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CRISPR-Based Biosensors for Plant Disease Diagnostics: Innovations, Applications, and Future Prospects

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ABSTRACT

Timely, accurate plant pathogen diagnostics are critical for mitigating crop losses and enhancing food security. Conventional methods (PCR, ELISA, LAMP) are effective but constrained by laboratory infrastructure, trained personnel, and multi-hour workflows, limiting field deployment. CRISPR–Cas biosensors—leveraging programmable target recognition and Cas12/Cas13 collateral cleavage—offer rapid, sensitive, and portable nucleic-acid detection. This review synthesizes plant-focused advances (2021–2025), covering molecular mechanisms; integration with isothermal amplification; and readouts spanning fluorescence, lateral-flow strips, and electrochemical sensing. We consolidate validated applications across viral, bacterial, and fungal pathogens, highlighting time-to-result (≈15–45 min), low per-test cost, and suitability for resource-limited settings. We also examine practical barriers, including plant matrix inhibitors, reagent stability, contamination control in amplificationcoupled assays, and fragmented regulatory pathways for agricultural diagnostics. Emerging solutions—microfluidic cartridges, lyophilized reagents, multiplexed panels using orthogonal Cas enzymes, and AI-assisted crRNA design—are accelerating translation toward ―sample-in, answer-out‖ platforms. We position CRISPR biosensors within precision agriculture and mobile surveillance ecosystems, emphasizing needs for standardized validation and field-level interoperability. Collectively, these developments indicate that CRISPR diagnostics are poised to become core components of decentralized, real-time plant health monitoring.

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Keywords: CRISPR-based biosensors, Plant disease diagnostics, Cas12/Cas13 detection

Introduction

Plant diseases contribute to approximately 20–40% of annual global crop losses, significantly impacting food security and agricultural economies (Savary et al., 2019). According to the Food and Agriculture Organization (FAO), plant diseases cost the global economy more than USD 290 billion annually, a figure exacerbated by climate change, global trade, and intensive farming practices (FAO, 2023). Effective management of plant diseases relies on timely and accurate detection of pathogens. However, conventional diagnostic methods such as PCR, ELISA, and LAMP often require centralized laboratories, trained personnel, and expensive equipment, limiting their application in resource-constrained agricultural settings (Lau & Botella, 2017). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)–Cas systems, originally known for gene editing, have been adapted into molecular diagnostics with high specificity, programmability, and sensitivity. Notably, platforms such as SHERLOCK (Cas13a-based), DETECTR (Cas12a-based), and HOLMES utilize the collateral cleavage activity of Cas enzymes to detect nucleic acids without the need for complex instrumentation (Gootenberg et al., 2017; Chen et al., 2018). These systems are now being integrated into biosensors, including lateral flow strips, fluorescent probes, and electrochemical readouts, enabling field-deployable solutions for real-time plant disease diagnostics.

Several studies have demonstrated CRISPR-based assays capable of detecting economically significant plant pathogens, such as *Tomato yellow leaf curl virus*, *Xanthomonas oryzae*, *Magnaporthe oryzae*, and *Ralstonia solanacearum*, with rapid turnaround and femtomolar-level sensitivity (Mahas et al., 2021). Despite these advancements, the adoption of CRISPR diagnostics in agriculture remains limited, partly due to challenges such as matrix interference, reagent stability, and lack of commercial validation. Additionally, while the literature is rich in clinical CRISPR diagnostic reviews, relatively few focus on plant pathology, and even fewer explore integration with digital agriculture technologies like AI-driven guide RNA design, smartphone-based surveillance, or Internet of Things (IoT) biosensors.

This review aims to fill that gap by synthesizing recent developments in CRISPR– Cas–based plant diagnostics, emphasizing biosensor formats, detection platforms, and their integration with precision agriculture. By analyzing case studies, signal detection modalities, pathogen-specific applications, and future prospects, this paper provides a comprehensive assessment of how CRISPR diagnostics can revolutionize plant disease management in the era of climate change and digital farming. Literature was sourced from peer-reviewed publications (2021–2025) using databases such as PubMed, Web of Science, and ScienceDirect, focusing on plant-focused CRISPR diagnostic tools, sensor integration, and field-deployable platforms.

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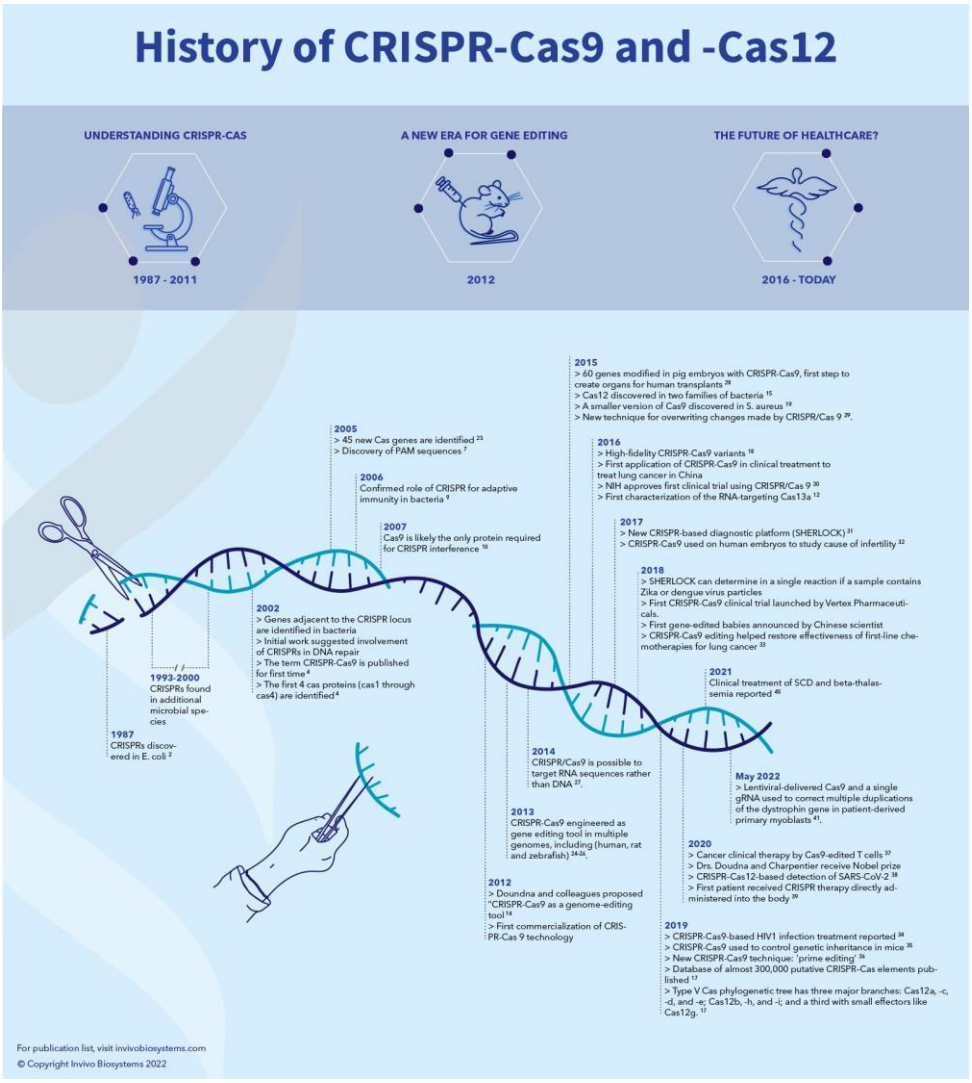


Figure 1. Timeline of CRISPR-Cas system development from discovery to modern diagnostic platforms. Key milestones include the initial discovery of CRISPR sequences in 1987, the development of Cas9 for genome editing (~2012), and the subsequent emergence of Cas12 and Cas13 diagnostics tools. Source: In Vivo Biosystems.

Section 2. Molecular Mechanisms and Detection Platforms Introduction to CRISPR-Based Molecular Diagnostics

CRISPR-Cas systems, originally part of prokaryotic adaptive immunity, have been engineered as robust, highly specific, and portable tools for detecting plant pathogens. The diagnostic utility of CRISPR is largely attributed to two key properties: (1) programmable recognition of nucleic acid targets using CRISPR RNAs (crRNAs), and (2) target-activated **collateral cleavage** of single-stranded nucleic acids, enabling signal

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amplification in biosensors (Yuan et al., 2022). These mechanisms make CRISPR-Cas diagnostics well-suited for field-level, real-time detection of plant diseases caused by viruses, bacteria, and fungi.

Table 1: Functional Overview of Key Cas Proteins

Cas Protein	Target Type	Collateral Cleavage?	Optimal Use	Example Diagnostic Platform
Cas9	dsDNA	No	Sequence-specific recognition	CRISPR-FDS, CRISPR-CHIP
Cas12a	dsDNA → ssDNA	Yes (ssDNA reporters)	DNA viruses, bacteria	DETECTR, HOLMES
Cas13a/b	ssRNA	Yes (ssRNA reporters)	RNA viruses	SHERLOCK
Cas14a	ssDNA (small)	Yes (no PAM required)	SNP detection, minimal samples	Cas14a-AMP, iSCAN

Cas12a and Cas13a have emerged as the leading tools for CRISPR-based diagnostics due to their robust collateral cleavage of synthetic reporter molecules after target recognition (Shanmugaraj et al., 2024), (Wang et al., 2023).

Mechanism of Detection

CRISPR-based diagnostics function through the following steps:

CrRNA-Guided Target Recognition: Cas proteins are programmed with synthetic crRNAs complementary to pathogen DNA or RNA.

Target Binding and Cleavage Activation: Cas12a/Cas13a bind to their target and become catalytically active.

Collateral Cleavage: Activated enzymes indiscriminately cleave nearby synthetic reporter molecules.

Signal Output: Cleaved reporters release a fluorescent, electrochemical, or colorimetric signal.

For example, in DETECTR (Cas12a + LAMP), detection of *Tomato Yellow Leaf Curl Virus* (TYLCV) is achieved in under 45 minutes with sub-femtomolar sensitivity (Mahas et al., 2021).

Integration with Amplification and Signal Platforms

Most CRISPR-Dx platforms combine nucleic acid amplification with biosensor output systems:

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Amplification Methods:
LAMP and **RPA** are commonly used isothermal methods compatible with Cas systems. These allow rapid amplification without thermocyclers, supporting field diagnostics (Li et al., 2023).

Signal Output Formats:
Fluorescence: Using FAM-quencher probes and handheld readers
Colorimetry: Via gold nanoparticles or pH changes
Lateral Flow Assays (LFAs): Paper-strip devices producing visible bands

Electrochemical Sensors: Emerging systems combining CRISPR with microelectrodes (Fu & Xianyu, 2023)
Recent innovations have included **multiplexing CRISPR reactions in microfluidic chips**, enabling simultaneous detection of multiple pathogens from the same sample.

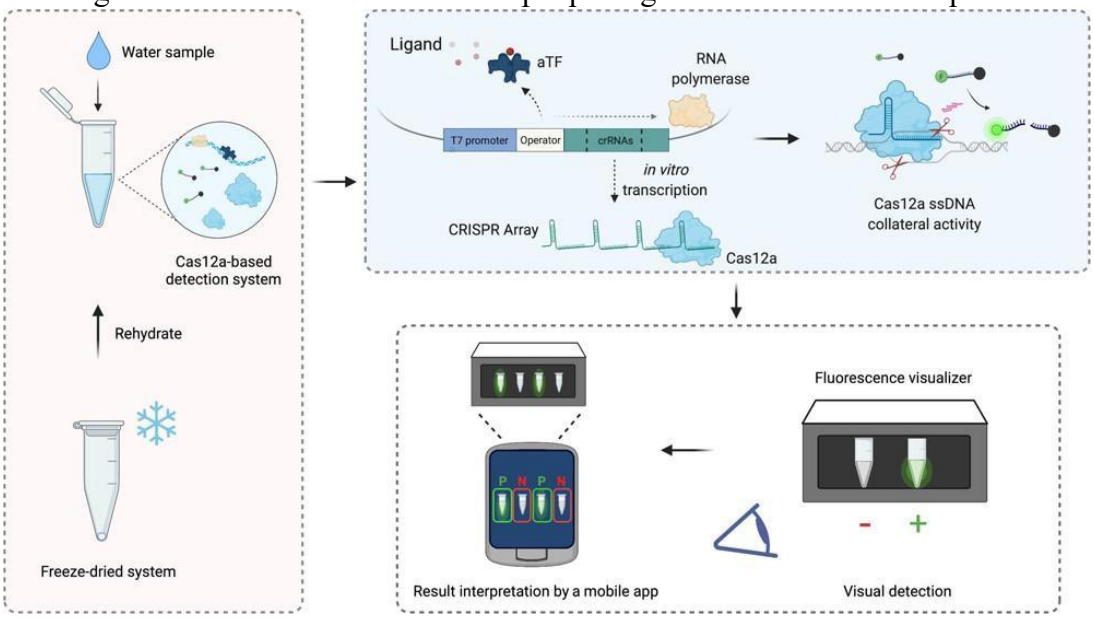


Figure 2. Workflow of a CRISPR-Cas12a–based field-deployable biosensor for pathogen detection.
The image illustrates a freeze-dried Cas12a detection system rehydrated with sample solution, transcription of CRISPR arrays, collateral cleavage activity leading to signal generation, and fluorescence-based result visualization. This figure was adapted from: Zhao, F., Wu, X., Qiao, J., et al. (2022). Analytical Chemistry, 94(11), 4617–4626.

Advantages and Challenges
Advantages:
High specificity from programmable crRNAs.

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Field-deployable with minimal instrumentation.
Rapid turnaround (15–45 minutes).
Affordable with potential for <$5/test in bulk settings.
Compatible with crude plant samples and visual outputs.

Challenges:

- Off-target collateral cleavage may produce background noise.
- Requires standardization across pathogens and plant matrices.
- Regulatory and commercialization pathways are still limited.
- Reagent stability (e.g., Cas protein lyophilization) for field kits remains under development.

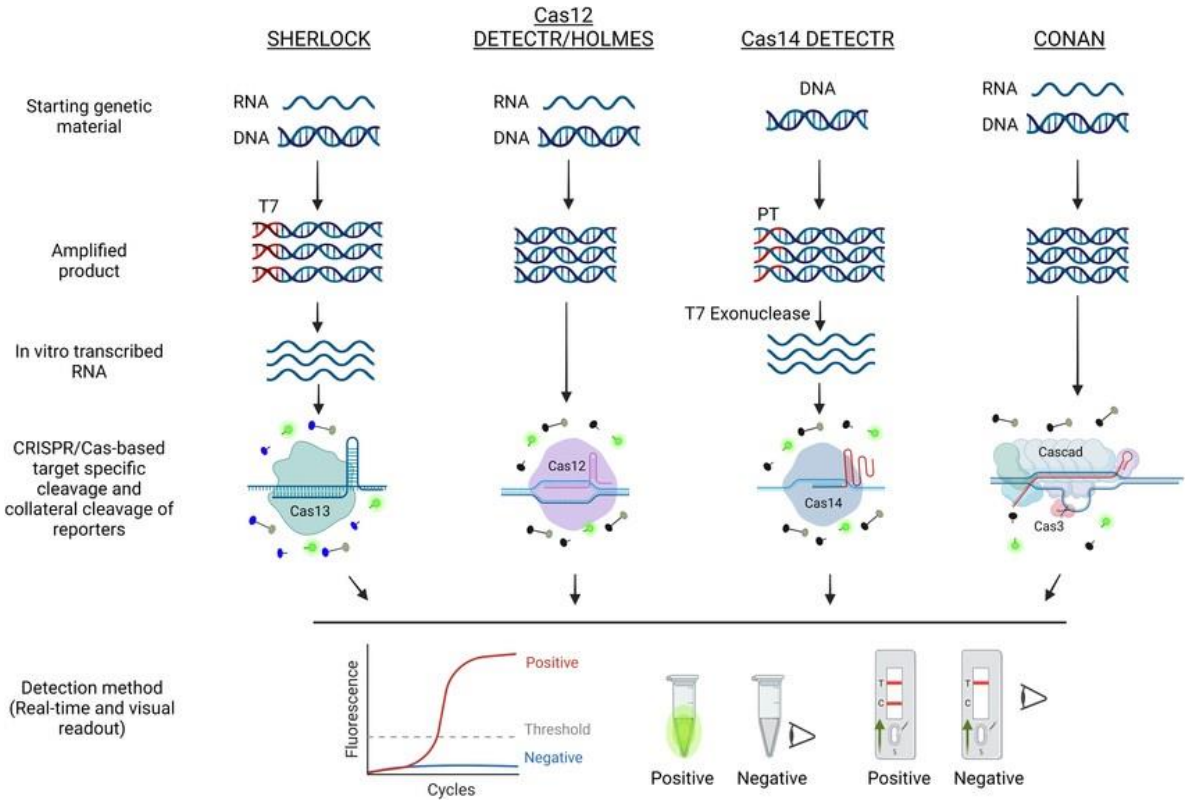


Figure 3. Conceptual comparison of traditional molecular diagnostics (e.g., PCR, ELISA) with CRISPR-based isothermal amplification platforms (e.g., SHERLOCK, DETECTR).
The figure illustrates the workflow differences in sample preparation, amplification steps, and detection methods, emphasizing the field-deployable nature and rapid visual outputs of CRISPR-based biosensors.
Adapted from Bhat, A., Aman, R., & Mahfouz, M. (2022). Onsite detection of plant viruses using isothermal amplification assays. Plant Biotechnology Journal, 20, Article e13871. https://doi.org/10.1111/pbi.13871

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Applications of CRISPR-Based Biosensors in Plant Disease Diagnostics CRISPR–Cas systems have transitioned from proof-of-concept studies to validated field applications for diverse plant pathogens. By merging nucleic acid recognition with signal amplification, CRISPR biosensors outperform conventional PCR and ELISA methods in terms of speed, sensitivity, portability, and cost-effectiveness. This section consolidates applications across viral, bacterial, and fungal pathogens, providing both an overview and detailed case studies.

Overview of Pathogen-Specific Applications
Validated CRISPR biosensors have been developed for several economically significant plant pathogens:

Viral Detection: ToLCNDV, TMV, and TYLCV have been detected using Cas12aLAMP and Cas13a-RPA systems (Mahas et al., 2021; Shanmugaraj et al., 2024).

Bacterial Pathogens: Xanthomonas oryzae and Ralstonia solanacearum in rice and tomato have been successfully identified using CRISPR assays with lateral flow strip visualization (Anbazhagan et al., 2024).

Fungal Pathogens: Initial demonstrations report Cas12a-based assays for Fusarium oxysporum and Alternaria solani from infected plant tissues.

Table 2. Comparison of CRISPR-Based vs. Traditional Diagnostic Methods for Plant Pathogens

Feature	Traditional Methods (PCR, ELISA)	CRISPR-Based Detection	Reference
Sensitivity	Moderate (10^3–10_5 copies)	High (femto- to attomolar levels)	Tian et al., 2021
Specificity	High, but strain-limited	Very high, singlebase resolution	Alon et al., 2021
Time to Result	2–6 hours	15–45 minutes	Dai et al., 2024
Equipment Requirement	Thermocyclers, lab setup	Minimal or portable devices	Mahas et al., 2022
Cost per Test	$10–$50	<$5 (paper-strip CRISPR sensors)	Mahas et al., 2022
Field Deployability	Low	High	Farrall et al., 2023

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Multiplexing Capability	Limited	Advanced (Cas12/13-based assays)	Hak et al., 2024
Cross-contamination Risk	Moderate	Low (one-pot, closed reactions)	Dai et al., 2024

Viral Pathogen Detection

CRISPR diagnostics are particularly effective against plant viruses due to their high sequence specificity:

ToBRFV in Tomato: A Cas13a-SHERLOCK assay enabled rapid detection in under 30 minutes, with >90% concordance to qPCR (Zhang et al., 2023).

Multiplexed Detection: Nanomaterial-enhanced Cas13a biosensors simultaneously detected TYLCV, CMV, and TSWV with distinguishable colorimetric outputs (Fu & Xianyu, 2023).

Field-Deployable Viral Platforms: Smartphone-integrated SHERLOCK and DETECTR systems achieved sub-femtomolar sensitivity for both single- and doublestranded viral genomes (Myhrvold et al., 2018).

Bacterial Pathogen Detection

CRISPR-based diagnostics have also been validated for bacterial phytopathogens of major agricultural concern:

Xylella fastidiosa: A Cas12a-LAMP assay achieved femtomolar sensitivity across infected citrus and olive tissues, with zero cross-reactivity during field trials (Farrall et al., 2023).

Ralstonia solanacearum (Bacterial Wilt): A one-pot RPA-Cas12a lateral flow system enabled rapid detection directly from soil suspensions and potato tubers, proving suitable for quarantine applications (Yang et al., 2023).

Fungal Pathogen Detection

Fungal diagnostics represent an emerging frontier for CRISPR biosensors:

Setophoma terrestris (Onion Pink Root Rot): An RPA-Cas12a platform reached 100× greater sensitivity than qPCR (0.01 pg/μL) with 98% field accuracy (Zhao et al., 2024).

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Leptosphaeria maculans (Phoma Stem Canker): A portable ―suitcase lab‖ achieved 100% diagnostic accuracy with a detection limit of 4.7 genomic copies (Lei et al., 2022).

Diaporthe spp. in Soybean: A Cas12a assay targeting the *tef1* gene distinguished closely related species with an 86% recovery rate in spiked seed powder (Sun et al., 2023).

Colletotrichum coccodes: A non-amplified Cas12a fluorescence platform detected pathogen DNA at 500 pM, representing a milestone in amplification-free diagnostics (Heathcote & Weckman, 2023).

Field Integration and Comparative Case Studies

The translation of CRISPR assays into portable diagnostic formats has accelerated in recent years:

Table 3. Field-Deployable CRISPR Biosensors for Plant Pathogen Detection

Pathogen Detected	Detection Platform	Limit of Detection	Time to Result	Field Tested?	Reference
Xylella fastidiosa	LAMP + Cas12a (DETECTR)	Femto-molar	10 min	Yes	Farrall et al., 2023
ToBRFV	Cas12a + gRNA	15–30 ng RT-PCR	15 min	Yes	Alon et al., 2021
Fusarium oxysporum (FOL)	One-pot LAMP-CRISPR/Cas12b	88.9 copies/reaction	45 min	Yes	Dai et al., 2024
Cucumber green mottle mosaic	Cas13a (RNA-targeting)	100× diluted sap	15 min	Yes	Hak et al., 2024
Microcystis aeruginosa (cyanobacteria)	EC-CRISPR on microgap electrode	0.089 pg/µl	<30 min	Yes	Kim et al., 2023

From viral to bacterial and fungal pathogens, CRISPR-Cas biosensors have matured into validated field diagnostics. Their accuracy, speed, and low cost position them as disruptive technologies in plant pathology. Continued innovation in reagent stabilization, multiplexed detection, and AI-assisted crRNA design will further cement their role in **precision agriculture and real-time plant health monitoring**.

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Challenges, Limitations, and Future Prospects of CRISPR-Based Biosensors in Plant Disease Diagnostics

Despite the significant progress made in developing CRISPR-based biosensors for plant pathogen detection, several key challenges limit their widespread adoption in real-world agricultural systems. These constraints span technical, infrastructural, biological, and regulatory domains, necessitating targeted innovations and coordinated policy development.

One of the foremost technical challenges lies in sample matrix complexity. Crude plant extracts often contain secondary metabolites, polysaccharides, and inhibitors that interfere with Cas enzyme activity and nucleic acid amplification. This biochemical noise can reduce assay sensitivity and reliability, especially when testing is performed outside of controlled laboratory conditions. While pre-treatment buffers and optimized extraction protocols are available, they increase the complexity and cost of field diagnostics, thereby limiting deployment in low-resource settings (Farrall et al., 2023). Additionally, most current CRISPR-based detection systems depend on preamplification steps such as recombinase polymerase amplification (RPA) or loopmediated isothermal amplification (LAMP). While these methods enhance sensitivity, they also introduce challenges related to time, contamination risk, and cost. Attempts to eliminate amplification steps have shown promise using nanomaterial enhancement or direct Cas12/Cas13 detection, but these non-amplified platforms still struggle to reach the limits of detection required for early-stage infections in plant tissue or soil (Heathcote & Weckman, 2023).

Table 4: Cost vs. Sensitivity Matrix of CRISPR vs Traditional Methods

Detection Method	Approximate Cost/Test	Limit of Detection	Time to Result	Reference
Conventional PCR	$10–20	~10^3 copies	2–4 hours	Kasfy et al., 2024
qPCR	$20–30	~10–100 copies	2–3 hours	Hak et al., 2024
LAMP	$5–10	~100 copies	30–60 minutes	Dai et al., 2024
CRISPR–Cas12/Cas13	$3–10	1–100 copies (single base)	15–45 minutes	Mahas et al., 2022; Farrall et al., 2023

Field deployment is further limited by infrastructural constraints. Although lateral flow assays (LFAs) and smartphone-integrated fluorescence readers have significantly improved the portability of CRISPR tools, many farms—especially in developing countries—lack access to even basic lab infrastructure such as centrifuges or pipettes. The absence of fully integrated, ―sample-in, answer-out‖ platforms remains a major hurdle. Moreover, availability of reagents, consistent supply chains, and technical

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training of field personnel remain highly variable across regions, reducing the operational readiness of these biosensors in real-time agricultural surveillance (Yang et al., 2023); (Sun et al., 2023).

The biological specificity of CRISPR systems is often cited as a strength; however, in complex field samples where multiple pathogens may co-exist, achieving true specificity becomes challenging. Suboptimal crRNA design can result in crossreactivity and false positives, particularly in multiplexed detection systems where multiple targets are tested simultaneously. Multiplex assays using orthogonal Cas proteins and AI-optimized guide RNA libraries are emerging as a solution but require further validation under realistic agricultural conditions (Fu & Xianyu, 2023). Quality control and batch-to-batch consistency of reagents remain another issue. The performance of lyophilized Cas proteins, freeze-dried amplification mixes, and lateral flow components can vary significantly across suppliers and storage conditions. Humidity and temperature fluctuations during transportation or field use can degrade enzyme activity, particularly when cold chain logistics are unavailable (Mahas et al., 2021).

From a regulatory perspective, CRISPR-based diagnostics occupy a gray area in most national and international plant protection frameworks. Unlike clinical diagnostics, which often have structured approval pipelines, the regulatory pathways for plant diagnostic tools remain fragmented. In many countries, diagnostic assays must be validated independently for quarantine, certification, or advisory use—a process that is time-consuming and resource-intensive. As a result, even highly promising CRISPR-based kits are not yet fully integrated into national surveillance or quarantine systems (Farrall et al., 2023); (Shanmugaraj et al., 2024).

Nevertheless, several technological trends offer hope for overcoming these limitations. Microfluidic-based platforms are enabling the integration of sample processing, amplification, and detection into compact, enclosed cartridges. Lyophilized reagents with ambient temperature stability are under active commercial development, eliminating the need for refrigeration. AI-enhanced bioinformatics tools now assist in crRNA design, improving both sensitivity and specificity across pathogen strains. Importantly, some groups are piloting systems that integrate CRISPR detection results directly with cloud-based decision support platforms, enabling real-time geospatial mapping of plant disease threats and automated risk advisories (Lei et al., 2022); (Yuan et al., 2022).

In summary, while CRISPR-based biosensors have demonstrated exceptional potential for transforming plant disease diagnostics, their real-world utility depends on addressing key challenges in sample handling, reagent stability, regulatory approval, and multiplexed specificity. If current technological trajectories continue, these biosensors are likely to evolve into core components of smart agricultural surveillance networks and biosecurity frameworks.

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Key Takeaways

- **CRISPR–Cas biosensors are transforming plant disease diagnostics**, offering rapid, sensitive, and field-deployable alternatives to traditional PCR, ELISA, and LAMP assays.
- **Cas12a and Cas13a platforms dominate current research**, with successful applications across viral, bacterial, and fungal pathogens in major crops.
- **Integration with isothermal amplification and portable readouts** (lateral flow strips, fluorescence, electrochemical sensors) enables affordable (<$5/test) and on-site detection.
- **Major challenges remain**: sample matrix interference, reagent stability, and limited commercialization/regulatory pathways.

Emerging solutions—microfluidics, lyophilized reagents, multiplexing, and AIdriven crRNA design—point toward scalable, real-time, precision agriculture tools. **Future outlook**: CRISPR diagnostics are positioned to become core components of **smart agricultural surveillance networks**, enhancing food security under climate change pressures.

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