CRISPR/Cas Toolkit: Unlocking Plant Defense Against Pathogens

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With the global population projected to exceed 9.8 billion by 2050, the demand for food will rise significantly to meet nutritional needs (Gosavi et al 2020). However, the widespread prevalence of plant pests and pathogens poses a serious threat to global food security, leading to the loss of 20% to 40% of crop yields annually worldwide. Climate change is a major environmental factor driving the evolution and spread of plant pathogens, resulting in the emergence of new diseases and the expansion of existing ones into new regions. As a result, crop diseases are becoming more frequent and severe, posing greater risks to agricultural systems (Chaloner et al 2021). Over the past decade, CRISPR/Cas-based genome editing has reshaped the landscape of plant pathology by enabling precise genetic interventions, advancing molecular diagnostics, and expediting the development of crops with enhanced disease resistance. CRISPR technology has opened up new avenues for identifying key defence-related genes in plants and enhancing crop resistance to pathogens, thereby contributing to future food security and sustainable agriculture (Wheately and Yang 2021).

CRISPR/CAS: a groundbreaking molecular toolkit for precise genome editing

The CRISPR/Cas system is an adaptive immune defence in bacteria that protects against viruses and other invading genetic material by precisely targeting and cutting their nucleic acids (Barrangou et al 2007; Brouns et al 2008). The RNA-guided nucleases of the CRISPR/Cas system exhibit sequence-specific binding and cleavage of nucleic acids upon viral invasion, thereby mediating antiviral interference and conferring adaptive immunity to the bacterial host. Through a process known as adaptation, bacteria acquire fragments of foreign genetic material and integrate them into their own genome at the CRISPR array locus, thereby establishing a molecular memory of prior invasions. Transcription and processing of the CRSIPR array results in the generation of CRISPR RNAs (crRNAs) that help guide Cas nucleases for the targeted and specific cleavage of nucleic acid sequences through complementary base-pairing (Brouns et al 2008: Marraffini and Sontheimer 2008).

Picture of CAS systems

Genome editing and CRISPR/CAS tool kit

A diverse array of CRISPR/Cas-based genome editing technologies has emerged, capitalizing on the versatile programmability and high target specificity of distinct Cas nucleases. These systems provide robust platforms for precise and efficient genetic manipulation across a broad spectrum of organisms (Wheatley and Yang 2021). The most conventional and widely employed application of genome editing is the creation of loss-of-function mutants. This approach utilizes sequence-specific nucleases, such as Cas9, to induce double-strand breaks (DSBs) at defined genomic loci. The resulting DNA lesions are predominantly repaired through the endogenous non-homologous end joining (NHEJ) pathway, an error-prone repair mechanism that ligates the broken DNA ends, often resulting in insertions or deletions (indels) that disrupt gene function. (Wheatley and Yang 2021). NHEJ is one of the most prevalent pathways for DNA repair.

In contrast, homology-directed repair (HDR) is a high-fidelity DNA repair mechanism that utilizes an exogenously supplied donor template containing homologous sequences flanking the double-strand break (DSB). This pathway facilitates accurate and template-guided replacement or correction of the damaged genomic region, enabling precise and predictable modifications at the target locus. HDR is particularly valuable for introducing specific nucleotide substitutions, gene insertions, or precise sequence corrections in genome engineering applications. (Pawelczak et al. 2018). HDR is one of the predominant forms of DNA repair in bacteria and many fungi.

CRISPR/CAS utilization in fundamental studies of plant-pathogen interactions

CRISPR/Cas toolkits are used mostly for targeted mutagenesis in both plants (hosts) and

pathogens. This enables researchers to investigate the functions of specific genes involved in plant immune responses and pathogen virulence mechanisms. The CRISPR/Cas allows genome editing and targeted mutagenesis in model plants (such as *Arabidopsis)* as well as agriculturally important cereals (like rice, wheat, etc.) and speciality crops. There are reports on CRISPR/Cas-mediated genome editing and targeted mutagenesis in fungi and oomycetes such as *Fusarium fujikuroi*, *Magnaporthe oryzae,* Phytophthora *capsici* etc., to understand how these pathogens cause disease. Disruption of pathogen effector genes such as RXLR effectors) is performed to study how their interactaction with plant resistance (R) genes. The utility of CRISPR/Cas 9 toolkits have been demonstrated for loss-of-function analysis and molecular characterization of the plant-microbe interactions during host-pathogen recognition. For example, CRISPR/Cas9 was used to knock out the *NRG1* gene in plants, revealing its role in a key immune signalling pathway. In rice, the *Ptr* gene role in broad-spectrum resistance to *Magnaporthe oryzae* was confirmed using CRISPR loss-of-function mutants. So in nutshell, CRISPR/Cas is fundamentally used to "turn off" genes in plants and pathogens to uncover the molecular details of how plants resist infection and how pathogens try to overcome this resistance.

CRISPR/CAS technology for genome editing to enhance plant disease resistance

Genome Engineering is referred as genetic modification of plants to enhance their resistant to diseases caused by bacteria, fungi, viruses, or insects, using CRISPR/Cas as a precise gene-editing tool. One of the most widely used approaches in engineering plant disease resistance with CRISPR/Cas involves knocking out susceptibility (S) genes or negative regulator of plant immunity For example knocking out the *MLO* gene in wheat and tomato—originally identified in barley—conferred resistance to powdery mildew. Similarly, deletion of the *EDR1* gene in wheat and the *DMR6* gene in tomato enhanced resistance to fungal and bacterial pathogens, respectively. Instead of editing the entire gene, scientists have targeted the transcription activator-like effector (TALE) binding elements within the promoter (on/off switch) to prevent pathogen-triggered gene expression. This strategy has been applied to the promoters of *CsLOB1* in citrus and *OsSWEET11*, *OsSWEET13*, and *OsSWEET14* in rice, providing resistance against multiple races of *Xanthomonas* pathogens (Oliva et al., 2019)

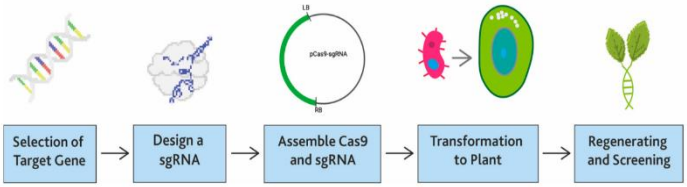

Fig. 1 Fundamental workflow of CRISPR/Cas9 technology for editing target genes.

CRISPR/CAS technology for plant detection and diagnostic applications

CRISPR/Cas systems, especially Cas12a and Cas13a, have been innovatively adapted for nucleic acid-based diagnostics in plant pathology due to their programmable specificity and collateral cleavage activities. Cas12a, guided by a CRISPR RNA (crRNA), targets double-stranded DNA sequences, while Cas13a targets single-stranded RNA. Upon binding to their respective target sequences, these nucleases become activated and exhibit non-specific, collateral cleavage of nearby single-stranded nucleic acids, which can be linked to a fluorescent or colorimetric reporters to generate a visible signal. To enhance sensitivity, these CRISPR-based detection systems are often coupled with isothermal amplification methods such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP), enabling detection of minute pathogen nucleic acid concentrations without the need for thermal cycling (Li et al., 2018). This approach has been successfully applied in the detection of agriculturally important plant pathogens, including *Magnaporthe oryzae*, *Candidatus Liberibacter asiaticus* (associated with citrus greening disease), and RNA viruses such as *Turnip mosaic virus* and *Cucumber mosaic virus*. CRISPR-based diagnostics not only provide high sensitivity and specificity but also offer field-deployable formats, such as lateral flow strips or portable fluorescence readers, enabling rapid, real-time disease surveillance and management in agriculture (Wheatley & Yang, 2021).

Conclusion and future perspectives

CRISPR/Cas-based diagnostics have emerged as transformative tools in plant pathology, enabling rapid, ultra-sensitive, and sequence-specific detection of pathogens. By utilizing the collateral cleavage activity of Cas12a and Cas13a, these systems—

especially when paired with isothermal amplification—facilitate field-deployable, real-time identification of viral, bacterial, and fungal agents. Future advancements aim to develop amplification-free systems, enhance multiplexing, and integrate portable biosensors for automated, on-site diagnostics. As challenges related to reagent stability, regulatory approval, and assay standardization are addressed, CRISPR-based platforms are poised to become integral to precision agriculture and global plant health surveillance (Wheatley & Yang, 2021)

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