investigation into the interaction between biotic elicitors showed that after exposure to both agents, susceptible tomato plants developed higher resistance to FORL infection.

To speed up crop germplasm improvement, geoinformatics and cloud-based, big data-driven applications are also being used. Crop germplasm that has improved tolerance to pathogens and abiotic stress and is compatible with various cropping systems and environmental conditions is needed (Roberts et al., 2021). We can now use data from these various sensor systems and link information about pathogens, pests, crop yield, soil fertility factors, water, etc. with climate factors to develop correlations using big data (defined as a combination of a variety of data, the velocity of data, and/or volume of data) management and analytics in combination with geographic information systems (GIS), which serves as the organizing principle for spatial data. The use of GMO-seed is occasionally an exception to grower adoption because GMO crops are frequently used in some regions of the world while being opposed in others (Bestelmeyer et al., 2020; Delgado et al., 2019) Thirty nations produce GMO crops, with five of those nations—the United States, Brazil, Argentina, Canada, and India—producing about 90% of the world's GMO crops (Van Acker et al., 2017). Additionally, GMO crops are not popular among many people due to perceived negative effects on human health and the environment (Jayaraman & Jia, 2012).

CONCLUSION

The world is becoming more advanced, but there are still opportunities for advancement in countries such as Nepal and others. Diagnosing plant pathogens accurately has always been a challenge for scientists, even with advances at the atomic level. So, advancements from conventional PCR to RT-PCR, as well as bio-sensors that use electrical impulses to detect plant pathogens, make it easier for plant pathologists to detect pathogens and require more research to delve more inside pathology world.

A crucial development in pathology is the ongoing observation of plant diseases in farmers' fields because it raises the possibility of addressing food insecurity. It is crucial to use a variety of integrated approaches for sustainable disease management in order to effectively address this challenge.

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