Article: RT1063

Biotica Research Today
Vol 4:8
2022
567
569

How to cite this article?
Rajadurai, 2022. Genome Editing System for Insect Pest Management. Biotica Research Today 4(8):567-569.

Genome Editing System for Insect Pest Management

Rajadurai Gothandaraman

Dept. of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu (641 003), India

Open Access

Corresponding Author
Rajadurai Gothandaraman
e-mail: rajaduraig89@gmail.com

Keywords
CRISPR/Cas, Genome editing, Pest management, pgSIT

Abstract

The recent advancement of gene-editing technologies such as Clustered Regularly Interspaced Short Palindromic Repeats and associated protein (CRISPR/Cas) has opened new doors for the development of novel pest control strategies. CRISPR/Cas provides stable genetic modifications within pest populations, allowing for basic exploratory research as well as support for efforts to suppress pest populations using gene drives. Highly efficient precision guided SIT (pgSIT) technology that can be deployed as eggs which exclusively give rise to sterile males. pgSIT is extremely robust at genetically sexing and simultaneously sterilizing the resulting progeny reproducibly with 100% efficiency.

Introduction

The global crop loss due to insect pests was estimated to be 10.8% during the post-green revolution period, costing billions of dollars (Dhaliwal *et al.*, 2015). Several insecticides have been reported to reduce crop pest infestation. However, long-term pesticide use has resulted in negative effects on non-target organisms, persistence in the environment, outbreaks of secondary pests, and the development of resistance in targeted pests. As a result, pesticides cannot be used as a long-term control method. Pest control objectives must be balanced with environmental concerns, such as the protection of pollinators and other beneficial species. The recent advancement of gene-editing technologies such as Clustered Regularly Interspaced Short Palindromic Repeats and associated protein (CRISPR/Cas) has opened new doors for the development of novel pest control strategies. CRISPR/Cas provides stable genetic modifications within pest populations, allowing for basic exploratory research as well as support for efforts to suppress pest populations using gene drives. The CRISPR/Cas9 system works through a ribonucleoprotein complex in which Cas9's target recognition lobe directs specific binding to target DNA by interacting with homologous sgRNA and the excision lobe cuts the DNA.

In insects, genome editing has been achieved by microinjecting freshly laid eggs (embryos) with various combinations of CRISPR reagents. The advanced genome editing tool is entitled as "prime editing". This novel edit technology is a 'search-and-replace' genome editing technology that mediates targeted insertions, deletions, all 12 possible base-to-base conversions, and combinations without requiring double stranded DNA breaks or donor DNA templates. Prime editors (PEs), initially exemplified by PE1, use a reverse transcriptase (RT) fused to an RNA-programmable nickase and a prime editing guide RNA (pegRNA) to copy genetic information directly from an extension on the pegRNA into the target genomic locus. PE2 uses an engineered RT to increase editing efficiencies, while

Article History
Received on: 02nd August 2022
Revised on: 07th August 2022
Accepted on: 08th August 2022

E-mail: *bioticapublications@gmail.com*

PE3 nicks the non-edited strand to induce its replacement and further increase editing efficiency. Prime editing offers much lower off-target activity than Cas9 at known Cas9 off-target loci, far fewer by-products and higher or similar efficiency compared to Cas9-initiated HDR, and complementary strengths and weaknesses compared to base editors.

Prime editing shows higher or similar efficiency and fewer by-products than homology-directed repair, have complementary strengths and weaknesses compared to base editing and induces much lower off-target editing than Cas9 nuclease at known Cas9 off-target sites. The CRISPR-Cas9 gene editing system has been known to produce extra cuts in wrong sections of DNA, which can interrupt cell function. Another type of gene editing that doesn't rely on DNA breaks and was thought to minimize inaccuracy is base editing, in which an enzyme can trade one DNA nuclease for another, but this strategy offers limited options as it can only make four of the 12 possible base pair changes.

Mechanism of CRISPR/Cas9

Cas9 has been widely used for genome editing in a variety of organisms since its discovery. Cas9, like engineered ZFNs and TALENs, is a programmable, sequence-specific endonuclease that is part of the CRISPR system's general structure. Cas9-mediated genome editing, like other nucleases, is accomplished in two steps: Cleavage of DNA followed by repair of DNA (Figure 1). The sgRNA directs Cas9 to a specific genomic locus, where Cas9 generates a DSB, triggering DNA repair *via* intrinsic cellular mechanisms such as non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ causes nearly random insertion and deletion mutations (*i.e.,* indels) at the DSB site, which can result in gene knockout (for example, by shifting the target gene's reading frame or mutating a critical region of the encoded protein). Through homologous recombination guided by a donor DNA template, HDR can be used to generate the desired sequence replacement at the DSB site, resulting in targeted gene deletion, mutagenesis, insertion, or gene correction. As a result, the CRISPR/Cas9 system provides an effective platform for sequence-specific genome editing, such as gene knockout, gene knock-in, and site-specific sequence mutagenesis and corrections (Wang *et al.,* 2016).

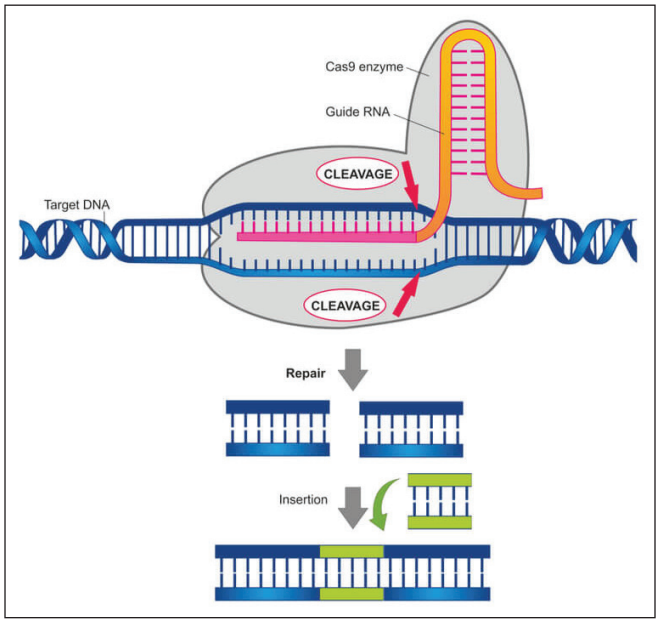

Figure 1: Mechanism of CRISPR/Cas9 (Photo courtesy: Fernández, 2021)

Applications of CRISPR Gene-Editing in Insect Pest Management

CRISPR/Cas9 application in insects is still in its early stages. It has been extensively reformed for various applications in model animals, which may shed light on potential applications in insects.

• ***Gene Drive:*** Gene drive is the increase in the frequency of specific genes caused by bias inheritance.

• ***Sequence-Specific Gene Regulation:*** CRISPR inference (CRISPRi) is a modified CRISPR/Cas9 system in which dCas9 paired with sgRNA can satirically inhibit transcription at the sgRNA base-pairing genomic locus.

• ***Genomic Imaging:*** By tagging dCas9 proteins with enhanced green fluorescent protein, the CRISPR/Cas9 system can be reconfigured as a live imaging system (EGFP).

• ***Population Suppression:*** The Cas9-based gene drive could be used for population suppression by targeting a gene that is necessary for survival in the wild but unimportant in a breeding facility (*e.g.*, a gene essential for vision). A Cas9-based gene drive could also be placed within a gene required for female development, survival, or fecundity.

• ***Insecticide Resistance Management:*** CRISPR/Cas9 could also be used to eliminate pesticide resistance. CRISPR/Cas9 can also be used to systematically knock down, knock out, or over-express specific targeted genes that cause insecticide resistance in insect pests.

Precision Guided SIT

The genetic-based sterile insect technique (SIT) is an effective and environmentally safe strategy to diminish populations of agricultural and horticultural insect pests. Highly efficient precision guided SIT (pgSIT) technology that can be deployed as eggs which exclusively give rise to sterile males. pgSIT functions by exploiting the precision and accuracy of CRISPR to simultaneously disrupt genes essential for female viability and male fertility to facilitate the release of eggs into the environment ensuring only sterile adult

males emerge (Bouyer and Vreysen, 2019). pgSIT is extremely robust at genetically sexing and simultaneously sterilizing the resulting progeny reproducibly with 100% efficiency. It will induce substantially greater population suppression than can be achieved by currently available self-limiting suppression technologies (Kandul *et al.,* 2019).

Conclusion

Molecular biotechnologies, which enable genetic improvement and modification across a wide range of insect pests, have become increasingly important pest management tools. CRISPR gene editing has the ability to change a specific gene of interest. It has been established and developed in both model and non-model insects. Highly efficient knockout and knock-in experiments have been carried out in model insects like Drosophila and silkworm, as well as non-model insects. Several well-designed systems based on CRISPR/Cas9 have been developed, including pgSIT, gene drive and regulation systems and DNA/RNA tracking systems, which will have a significant impact on functional studies and pest control.

References

Bouyer, J., Vreysen, M.J.B., 2019. Concerns about the feasibility of using "precision guided sterile males" to control insects. *Nat.Commun.* 10, 3954. DOI: 10.1038/s41467-019-11616-9.

Dhaliwal, G.S., Jindal, V., Mohindru, B., 2015. Crop losses due to insect pests: Global and Indian Scenario. *Indian Journal of Entomology* 77(2), 165- 168.

Fernández, C.R., 2021. CRISPR-Cas9: The Gene Editing Tool Changing the World. Available at: https://www.labiotech.eu/in-depth/crispr-cas9-review-gene-editing-tool/. Accessed on: 29 July 2022.

Kandul, N.P., Liu, J., Wu, S.L., Marshall, J.M., Akbari, O.S., 2019. Transforming insect population control with precision guided sterile males with demonstration in flies. *Nat. Commun.* 10, 84.

Wang, H., Russa, M.L., Lei, S.Q., 2016. CRISPR/Cas9 in Genome Editing and Beyond. *Annual Review of Biochemistry* 85, 227-64.