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# CRISPR-Cas-based Detection of Plant Pathogens

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## Abstract

Climate change is causing unforeseen alterations in pathogenic agents, contributing to the emergence of novel pathogen variants that can devastate crops. To overcome this, timely phytopathogen detection is crucial. CRISPR-Cas technology has recently emerged as a versatile and adaptable tool, well-suited for detecting a broad range of plant pathogens. This helps in developing disease management strategies and treatment plans. Among the different Cas variants, Cas12a and Cas13a are widely used for phytopathogen detection. Usually, the CRISPR-Cas system is followed after recombinase polymerase amplification or loop-mediated isothermal amplification, and the specific binding of the Cas protein to nucleic acid (Target) is detected as a measurable fluorescent or electrochemical signal. The advantages of CRISPR-Cas technology include high specificity, sensitivity, rapid results, *etc*. As CRISPR-Cas technology advances and becomes more accessible, integrating it into plant pathology holds great promise for improving food security and sustainability.

**Keywords:** CRISPR-Cas, Detection, Disease, Plant Pathogens

## Introduction

CRISPR (Clustered regularly interspaced short palindromic repeats)-Cas technology has come to fore as a potent tool in the field of plant pathology and agriculture, revolutionizing the detection and management of phytopathogens. This innovative approach fosters the precise detection of DNA or RNA sequences of plant pathogens. One of the primary advantages of CRISPR-Cas technology in phytopathogen detection is its unparalleled sensitivity and specificity. The guide RNAs (gRNAs) can be tailored to the unique genetic signatures of pathogens, ensuring accurate identification even at very low pathogen concentrations which is crucial for early disease detection and intervention, reducing the risk of crop damage and yield loss. The speed at which CRISPR-Cas systems can deliver results is another significant advantage. Many CRISPR-based pathogen detection assays provide rapid outcomes, making them ideal for on-site, point-of-care testing. This timeliness allows for immediate decision-making in the field, such as implementing targeted treatment measures or quarantine protocols to prevent disease spread. In terms of sustainability, CRISPR-Cas technology aligns with environmentally friendly practices. Its precision in detection reduces the need for broad-spectrum chemical treatments, which can have detrimental effects on ecosystems. This article explores the current advancements in CRISPR-Cas-based detection of plant pathogens, delving into its mechanisms and shedding light on the promising future prospects in this field.

## An Overview of CRISPR-Cas

CRISPR-Cas, found in the majority of bacteria and archaea, represents an adaptive immune system, shielding these organisms from potential infections by phages, viruses and other extraneous genetic entities. Francisco Mojica was the first scientist to hypothesize the function of CRISPR loci, working on *Haloferax mediterranei*. However, Ishino *et al.* in 1987 first discovered CRISPR in the DNA sequence of *Escherichia coli*. Later in the year 2007, Rodolphe Barrangou and Philippe Horvath provided experimental evidence about the CRISPR system's mechanism of action while working on *Streptococcus thermophilus*. The CRISPR-Cas system is currently categorized into two classes and six types, with further subdivisions into subtypes (Figure 1). In Class 1 systems, representation consists of a complex comprising multiple proteins, whereas in Class 2, it is embodied by a single multidomain protein (including Cas9, Cas12 or Cas13) (Koonin and Makarova, 2019). The advent of CRISPR-Cas RNA and DNA editing technologies has revolutionized

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ave revolutionized genome modification, propelling advancements in various fields including agriculture. Various CRISPR-Cas systems are widely employed to generate double-strand breaks (DSBs) at precise genome locations in various species. Notably, Class 2 CRISPR immune systems are simpler than Class 1, making them more accessible for repurposing in genome editing tool development. As a result, three prominent Class 2 effectors, *viz.*, Cas12, Cas13 and Cas9, have gained immense popularity for precise cleavage of both RNA and DNA. Among these three, Cas9 and Cas12 effectors function as DNA-targeting endonucleases, whereas Cas13 primarily targets RNA. To put it plainly, these effectors can attach to and cleave specific nucleic acids guided by RNA.

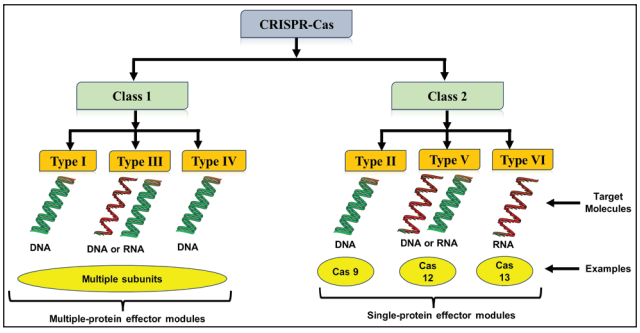


Figure 1: CRISPR-Cas System classification

## How does the CRISPR-Cas9 Work?

A specific guide RNA (gRNA) is designed to match the target DNA sequence where the desired genetic modification will occur. The gRNA is combined with the Cas9 protein to form a complex. The gRNA serves as a guide to direct Cas9 to the target DNA sequence. The Cas9-gRNA complex scans the DNA within the cell to find a sequence that matches the gRNA. Once the complementary DNA sequence is found, Cas9 cuts both strands of the DNA at the targeted location, creating a double-strand break (DSB). The innate DNA repair mechanisms of the living cell, such as non-homologous end joining (NHEJ) or homology-directed repair (HDR), attempt to repair the DSB NHEJ repair and may introduce small insertions or deletions, disrupting the target gene's function. HDR repair can be utilized to introduce specific desired genetic changes at the DSB site. The gene modifications achieved through CRISPR-Cas9 can result in gene knockout (disruption of gene function) or precise gene editing (*e.g.*, correction of disease-causing mutations or introduction of specific alterations) (Figure 2).

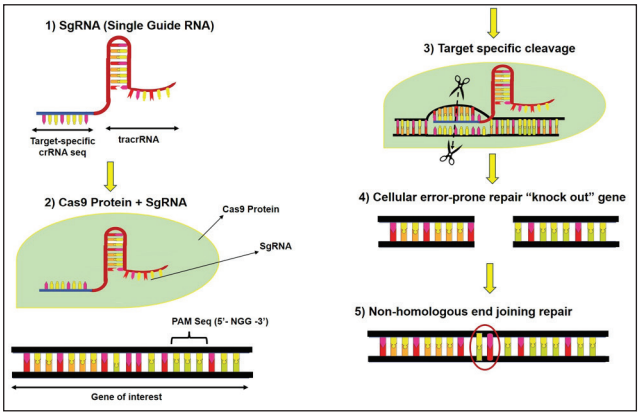


Figure 2: Mechanism of CRISPR-Cas9 system

## CRISPR-Cas in Plant Pathogen Detection

Accurate diagnosis is the first step in effectively managing crop diseases. It enables the identification of the specific pathogens or factors responsible for crop diseases, which in turn allows for the implementation of appropriate control measures and thus prevents unnecessary costs associated with disease management and reduces the environmental impact of agricultural practices involving harmful chemicals. Owing to the high specificity of Cas protein, they are considered a potential candidate in pathogen detection. Moreover, the assay can be performed in less than 2 hours. Recently, numerous high-efficiency point-of-care nucleic-based system detection systems with high reliability and sensitivity (picomolar range) have been developed. Several Cas-variants-based have been used to develop detection tools. Such techniques basically depend on the DNA extraction of pathogens. And later the pathogen-specific DNA motif Cas protein bind involving harmful chemicals which is detected *via* a certain signal such as a visual signal as in lateral flow assay, or a fluorescent or electrochemical signal (Wang *et al.*, 2020). Among different variants of Cas-variants, detection methods based on both Cas12a and Cas13a are widely exploited as they leverage the precision of Cas nucleases to identify a specific target sequence within pathogen nucleic acids. Before introducing Cas nuclease, isothermal amplification is performed using recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP). Then, specific nucleotide binding is detected through a measurable signal (Figure 3). Furthermore, Cas-based techniques are adaptable for use with lateral flow assays employing immunostrips (Figure 3). When compared to traditional nucleic acid detection methods like a digital droplet, PCR, qPCR, *etc.*, Cas-based detection permits considerably lower sample inputs, offering reliable detection with sensitivity ranging from femtomolar ($10^{-15}$) to zeptomolar ($10^{-21}$), depending on the specific techniques employed (Li *et al.*, 2019).

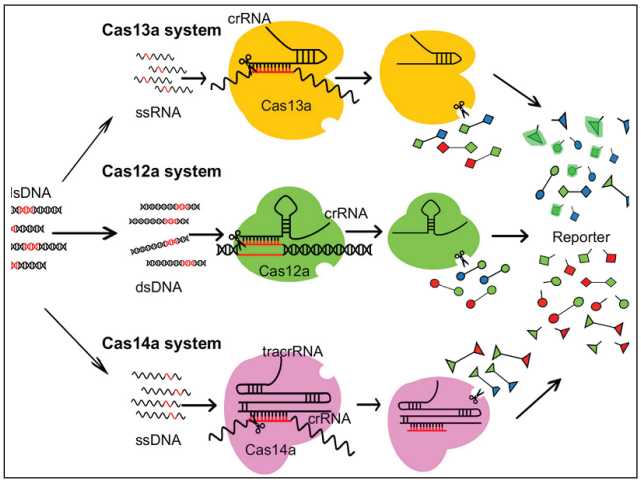


Figure 3: Schematic representation of detection of pathogen nucleic acid using CRISPR Cas system (Adapted from Wang *et al.*, 2020)

Kang *et al.* (2021) demonstrated Cas12a-based detection of the wheat blast caused by the *Magnaporthe oryzae Triticum* (MoT) pathotype. They targeted two specific

sequences with Cas12a protein *viz.*, MoT-6098 and MoT-6099 sequences after LAMP amplification and observed rapid and highly specific detection of wheat blast. Another study involved targeting two *M. oryzae* genes using Cas12a-based DNA. The target genes could be easily detected with high specificity using a fluorescent reporter after RPA for 14 crRNAs (Zhang *et al.*, 2020). The same group of researchers combined Cas-12a with filter-paper-based DNA purification and RPA for target gene detection of pathogens *via* lateral flow assay (Zhang *et al.*, 2021). A Cas-12a-based combined with RPA multiplex detection system was optimized for the detection of RNA virus and viroid from crude extract, *viz*., *Apple chlorotic leaf spot virus* (ACLSV), *Apple scar skin viroid* (ASSVd), *Apple necrotic mosaic virus* (ApNMV), *Apple stem pitting virus* (ASPV) and *Apple stem grooving virus* (ASGV) (Jiao *et al.*, 2021). The methods were proved to be simpler and rapid, taking less than hours. Another study conducted by Aman *et al.* (2020) demonstrated an engineered CRISPR-Cas assay that involved preamplification using RT-RPA for the detection of economically significant RNA viruses, namely Potato virus X and Y, along with *Tobacco mosaic virus* in less than 20 min.

However, the major setbacks of CRISPR-Cas-based detection are the cumbersome sample preparation steps, shortfall in multiplexing and single nucleotide specificity which means a mutation in a single nucleotide can result in false negative results (Zhang *et al.*, 2020).

## Conclusion

In conclusion, the application of CRISPR-Cas technology in the detection of plant pathogens represents a significant advancement in plant pathology. This innovative approach offers rapid and highly specific detection of pathogenic agents, enabling early intervention and precise disease management. Cas-based techniques, particularly Cas12a and Cas13a, offer highly sensitive, rapid and precise identification of plant pathogens, crucial for timely intervention and minimizing the environmental and economic impacts of crop diseases. The ability to tailor CRISPR-Cas systems for various plant pathogens enhances the resilience of crops and promotes sustainable agricultural practices. As we continue to explore and refine this method, it holds immense promise for safeguarding global food security and addressing the challenges posed by plant diseases.

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