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CRISPR/Cas9 Mediated Resistance: A Sustainable Approach Against Plant Viruses

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Plant diseases caused by a wide range of pathogens, including fungi, bacteria, viruses and nematodes, pose significant challenges to global agriculture by reducing crop yield and quality (Strange and Scott, 2005). Plant viral diseases represent one of the major constraints to global agricultural productivity, leading to significant yield losses and economic damage. Conventional approaches for managing plant viral infections, including cultural practices, chemical treatments, and conventional breeding for resistance, have shown limited success due to the high mutation rates, wide host range and rapid adaptability of plant viruses (Zewdu *et al*., 2023). Moreover, the lack of effective antiviral chemicals and the time-consuming nature of traditional breeding highlight the urgent need for innovative and precise strategies to enhance crop resilience against viral pathogens.

The advent of genome editing technologies has revolutionized plant biotechnology, among which the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 system has emerged as a powerful and versatile tool. Derived from the adaptive immune system of prokaryotes, CRISPR/Cas9 enables targeted modifications in plant genomes with high efficiency and specificity. In the context of plant viral disease management, CRISPR/Cas9 has been successfully employed to engineer virus-resistant plants either by directly targeting viral genomes or by modifying host susceptibility factors essential for viral replication and movement (Shahriar *et al*., 2021).

Recent studies have demonstrated the application of CRISPR/Cas9 in conferring resistance against DNA viruses such as *Geminivirus*, as well as RNA viruses through host genome editing strategies (Wani *et al*. 2023). This technology not only offers durable resistance but also provides an opportunity to overcome the limitations associated with conventional methods. As a result, CRISPR/Cas9 is increasingly being recognized as a transformative approach for sustainable crop protection and global food security.

Mechanism of CRISPR/Cas9 in Plant Virus Disease Management

The CRISPR/Cas9 system, originally discovered as an adaptive immune mechanism in bacteria and archaea, functions through a RNA-guided endonuclease (Cas9) that introduces double-stranded breaks (DSBs) at specific DNA target sites. In genome editing, this system is reprogrammed using a synthetic single guide RNA (sgRNA), which directs Cas9 to complementary sequences in the host or viral genome adjacent to a protospacer adjacent motif (PAM). Once the DNA is cleaved, the host cell's repair pathways, primarily non-homologous end joining (NHEJ) or homology-directed repair (HDR) introduce mutations that can disrupt gene function or modify sequences in a precise manner.

In plant viral disease management, the CRISPR/Cas9 system operates mainly through two approaches: direct targeting of viral genomes and modification of host susceptibility genes. In the first approach, sgRNAs are designed to recognize and cleave conserved regions of viral genomes, particularly DNA viruses such as *Geminiviruses*. The cleavage prevents viral replication and accumulation, thereby conferring resistance to the host plant. In the second approach, host genes that are essential for viral replication, translation, or movement (for example, eIF4E or DNA polymerase-interacting proteins) are disrupted by Cas9-mediated editing. This strategy renders the plant resistant without significantly affecting its normal physiological functions.

Moreover, CRISPR/Cas9 offers flexibility to simultaneously target multiple sites within viral genomes or multiple host factors, reducing the risk of resistance breakdown caused by viral mutations. With its high specificity and adaptability, the CRISPR/Cas9 mechanism provides a powerful platform to engineer durable resistance against a wide range of plant viruses.

CRISPR/Cas9 in Plant Virus Disease Management

The use of CRISPR/Cas9 to destroy invading viruses was first used in 2015 to develop plant resistance to DNA viruses belonging to the *Geminiviruses* (Ali *et al*., 2015; Baltes *et al*., 2015; Ji *et al.*, 2015). Following that success, many researchers began experimenting with this method, reporting on the effectiveness of CRISPR/Cas9 technology in developing viable virus resistance in a variety of plants (Zaidi *et al*., 2016).

Table 1: Successful examples of CRISPR/Cas9 mediated plant virus disease management

Virus	Plant	Target	Type of virus	Reference
CLCuD	*N. benthamiana*	OG	DNA	Mubarik *et al.* (2021)
CLCuKoV-Bur	*N. benthamiana*	IR	DNA	Hamza *et al*. (2021)
TYLCV	*Solanum lycopersicum*	SlPelo and SlMlo1	DNA	Pramanik *et al.* (2021)
TuMV	*N. benthamiana*	CasRx	RNA	Cao *et al.* (2021)
TMV	*N. benthamiana*	CasRx	RNA	Cao *et al.* (2021)
CMV	*N. benthamiana*	CasRx	RNA	Cao *et al.* (2021)
WDV	*Hordeum vulgare*	MP and CP	DNA	Kis *et al.* (2019)
PepMoV	*S. lycopersicum*	eIFs	RNA	Yoon *et al.* (2020)
BBTV and BSV	*Musa* spp.	PDS gene	DNA	Tripathi *et al.* (2021)
SMV	*Gylcine max*	GmF3H1, GmF3H2 and GmFNSII-1	RNA	Zhang *et al*. (2020)
TYLCV	*S. lycopersicum*	CP and IR	DNA	Faal *et al*. (2020)

Advantages of CRISPR/Cas9 Over Conventional Methods

- **High Precision and Specificity:** CRISPR/Cas9 enables targeted editing of viral genomes or host susceptibility genes with nucleotide-level accuracy, unlike conventional breeding, which introduces large genomic changes through crossing.
- **Rapid Development of Resistance:** Traditional breeding for virus resistance is time-consuming (often requiring several years of backcrossing). CRISPR/Cas9 allows the direct and quick development of resistant varieties within a single generation.
- **Broad Applicability:** A single CRISPR/Cas9 system can be customized with different sgRNAs to target diverse viruses or multiple viral strains simultaneously, which is not feasible with conventional resistance genes.
- **Durable Resistance:** Multiplex targeting of conserved viral sequences or editing of host factors provides long-lasting resistance and reduces the likelihood of viral escape mutants.

- **Non-reliance on Pesticides:** Unlike vector management using insecticides, CRISPR/Cas9-mediated resistance is environmentally friendly and sustainable, reducing chemical inputs in agriculture.
- **Overcoming Natural Resistance Limitations:** Many crop species lack naturally occurring resistance genes against viruses. CRISPR/Cas9 circumvents this limitation by introducing resistance directly, even where no natural resistance source exists.
- **Integration with Modern Breeding Programs:** Edited plants can be seamlessly integrated into marker-assisted breeding or other molecular breeding approaches, accelerating the pipeline for resistant variety release.

Limitations of CRISPR/Cas9 Compared to Conventional Methods

While CRISPR/Cas9 has emerged as a revolutionary tool for developing virus-resistant plants, several limitations hinder its broad application when compared with conventional management strategies such as resistant variety breeding, cross-protection or vector control.

- **Off-target Effects:** Cas9-sgRNA complexes may cleave unintended genomic regions, leading to undesirable mutations that can affect plant growth, development, or yield. This risk is less pronounced in conventional breeding, where genetic changes are more predictable.
- **Viral Escape Mutants:** Plant viruses, especially RNA viruses, have high mutation rates. Single-site targeting with CRISPR/Cas9 may allow viruses to evolve escape variants, whereas conventional approaches like polygenic resistance often provide more durable protection.
- **Limited Direct Application to RNA Viruses:** CRISPR/Cas9 primarily targets DNA. RNA viruses, which form the majority of plant viruses, cannot be directly targeted using Cas9 and require indirect strategies (editing host susceptibility genes). Conventional methods, such as cross-protection, can be directly used against RNA viruses.
- **Delivery Challenges:** Efficient delivery of CRISPR/Cas9 components into plant cells remains complex, often requiring tissue culture, Agrobacterium-mediated transformation, or viral vectors. Conventional approaches (e.g., resistant cultivar development, cultural practices) are easier to implement in the field.
- **Regulatory and Biosafety Concerns:** Genome-edited crops face strict biosafety regulations, and public acceptance remains limited in many countries. Conventional methods such as crop rotation, resistant varieties, or chemical/vector control face fewer regulatory barriers.
- **Polygenic Nature of Resistance:** Many plant–virus interactions involve multiple host genes and pathways. CRISPR/Cas9 editing of a single gene may not provide complete or long-term resistance, unlike traditional breeding where multiple resistance genes can be introgressed simultaneously.

Conclusion

Plant viral diseases cause significant yield losses and pose serious challenges to sustainable agriculture. Traditional approaches, including cultural practices and conventional breeding, often fail to deliver durable resistance because of the rapid mutation rates and adaptability of viruses. CRISPR/Cas9 genome editing has emerged as a precise and versatile tool for developing virus-resistant plants. By directly cleaving viral genomes or disrupting host susceptibility genes, this system provides an efficient and targeted strategy for managing viral infections. Successful applications in crops like tomato, potato, and cucumber demonstrate its potential to provide long-lasting resistance. Multiplex targeting further enhances durability by minimizing viral escape. However, challenges such as off-target effects, viral evolution, and regulatory considerations must be addressed before large-scale deployment. Looking ahead, advanced CRISPR variants such as Cas12, Cas13 and base or prime editing promise even greater potential. Overall, CRISPR/Cas9 offers a transformative approach to securing global food production.

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