

CRISPR-cas9 genome editing: a molecular wonder in pest management

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Abstract

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated system) with its scientific advancements, uniqueness, specificity for organisms & easy handling, has become a revolutionary and improvised modern genome editing technology, all across the world especially being designed to alter the genomic sequences inside the living organism at a particular locus to get the desired result with higher accuracy in case of pest and disease control. It is meant for inducing a gene expression or suppressing a genetic expression against particular traits like insecticide resistance. These genetic alterations/ changes make the pests susceptible to various insecticides, and fungicides as well as compromising the reproductive fitness, & being an obstacle in the metamorphic growth of the pest, including hindrance in some other parameters.

Keywords: CRISPR/Cas9, genome editing, gene expression, locus, insects

Introduction & Review

Insect pests are part of a diversified cropping ecosystem, causing severe damage alongside huge crop loss. 20 yrs. Ago crop protection completely relied upon chemicals and conventional breeding approaches. Still, its negative impact on the environment as well as natural enemies was observed very clearly (Ijaz *et al.*, 2023), due to which later on after some period, the world witnessed the magic of *Bt* transgenic crops for resistant breeding programs, which also become resistant to the insect pests after being used vigorously & rapidly (Ahmed, 2023). The goal of the first sequence-specific DNA modification in *Nicotiana tabacum*'s DNA was to achieve a superior trait in 1988 (Paszkowski *et al.*, 1988). This led to the need for more advanced genome editing techniques, which were eventually met with the development of numerous effective and specialized tools, including CRISPR-Cas, TALENs, ZFNs, and RNA interference (RNAi).

In 2012 Emmanuelle Charpentier, Jennifer Doudna, and Fang Zhang invented it and received the Nobel Prize (Chemistry) for it in 2020 (Novel Media, 2020). 2013 saw the effective use of CRISPR-Cas in plants in a lab setting. This achievement opened up new avenues for researchers to investigate the CRISPR-Cas system for future applications and to

enhance their knowledge of RNA-mediated cell functions (Majhi *et al.*, 2022). The CRISPR/Cas system is a popular and simple method for genetic modifications because of the inclination for genome modification for gene knockdown techniques, transgene integration, nucleotide substitution, transcription regulation, etc. (Sushmita *et al.*, 2021; Tang *et al.*, 2017). A workable method for creating insect-resistant plants that will support sustainable agriculture is CRISPR-Cas gene editing (Moon *et al.*, 2022). Using this viewpoint technology, it might be feasible to generate insect resistance by altering effect or target interactions, eliminating genes that are prone to infection, disabling the defensive hormone's detrimental effects, and employing other techniques (Gantz & Akbari, 2018).

Methodology of CRISPR-Cas9

In bacteria and archaea, CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins) is an adaptive defense mechanism that helps them to precisely identify and break down foreign intracellular DNA (Sorek *et al.* 2013). Cas9 acts as a DNA endonuclease for the CRISPR/Cas system mostly observed in *Streptococcus pyogenes*. The DNA sequence selectivity of Cas9 is established through small linked RNAs (crRNA and tracrRNA), & they are frequently combined to create a single RNA called a single guide RNA, or sgRNA in lab terminology (Sorek *et al.* 2013). According to Gao (2021), because CRISPR-Cas may be used to edit numerous genes in a single run, it is far more flexible and user-friendly than other tools such as MNs, ZFNs, and TALENs, etc.

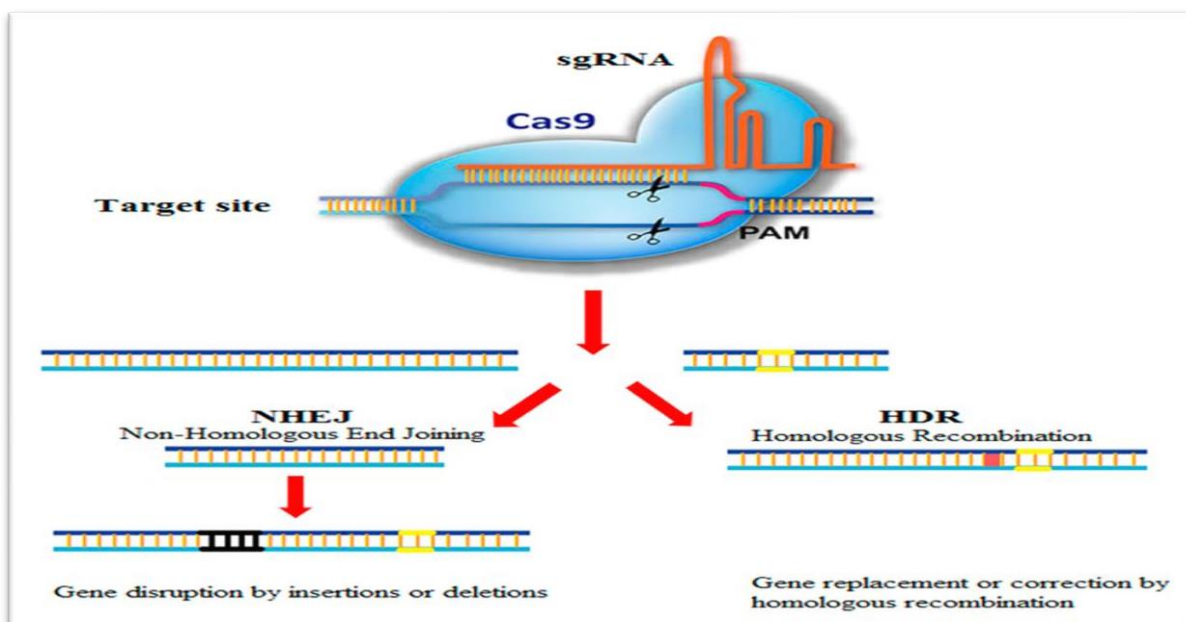


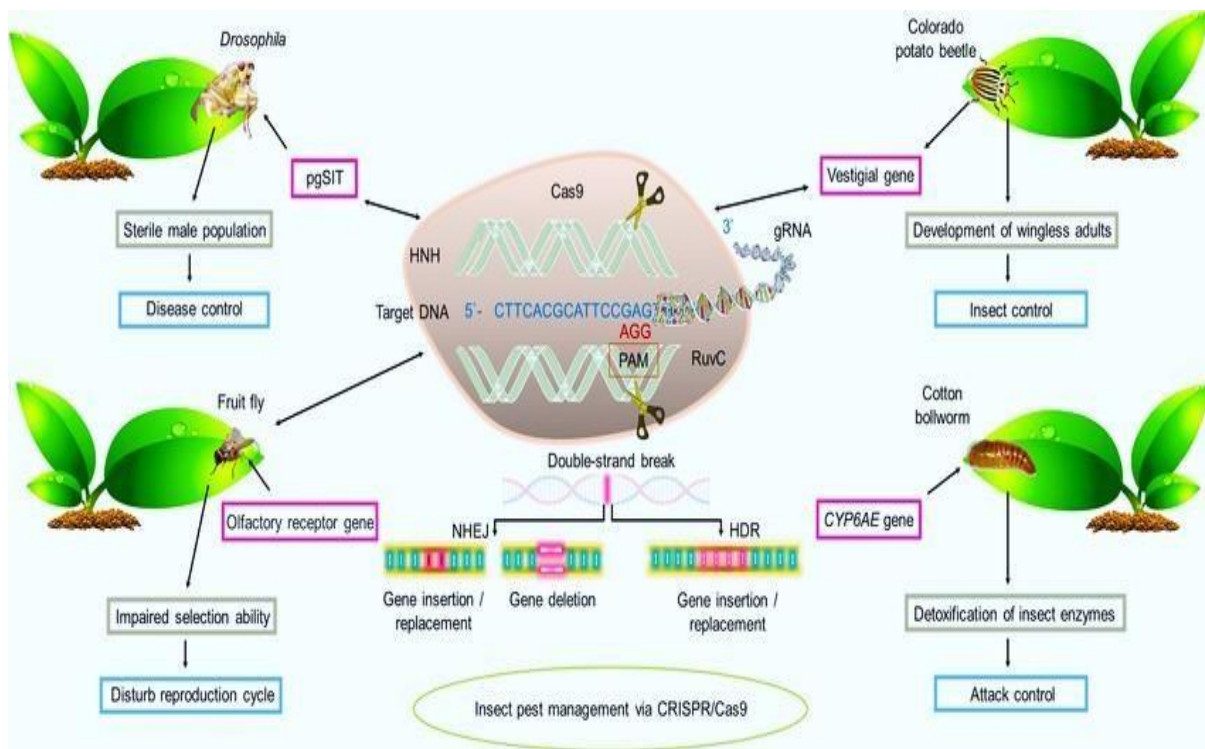
Fig 1. Diagrammatic representation of CRISPR-Cas9 genome editing system (Tavakoli *et al.*, 2021)

It has 3 types *i.e.*, Type I, II & III Cas systems (Hillary & Cesear, 2023). But the most used in plant protection is the type II system having Cas9 enzyme. This system is adapted for genome editing, targeting particular DNA sequences to create double-stranded breaks (DSBs)

by the Cas9 enzyme through a guide RNA (sgRNA) (Wu *et al.*, 2018). Here the DSBs which are created by the Cas9 have the role of a catalyst or molecular scissors. The Cas9 enzyme movement to a particular location in the genome is made possible by small guide RNAs (sgRNA), which allow the substitution of a new sequence with the old one (Sichani *et al.*, 2023). Cell repair mechanisms then introduce targeted genetic modifications or disrupt specific genes in a precise manner across various organisms, including insects and other pathogens.

Role of CRISPR-Cas9 in insect-pest management

This advanced technology has been highly successful in recent years being implemented in several pest management strategies, which are environmentally safe as well as easily being implemented with higher success rate. Studies by Lamb *et al.* (2016) & Char *et al.* (2017) have demonstrated that the growing use of gene-editing tools as an effective means of genome-editing offers a multitude of opportunities that may impact important characteristics *viz.*, biotic stress resistance like pest control. Some of the successful examples being stated below studied by various researchers;



✚ Zhang *et al.* (2023) used the CRISPR-Cas23a technology to prevent the *Locusta migratoria* from receiving pheromones by suppressing its odorant receptor, which became an effective technology.

✚ In the fruit fly spp. *Bactrocera dorsalis*, the CRISPR-Cas9 induced mutations in the transformer gene resulting in defective growth in reproductive organs and higher male production, helping with pest management (Zhao *et al.*, 2019).



- ✚ The *NPC1b* gene meant for cholesterol uptake from the external diet, was edited using CRISPR-Cas9 by Li & Jing (2020) to inhibit the bollworm, *Helicoverpa armigera* larvae’s growth, which leads to pest control.
- ✚ The CpomOR1 gene which is meant to trigger egg viability and production was altered to control the devastating pest, Codling Moth *Cydia pomonella* (Lepidoptera: Tortricidae) in apples.
- ✚ CRISPR/Cas9-mutated white (w-) gene in *Drosophila suzukii* resulted in a reproductive backlash, due to low pigmentation in the testis sheath (Sim et al., 2019; Ahmed et al., 2019) and could be used for fruit fly management.

Advantages and disadvantages of this novel technology

Advantages	Disadvantages
Specific in nature	Need expertise to handle the process
Easy-to-go process	Chances of unwanted mutation
Higher success rate	Limitations at the success rate
Significant result in a single run	-----
Highly advanced Technique	-----

Conclusion

In the field of agricultural entomology, this technology has become a boon in recent years with higher genome editing potential in the case of the pest control arena. CRISPR-Cas9 technology emphasizes more to rely on environment-friendly methods rather than unhealthy chemical pesticides, which have more detrimental effects. This technology is way more important as the gene knockout or knockdown process has zero negative effects on natural enemies. At the same time, this CRISPR technology targets only the gene inside without any outside morphological characters making it one of the smoothest and finest genomes editing technologies ever to be produced in the field of biotechnology for crop protection.

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