BioThink, Vol -2, Issue -1
ISSN: 3048-7943

CRISPR/Cas in Enhancing Crop Resistance Against Viral Pathogens

MADHAIYAN SAKTHIVEL[1], SENTHILKUMAR S[1], KARAN SATHISH[2*] AND MARIMUTHU ELANGOVAN[3]

G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India- 263145,
ICAR-Indian Agricultural Research Institute, New Delhi, India- 110012
Corresponding author E-mail: karansathish555@gmail.com

Introduction

Plants are continuously being exposed to various pathogens including bacteria, fungi, viruses and nematodes, which seriously threaten agriculture crops worldwide. One of the major causes contributing to the loss of agricultural production is virus diseases, estimated to reduce the crop yield by 10–15% each year. Target-specific genome editing has emerged as a modern approach to address these challenges by modifying genes to improve crop resistance and productivity. In response to serious damage caused by viral diseases, there is an urgent necessity of improving resistance in host plants against diverse plant viruses, and the CRISPR/Cas9 system is considered a highly promising genome-editing method to improve plant resistance against different viral pathogens. This technique works by creating double-stranded breaks in DNA at specific genomic sites, repaired through pathways like non-homologous end-joining (NHEJ), which can introduce beneficial mutations. CRISPR's ability to modulate target genes related to metabolism, immunity, and stress tolerance has the potential to revolutionize agricultural biotechnology by producing crops with improved yields and resilience.

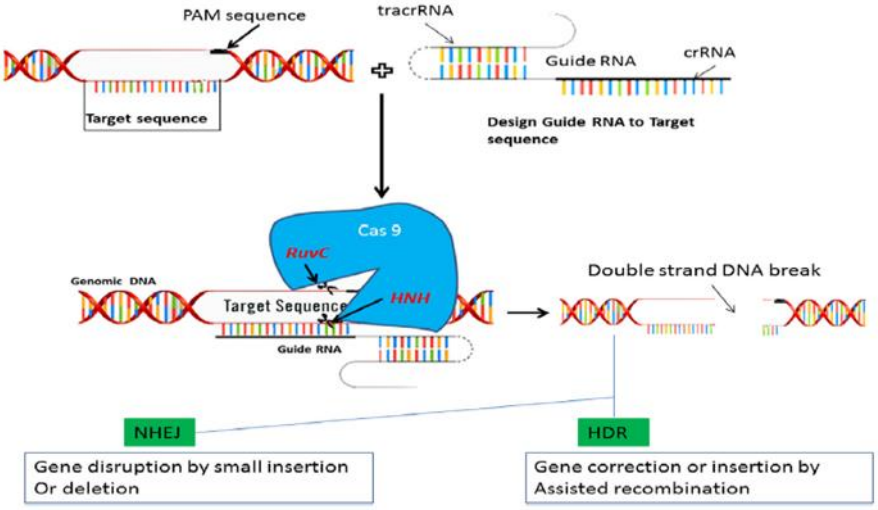
CRISPR/cas9 system: structure and its mechanism

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins form the CRISPR/Cas system, which evolved in archaea and bacteria as an adaptive immune system against invading foreign nucleic acids originating from viral or plasmid pathogens. The CRISPR/Cas9 system is composed of a single guide RNA (sgRNA) and Cas9, a double-

strand nuclease. sgRNA is a complex of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA or trRNA). This complex introduces site-specific double-stranded breaks (DSBs) in DNA, guided by a 20-base-pair sequence adjacent to a Protospacer Adjacent Motif (PAM) sequence (5′-NGG), critical for Cas9 activity. The CAS9 endonuclease contains two evolutionary preserved regions: a RuvC like nuclease domain and an HNH nuclease domain, both showing a difference in their nuclease activity. It has been experimentally shown that the HNH nuclease domain nicks the DNA strand which is complementary to the crRNA, while the RuvC-like nuclease domain nicks the non-complementary DNA strand **(Fig 1).**

Various vectors, like pRGE31 and lentiCRISPR v2, enable CRISPR/Cas9 applications in plants. These vectors are engineered with essential components (promoters, selection markers, Cas9 coding sequence) for genome editing. By introducing the guide RNA through Agrobacterium-mediated transformation, Cas9 cleaves the target DNA, activating repair pathways like error-prone non-homologous end-joining (NHEJ). This process induces insertions or deletions, leading to frameshift mutations and precise gene editing in plants.

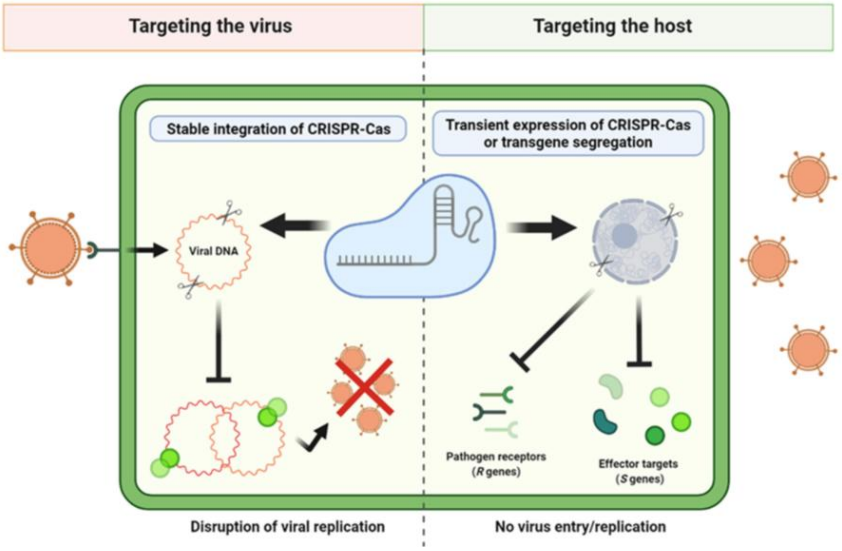
Fig. 1. CRISPR/Cas9 system with structure and mechanism



Enhancing Plant Virus-Resistance Strategies Using CRISPR/Cas9

The CRISPR/Cas9 technology allows for the development of a broader range of CRISPR variants useful for different applications and has been successfully demonstrated to engineer virus-resistant crop cultivars. However, gene disruption is one of the most common applications of the CRISPR/Cas9 tool and helps to overcome the error-prone behaviour of cellular NHEJ (DNA-repair machinery). CRISPR/Cas9 technology enables virus-resistant crop development by disrupting susceptibility (S) genes through targeted insertions/deletions (InDels), causing frameshift mutations that alter plant-virus interactions. This approach can also target promoter regions to block gene expression and effector binding, which results enhancing resistance. Deleting chromosomal fragments near S gene clusters offers durable resistance across hosts. In addition to virus resistance, CRISPR-mediated gene insertion opens an avenue to study important S genes. The functional analysis of susceptibility genes enables us to understand the regulation of gene expression. This tool can replace the improper and poorly performing R genes from cultivated crop species with a functional R gene from a virus-resistant cultivar via multiplexed homology directed repair (HDR) methodology. This approach is beneficial to introduce only specific mutations associated with virus resistance traits instead of replacing the whole gene. The CRISPR–Cas technology against virus infection is based on two different strategies: (a) Editing the viral genome, (b) modifying host factors essential in the virus life cycle (Fig 2).

Fig 2. Development of CRISPR–Cas-mediated virus resistance in plants



(a) Editing the viral genome

CRISPR–Cas editing for disease resistance have focused on targeting single-stranded DNA viral genomes, particularly the Geminiviridae family, which cause huge losses in agricultural production among relevant crop families including Cucurbitaceae, Euphorbiaceae, Solanaceae, Malvaceae, and Fabaceae. Initial efforts have focused to targeting coding and noncoding regions of Beet severe curly top virus (BSCTV) and editing the viral replication initiator protein and CP or the conserved intergenic region in Tomato yellow leaf curl virus (TYLCV), Beet curly top virus (BCTV), and Merremia mosaic virus (MeMV), achieving disease resistance in *Arabidopsis thaliana* and *Nicotiana benthamiana*. However, the disruption of the intergenic region led to durable, broad-spectrum resistance against multiple viruses without viral variant escapes.

On the other hand, protection against RNA viruses was deemed unfeasible with DNA-targeting Cas9, became possible by expressing RNA-specific nucleases like *Francisella novicida* (FnCas9) and *Leptotrichia wadei* (LwaCas13a), a notable reduction in viral accumulation of Cucumber mosaic virus (CMV) and Turnip mosaic virus (TuMV) in *A. thaliana* and *N. benthamiana*, respectively.

(b) Editing the host plant genome

CRISPR–Cas can achieve durable disease resistance by targeting Resistance (R) and/or Susceptibility (S) genes that are present within the host plant genomes. The introduction of dominant or semidominant Resistance (R) genes into elite cultivars via breeding, which recognize pathogen effectors that alter molecular processes within the plant to support pathogen growth but often have a narrow spectrum and are prone to pathogen adaptation. S genes encode either negative regulators of immunity or host proteins involved in plant endogenous pathways that, when controlled by the pathogen, lead to the suppression of defense mechanisms, inactivation of pathogen propagation, reducing symptoms or preventing infection entirely. Translation of viral RNA is a crucial step in a pathogen's life cycle before viral replication can occur, and it relies completely on the host translational machinery. Therefore, the eukaryotic translation initiation factors eIF4E and eIF(iso)4E which are hijacked by viral genome-linked proteins (VPg) from potyviruses, has showed eIF4Es-mediated resistance against potyviruses in various crops including lettuce, pepper and wild tomato.

Plant viruses move cell to cell through plasmodesmata by using movement proteins that increase the plasmodesmata size exclusion limit and form microtubules. Both cell-to-cell and long-distance movement of viruses are mediated by viral proteins and host factors. Host plasmodesmata-located proteins (PDL1, PDL2, and PDL3) assist in the systemic movement of viruses like Grapevine fanleaf virus and Cauliflower mosaic virus. Disrupting interactions between these proteins and viral movement proteins reduces microtubule formation, delays infection, and leads to milder symptoms in host plants.

Advantages of CRISPR/Cas system over the related conventional techniques

1. Precision and Efficiency

CRISPR/Cas allows highly specific targeting of genetic sequences, minimizing off-target effects. It can edit genes with greater efficiency and accuracy compared to techniques like zinc-finger nucleases (ZFNs) or

transcription activator-like effector nucleases (TALENs).

2. Versatility

The system can target virtually any DNA sequence by simply redesigning the guide RNA. It is adaptable to multiple organisms and applications, including gene knockout, knock-in, or regulation.

3. Multitarget Capability

CRISPR can simultaneously target multiple genes or loci using multiplexed guide RNAs, enhancing its efficiency in complex edits

4. Faster Development

Its rapid design and implementation shorten the time required to achieve desired genetic modifications compared to conventional breeding or older editing methods.

Lastly, the CRISPR research community's open-access approach, including resources like plasmids (e.g., Addgene), gRNA design tools, and expert forums, has fostered widespread adoption and accelerated advancements in understanding and applying the technology.

Conclusion

The CRISPR/Cas9 system has revolutionized plant biotechnology by offering precise, efficient, and versatile genome-editing capabilities. It has proven instrumental in engineering virus-resistant crops by targeting viral genomes and host susceptibility genes, enhancing plant immunity and resilience. Its simplicity, cost-effectiveness, and adaptability, combined with the research community's open-access initiatives, have accelerated its development and application. As an advanced tool, CRISPR/Cas9 holds immense potential for addressing agricultural challenges and ensuring global food security.

References

Chen, F., Chen, L., Yan, Z., Xu, J., Feng, L., He, N., Guo, M., Zhao, J., Chen, Z., Chen, H., Yao, G. and Liu, C. (2024). Recent advances of CRISPR-based genome editing for enhancing staple crops. *Frontiers in Plant Science*, 15, 1478398.

Majeed, U., Yaqoob, U., Qazi, H. A., Ahmad, S. and John, R. (2018). CRISPR/Cas system as an emerging technology to enhance plant viral

immunity. *Physiological and Molecular Plant Pathology*, 103, 107-113.

Robertson, G., Burger, J. and Campa, M. (2022). CRISPR/Cas-based tools for the targeted control of plant viruses. *Molecular Plant Pathology*, 23(11), 1701-1718.

Shahriar, S. A., Islam, M. N., Chun, C. N. W., Rahim, M. A., Paul, N. C., Uddain, J. and Siddiquee, S. (2021). Control of plant viral diseases by CRISPR/Cas9: resistance mechanisms, strategies and challenges in food crops. *Plants*, 10(7), 1264.

Uranga, M., & Daròs, J. A. (2023). Tools and targets: The dual role of plant viruses in CRISPR–Cas genome editing. *The Plant Genome*, 16(2), e20220.