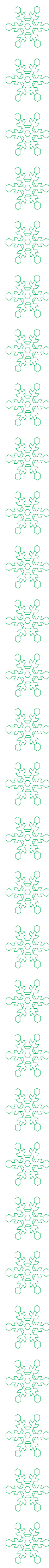

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CRISPR-Cas Mediated Diagnostics and Genome Editing Approaches for Sustainable Management of Tomato Pathogens

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Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops worldwide; however, its productivity is significantly constrained by diverse fungal, bacterial, and viral pathogens. Emerging pathogens such as Tomato brown rugose fruit virus (ToBRFV) have intensified the need for rapid, accurate, and field-deployable diagnostic systems. Conventional pathogen detection methods including ELISA, PCR, and RT-qPCR are highly sensitive but require sophisticated laboratory infrastructure and skilled personnel. In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated systems have emerged as transformative tools for plant disease diagnostics and crop improvement. CRISPR-Cas12 and Cas13 systems possess collateral cleavage activity that enables highly sensitive nucleic acid detection, thereby facilitating the development of rapid biosensors and portable point-of-care diagnostic platforms. Furthermore, integration of artificial intelligence (AI) with CRISPR technologies has improved guide RNA design, assay specificity, and off-target prediction. In addition to diagnostics, CRISPR-mediated genome editing has enabled targeted modification of susceptibility genes and development of disease-resistant tomato cultivars. This review summarizes recent advances in CRISPR-Cas diagnostics, AI-assisted guide RNA design, biosensor integration, and genome editing approaches for sustainable management of tomato pathogens. Current challenges, biosafety concerns, and future prospects of CRISPR-enabled technologies in precision agriculture are also discussed.

Keywords: CRISPR-Cas systems; Tomato pathogens; Cas12; Cas13; ToBRFV; Genome editing; Biosensors; Artificial intelligence; Guide RNA; Sustainable agriculture

Introduction

Tomato (*Solanum lycopersicum* L.) is among the most widely cultivated vegetable crops globally because of its nutritional value, culinary importance, and industrial applications. Tomato fruits are rich in vitamins, antioxidants, minerals, and lycopene, contributing significantly to human health and food security. Despite its economic importance, tomato production is severely affected by numerous fungal, bacterial, and viral pathogens that cause substantial yield losses and deterioration in fruit quality.

Major fungal pathogens affecting tomato include *Alternaria solani*, the causal agent of early blight; *Fusarium oxysporum* f. sp. *lycopersici*, responsible for Fusarium wilt; and *Botrytis cinerea*, which causes gray mold disease. Important bacterial pathogens include *Ralstonia solanacearum* and *Xanthomonas* spp., whereas viral pathogens such as Tomato yellow leaf curl virus (TYLCV) and Tomato brown rugose fruit virus (ToBRFV) have emerged as major global threats to tomato cultivation (Gavrish et al. 2025).

Traditional diagnostic techniques such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and quantitative PCR (qPCR) are widely used for

pathogen identification. Although these methods provide high sensitivity and specificity, they are labor-intensive, time-consuming, and dependent on sophisticated laboratory infrastructure, thereby limiting their utility in field conditions (Paul and Sahoo 2025). Consequently, there is an urgent need for rapid, accurate, portable, and cost-effective diagnostic tools for early pathogen detection and effective disease management.

The emergence of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-associated technologies has revolutionized molecular biology and agricultural biotechnology. Initially identified as adaptive immune systems in bacteria and archaea, CRISPR-Cas systems are now extensively utilized for genome editing and molecular diagnostics (Karimi et al. 2025). Among various CRISPR-associated proteins, Cas12 and Cas13 nucleases have attracted considerable attention due to their collateral cleavage activities, which enable highly sensitive nucleic acid detection.

In addition to diagnostics, CRISPR-mediated genome editing has opened new avenues for developing disease-resistant tomato cultivars through targeted modification of susceptibility and resistance genes. Recent integration of artificial intelligence (AI) with CRISPR technologies has further enhanced guide RNA optimization, assay specificity, and off-target prediction, thereby improving the efficiency of both diagnostics and genome editing applications (Thakur et al. 2025). Therefore, CRISPR-Cas systems represent promising next-generation tools for sustainable management of tomato pathogens and precision agriculture.

CRISPR-Cas Systems

CRISPR-Cas systems are broadly classified into two major classes based on the structure of their effector complexes. Class 1 systems utilize multi-protein effector complexes, whereas Class 2 systems employ single effector proteins such as Cas9, Cas12, and Cas13 (Thakur et al. 2025). Due to their simplicity and high efficiency, Class 2 systems are predominantly used in genome editing and molecular diagnostics.

Cas9 nucleases target double-stranded DNA and are extensively utilized for genome editing applications. In contrast, Cas12 proteins recognize DNA targets and subsequently exhibit nonspecific collateral cleavage of nearby single-stranded DNA reporters. Similarly, Cas13 nucleases specifically target RNA molecules and demonstrate collateral cleavage of surrounding RNA reporters upon target recognition (Karimi et al. 2025). These unique biochemical properties make Cas12 and Cas13 highly suitable for pathogen detection platforms.

The CRISPR mechanism generally involves three major stages: spacer acquisition, crRNA biogenesis, and target interference. Guide RNAs direct Cas proteins toward complementary nucleic acid sequences, resulting in highly specific cleavage of target molecules (Paul and Sahoo 2025). Recent advances in engineered Cas variants and guide RNA optimization have significantly enhanced editing precision and diagnostic sensitivity in agricultural systems.

CRISPR-Based Diagnostics for Tomato Pathogens

CRISPR-based diagnostic systems have emerged as highly promising alternatives to conventional molecular assays because of their rapidity, sensitivity, portability, and field applicability. Among various CRISPR platforms, Cas12- and Cas13-based systems are extensively used for detecting plant pathogens.

Cas12-Based Detection Platforms

CRISPR-Cas12 diagnostics rely on collateral cleavage activity triggered after recognition of target DNA sequences. Activated Cas12 indiscriminately cleaves nearby single-stranded DNA reporter molecules, generating detectable fluorescent or colorimetric signals (Detection of ToBRFV through CRISPR-Cas12a and CRISPR-Cas9 systems 2025).

The DETECTR platform combines recombinase polymerase amplification (RPA) with Cas12-mediated cleavage to achieve ultrasensitive pathogen detection. Recent studies have successfully utilized CRISPR-Cas12a assays for rapid detection of Tomato brown

rugose fruit virus (ToBRFV) in infected tomato plants through fluorescence-based and lateral flow systems (Karimi et al. 2025).
Advantages of Cas12-based diagnostics include:

- High sensitivity and specificity
- Minimal equipment requirement
- Rapid detection capability
- Compatibility with portable biosensors
- Field-level applicability

However, reagent stability, contamination risk, and assay standardization remain major challenges limiting widespread commercialization (CRISPR revolution 2025).

Cas13-Based Detection Platforms

CRISPR-Cas13 systems are particularly effective for detecting RNA viruses infecting tomato crops. The SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking) platform integrates isothermal amplification with Cas13-mediated collateral cleavage for ultrasensitive RNA detection (Karimi et al. 2025).

Recent studies integrating artificial intelligence with CRISPR-Cas13a systems demonstrated highly accurate detection of ToBRFV using optimized guide RNAs targeting conserved viral genomic regions. AI-assisted computational pipelines improved crRNA specificity and minimized off-target interactions, thereby enhancing assay reliability and sensitivity (Karimi et al. 2025).

Cas13-based systems provide several advantages:

- Direct RNA targeting
- Attomolar sensitivity
- Rapid detection
- Compatibility with smartphone-assisted biosensors
- Point-of-care applicability

Integration of Cas13 diagnostics with microfluidic devices and portable fluorescence readers further expands their utility in field-based pathogen surveillance.

AI-Assisted Guide RNA Design

Guide RNA design is one of the most critical determinants of CRISPR specificity and efficiency. Improperly designed guide RNAs may lead to off-target cleavage and reduced assay accuracy. Consequently, artificial intelligence (AI) and machine learning approaches are increasingly being employed to optimize guide RNA sequences for both diagnostics and genome editing applications (Thakur et al. 2025).

AI-assisted platforms analyze nucleotide composition, mismatch tolerance, thermodynamic stability, and target accessibility to predict highly efficient guide RNAs. Deep learning algorithms further facilitate prediction of off-target binding sites and improve editing precision (Karimi et al. 2025).

Recent investigations combining AI pipelines with CRISPR-Cas13a diagnostics demonstrated enhanced detection efficiency for ToBRFV in tomato samples, highlighting the enormous potential of integrating machine learning with CRISPR technologies for sustainable disease management.

Biosensors and Point-of-Care CRISPR Diagnostics

Integration of CRISPR systems with biosensors has accelerated the development of portable pathogen detection devices for agricultural applications. CRISPR-based biosensors utilize fluorescence, electrochemical signals, or lateral flow readouts for rapid and real-time pathogen monitoring (CRISPR revolution 2025).

Several biosensor platforms have been developed, including:

- Fluorescent biosensors
- Electrochemical biosensors
- Smartphone-assisted detection systems
- Paper-based lateral flow devices

- Microfluidic CRISPR chips

Smartphone-integrated CRISPR detection systems enable rapid pathogen identification directly in field conditions without sophisticated laboratory infrastructure. Such technologies hold significant promise for precision agriculture and early disease surveillance.

CRISPR-Mediated Genome Editing for Disease Resistance

CRISPR-Cas9-mediated genome editing has emerged as a promising strategy for developing disease-resistant tomato cultivars. Unlike conventional breeding approaches, CRISPR enables precise modification of susceptibility and resistance genes associated with pathogen infection (Wang et al. 2025).

Several susceptibility genes have been successfully edited in tomato, including:

- *SlMlo1*
- *DMR6*
- *eIF4E*
- *SlPelo*

Editing these genes has resulted in enhanced resistance against fungal, bacterial, and viral pathogens. Multiplex genome editing further enables simultaneous targeting of multiple genes to achieve durable and broad-spectrum resistance (Wang et al. 2025).

Advanced approaches such as base editing and prime editing provide greater precision by enabling nucleotide substitutions without generating double-stranded DNA breaks. Furthermore, nanoparticle-mediated delivery systems and ribonucleoprotein complexes are being explored to produce transgene-free edited plants with improved biosafety profiles (Thakur et al. 2025). Table 1 depicts Comparative Analysis of Conventional and CRISPR-Based Diagnostic Platforms for Tomato Pathogen Detection.

Table 1. Comparative Analysis of Conventional and CRISPR-Based Diagnostic Platforms for Tomato Pathogen Detection

Feature	Conventional PCR/RT-PCR	CRISPR-Cas12 Diagnostics	CRISPR-Cas13 Diagnostics	Citations
Target molecule	Detects DNA and RNA targets; RNA requires reverse transcription	Targets DNA molecules	Targets RNA molecules directly	Paul and Sahoo (2025); Karimi et al. (2025)
Detection mechanism	Thermal cycling-based nucleic acid amplification	Collateral cleavage of ssDNA reporters after target recognition	Collateral cleavage of ssRNA reporters after RNA target binding	Detection of ToBRFV through CRISPR-Cas12a and CRISPR-Cas9 systems (2025); Karimi et al. (2025)
Detection time	Approximately 3–6 h	Less than 1 h	Less than 1 h	Paul and Sahoo (2025); CRISPR revolution (2025)
Sensitivity	High	Very high	Very high	Karimi et al. (2025)
Specificity	High but prone to primer mismatch	Very high because of programmable gRNAs	Excellent specificity for RNA targets	Thakur et al. (2025); Karimi et al. (2025)

Equipment requirement	Requires sophisticated laboratory equipment	Minimal instrumentation required	Compatible with portable biosensors	Paul and Sahoo (2025); CRISPR revolution (2025)
Field applicability	Limited	Excellent	Excellent	Karimi et al. (2025); CRISPR revolution (2025)
Point-of-care suitability	Poor	Good	Excellent	Karimi et al. (2025)
RNA virus detection	Requires RT step before amplification	Detects indirectly after cDNA synthesis	Direct RNA detection	Paul and Sahoo (2025); Karimi et al. (2025)
Multiplex capability	Moderate	High	High	Thakur et al. (2025)
Cost effectiveness	Moderate to high operational cost	Relatively cost-effective	Cost-effective for rapid surveillance	CRISPR revolution (2025)
Need for skilled personnel	Requires trained laboratory personnel	Minimal technical expertise needed	User-friendly for field applications	Paul and Sahoo (2025)
Portability	Poor portability	Portable	Highly portable	CRISPR revolution (2025)
Biosensor integration	Limited integration	Extensive integration with lateral-flow and fluorescence biosensors	Extensive integration with smartphone and microfluidic biosensors	CRISPR revolution (2025); Karimi et al. (2025)
Detection of emerging pathogens	Requires redesign of primers	Rapid adaptation possible using programmable guide RNAs	Highly adaptable for evolving RNA viruses	Thakur et al. (2025); Karimi et al. (2025)
Application in tomato pathogens	Detection of TYLCV, ToBRFV, bacterial pathogens	Applied for ToBRFV and bacterial pathogen detection	Effective against ToBRFV and RNA viruses	Detection of ToBRFV through CRISPR-Cas12a and CRISPR-Cas9 systems (2025); Karimi et al. (2025)
Major limitations	Time-consuming and laboratory-dependent	Reagent stability and standardization issues	Off-target collateral activity concerns	CRISPR revolution (2025); Karimi et al. (2025)

Challenges and Biosafety Concerns

Despite remarkable progress, several challenges limit large-scale implementation of CRISPR technologies in agriculture. Off-target mutations remain a major concern in genome editing applications. Additional limitations include:

- High reagent costs
- Limited field stability
- Regulatory uncertainty
- Low delivery efficiency
- Public acceptance issues

Furthermore, biosafety concerns related to ecological impacts and unintended genetic modifications require comprehensive evaluation before commercialization of CRISPR-edited crops (Thakur et al. 2025).

Future Perspectives

Future research should focus on integrating CRISPR technologies with artificial intelligence, nanotechnology, biosensors, and IoT-enabled precision agriculture systems. Multiplex CRISPR platforms capable of simultaneously detecting multiple pathogens will significantly improve disease surveillance and crop protection strategies.

AI-driven guide RNA optimization and predictive disease modeling may accelerate development of next-generation diagnostic systems. Advances in prime editing, epigenome editing, and transgene-free genome editing are also expected to facilitate sustainable crop improvement with minimal biosafety concerns.

The convergence of CRISPR technology, machine learning, and precision agriculture has the potential to revolutionize sustainable management of tomato pathogens and strengthen global food security.

Conclusion

CRISPR-Cas systems have emerged as powerful tools for rapid pathogen diagnostics and precise genome editing in tomato crops. Cas12- and Cas13-based diagnostic platforms provide highly sensitive, rapid, and field-deployable alternatives to conventional molecular assays. Simultaneously, CRISPR-mediated genome editing enables development of disease-resistant tomato cultivars through targeted modification of susceptibility genes.

Integration of artificial intelligence, biosensors, and portable diagnostic platforms has further enhanced the applicability of CRISPR technologies in sustainable agriculture. Although challenges related to biosafety, regulation, and off-target effects remain, continuous technological advancements are expected to accelerate commercialization and large-scale adoption of CRISPR-enabled plant disease management systems.

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