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Harnessing microfluidic
tools for early plant pathogen detection

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Introduction

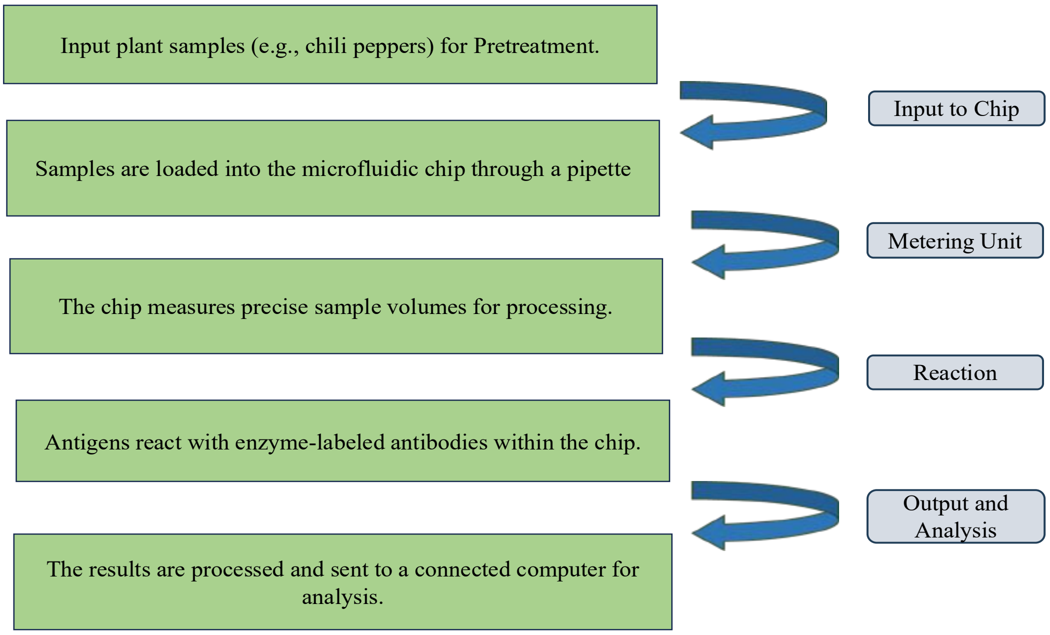
The increasing global population, projected to surpass 9.7 billion by 2050, is driving the demand for increased food production. This demand is exacerbated by concerns including farmland degradation and global warming, as well the rise of diseases of plants that threaten crop yields. The Food and Agriculture Organization (FAO) estimates that plant diseases cause annual losses of 13-22% of major crops, including rice, wheat, and maize. This translates to financial setbacks of around $220 billion globally and affects over eight hundred million individuals.

To meet these challenges, swift detection and control of plant diseases are critical. Traditional methods of plant disease control, such as visual assessments, generally unsuccessful enough that require technical abilities. During the past several years, microfluidic technology, also known as lab-on-a-chip (LOC) systems, It's become as a powerful instrument enabling quick processing, accurate detection of plant diseases caused by bacteria, fungi, and viruses. Microfluidics has revolutionized sample separation and detection by miniaturizing and integrating various analytical processes onto a single chip.

Principles and Mechanisms of Microfluidics

Microfluidics involves fluid modulation in structures with micrometer-scale proportions. This technology is categorized into three main types: continuous flow, droplet-based, and digital microfluidics. Continuous flow systems use permanently etched microchannels to control fluid movement, while droplet-based systems generate droplets using immiscible fluids. Digital microfluidics, on the other hand, moves and controls distinct particles on an electrode array that is flat and propelled by electrostatic forces.

Microfluidics provides accurate command over managing samples and fluid dynamics, enabling the integration of complex processes such as enzyme-linked immunosorbent assays (ELISA) and polymerase chain reactions (PCR) on a single platform. For example, ELISA can be miniaturized to detect plant pathogens with high sensitivity by leveraging microfluidic systems, which offer faster reaction times, reduced reagent consumption, and simplified workflows.



Input plant samples (e.g., chili peppers) for Pretreatment.

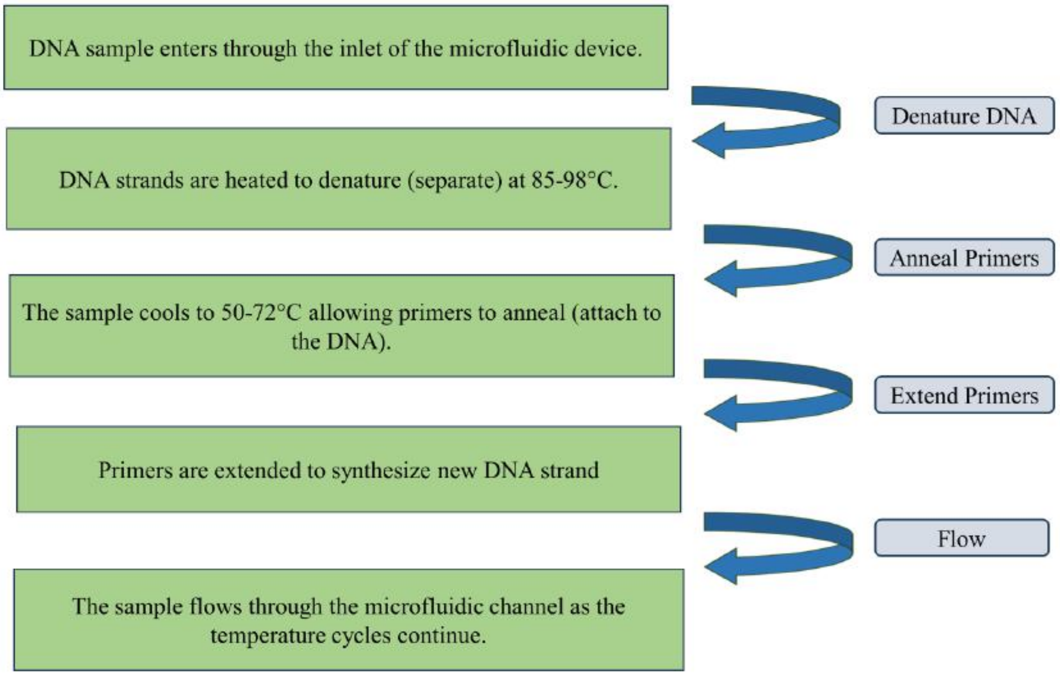
Samples are loaded into the microfluidic chip through a pipette

The chip measures precise sample volumes for processing.

Antigens react with enzyme-labeled antibodies within the chip.

The results are processed and sent to a connected computer for analysis.

Fig:1 Enzyme-linked immunosorbent assay (ELISA) revealed LOC



DNA sample enters through the inlet of the microfluidic device.

DNA strands are heated to denature (separate) at 85-98°C.

The sample cools to 50-72°C allowing primers to anneal (attach to the DNA).

Primers are extended to synthesize new DNA strand

The sample flows through the microfluidic channel as the temperature cycles continue.

Fig:2 Polymerase chain reaction (PCR) revealed LOC

Microfluidic Methods for Detecting Plant Diseases

The details various microfluidic-based methods for detecting plant diseases, including immunological methods, nucleic acid testing, morphological detection, and others. Below is an overview of each approach.

Immunological Methods: Immunological methods, such as ELISA, have been widely used for detecting plant pathogens. ELISA involves immune and enzymatic reactions to detect pathogens based on colorimetric responses. Microfluidic platforms can miniaturize and automate ELISA, resulting in enhanced sensitivity and efficiency. For instance, detecting watermelon viruses with a microfluidic ELISA sandwich demonstrated 2-

12.5 times greater sensitivity than conventional methods.

Recent advancements include smartphone-enabled colorimetric ELISA (c-ELISA), which offers both an intuitive application and a completely automated sensing technique. These technologies are paving the way for more accessible and rapid plant disease diagnostics.

Nucleic Acid Testing: Nucleic acid-based testing methods, particularly PCR, are highly specific and effective for pathogen detection. Microfluidic platforms can integrate PCR for on-chip DNA amplification, reducing contamination risks and processing times. One study developed an ongoing DNA target identification method using microfluidic biodevices, enabling rapid analysis of pathogens like gray mold.

Additionally, advancements in loop-mediated isothermal amplification (LAMP) and real-time convective PCR have further streamlined nucleic acid testing on microfluidic platforms. CRISPR-Cas systems, which leverage unique sequence-binding properties, are also being integrated with microfluidics to provide high sensitivity and specificity for plant disease detection.

Morphological Detection: Conventional techniques for morphological analysis, such as a close inspection under a microscope and staining, play a role in identifying fungal pathogens. However, these methods are often limited by low throughput and the need for expert interpretation. Microfluidic platforms, combined with microscopic imaging, have enabled observation to be feasible fungal growth throughout time. These systems can be used to study the single-cell development kinetics and to conduct filamentous fungal screening at high rates.

Microfluidics has furthermore applied to research fungal interactions Moreover, at the single hyphal scale, enzyme production. For example, this platform was developed to study the interactions between fungi causing diseases like soybean sudden death syndrome etc.

Other Methods: Beyond immunological, nucleic acid, and morphological methods, novel microfluidic devices are being developed for plant disease detection. These include fluorescence-based detection systems, wireless standalone devices, and disposable microfluidic devices for rapid parallel readings of virus variants. Such innovations are contributing to the development of more portable and user-friendly diagnostic tools for agriculture.

Materials Used in Microfluidic Devices

The choice of materials in microfluidic devices is critical for optimizing performance. Early microfluidic devices were made from inorganic materials like glasses and silica, but these components are becoming less optimal. due to limitations in functionalization and integration. Today, polymer materials such as polydimethylsiloxane (PDMS) are widely used for lab-on-a-chip applications. PDMS is favored for its accessibility, low poisoning, and optical transparency, making it suitable for microorganism culture and detection.

Other materials, such as paper and hydrogels, are also being explored for microfluidic applications. Paper-based microfluidic devices (μPADs) are cost-effective, portable, and suitable for resource-limited environments. Hydrogels, in which materials that are 3D porous and heavily hydrated, being used for microbiological culture and on-chip examination.

Applications in Plant Disease Detection

Microfluidics has shown great potential for identifying diseases of plants brought on by fungi, viruses, and bacteria. The document highlights various applications of microfluidic devices in agriculture:

Fungal Detection: Fungi account for 80% to 70% of infections that pose a risk global creating diseases in plants to ensure food security, making accurate identity is essential for effective treatment. Modern microfluidic techniques help detect early disease biomarkers like hormones in plants and unstable organic substances, aiding in early diagnosis. Azelaic acid (AzA) and salicylic acid (SA) are key metabolites to identify plant diseases, as demonstrated by an investigation where microbeads immobilized with tyrosinase enabled real-time detection of AzA, revealing a significant increase in infected grape samples. An additional is jasmonic acid (JA) important metabolic marker. Microfluidic systems can detect these three hormones with high sensitivity.

Advances in image analysis and spectrum identification, advanced spore identification through statistical modelling. Imaging without lenses for diffraction and microfluidics enable quick identification of fungal spores like *Botrytis cinerea* in greenhouse environments. Additionally, active gas-driven microbial separation methods have improved spore enrichment efficiency up to 94%. Electrochemical impedance spectroscopy (EIS) has also been used to detect pathogens like *Sclerotinia sclerotiorum* in rapeseed. Despite challenges in nucleic acid detection for fungal spores due to their thick cell walls, microfluidic devices show promise in addressing these limitations by culturing spores until mycelium growth is achieved.

Viral Detection: Monitoring Plant illnesses brought on by viruses are crucial due to the capacity to trigger severe signs and symptoms, significantly reduce crop productivity and caliber, and lead to substantial financial setbacks. Infections with viruses are difficult in order to avoid and can have severe impacts on agricultural production. Quick and accurate detection of contaminated crops is consequently essential, highlighting the demand for quick, affordable, on-site viral detection tools. A small-sized biosensor with flow sensing has been developed for the quick detection of banana bunchy top virus, combining both conventional immunoassay and chromatography with 0.13 aM as the detection limit, ten periods more sensitive as opposed to electrophoresis. For detecting Citrus tristeza virus (CTV), an ultrasensitive electrochemical detection method utilizing antibody fixation on electrodes offers an effective approach with a detection limit of 0.3 fg/mL. However, there exist still a few compact techniques that integrate virus gathering and identifying, with the majority requiring off-chip preprocessing and more stages in visualizing, posing challenges to the execution of point-of-care testing (POCT) in agriculture.

Bacterial Detection: An electrochemically integrated microfluidic immune sensor can effectively detect *Xanthomonas arboricola* in walnuts., while an additional electrochemical immunosensor efficiently detects *Pectobacterium atrosepticum* in potatoes using impedance theory. Microfluidic modules and microelectrode arrays are combined in the Lab-on-a-chip LOC) platform., offers high sensitivity at a low cost, outperforming traditional ELISA and PCR methods. For detecting Olive Quick Decline Syndrome, a similar LOC microelectrode achieved sensitivity that is 7.5 times higher than ELISA. Recent advances in point-of-care testing (POCT) microfluidic technology focus on improving sensitivity, cutting down on detection time, and optimizing extract enrichment, but integrating separation, detection, and visualization remains a challenge.

Future Prospects and Challenges

Microfluidic technology holds great promise for advancing plant disease diagnostics and improving global food security. However, challenges remain in scaling up these technologies for widespread use. Issues such as user interface simplicity, integration with portable devices, and cost-effective manufacturing need to be addressed to make microfluidic systems more accessible to farmers and agricultural professionals.

Additionally, integrating microfluidic devices with digital technologies like the Internet of

Things (IoT) and artificial intelligence (AI) could further enhance their capabilities. For example, image sensors CMOS have been employed to identify signals produced by immunological response systems, enabling real-time monitoring of plant diseases. AI and machine learning algorithms can also be applied to analyze data from microfluidic devices and provide actionable insights for disease management.

Conclusion

Microfluidics represents a significant advancement in the early detection of plant diseases, offering rapid, sensitive, and cost-effective diagnostic tools. Using microfluidic technology in conjunction with other scientific disciplines, such as materials science and digital technologies, is paving the way for innovative solutions to global food security challenges. As research in this field continues to evolve, microfluidics will play an increasingly important role in ensuring the health and productivity of crops worldwide.

This article incorporates key insights from the document, providing a comprehensive overview of recent advancements in microfluidics for plant disease detection. It addresses the principles, methods, materials, and applications of microfluidic technology, along with potential future directions.

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