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Advancements in Genome Editing for Insect Control - A Comprehensive Review

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ABSTRACT

Genome editing technologies have revolutionized the field of insect control, offering promising strategies for combating insect-borne diseases, agricultural pests and invasive species. This review aims to provide a comprehensive overview of the recent advancements in genome editing techniques and their applications in insect control. We discuss the principles and applications of CRISPR-Cas 9, TALENs, and ZFNs, highlighting their potential for precise and efficient genome modifications in insects. Additionally, we explore various insect control strategies, including genetic sterilization, gene drives and population suppression, enabled by genome editing. Furthermore, we delve into the ethical considerations and regulatory challenges associated with the use of genome editing in insect control. Overall, this review aims to shed light on the current state-of-the-art in genome editing for insect control and its implications for addressing pressing global challenges.

Keywords Genome editing, CRISPR-Cas 9, TALENs, ZFNs, *Drosophila melanogaster*, DNA endonuclease.

INTRODUCTION

Insect geneticists have long relied on genome modification technologies to unravel the mysteries of insect biology. Early techniques involved the use of chemical mutagens and radiation to induce random genetic changes. While these methods were effective to some extent, they lacked precision and control. During the early 1980s, transposon-based technologies emerged as a significant advancement in insect genetics. Transposons or jumping genes are DNA sequences capable of moving from one location to another within the genome. These transposons could be harnessed to induce DNA breaks and stimulate the repair processes within the insect genome. A major breakthrough in insect genome editing came with the development of targeted sequence modification or replacement methods. Gloor *et al.* (1991) described a technique that relied on double-strand DNA break-induced homologous recombination. This process allowed the creation of new alleles of selected genes

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in insects like *Drosophila melanogaster*. However, this technology was initially inefficient and limited in its utility. Researchers soon developed improved variations of the original method, leading to highly versatile gene-editing methods. Rong and colleagues described techniques that allowed editing of potentially any gene in the *Drosophila melanogaster* genome. These advancements were instrumental in expanding the applications of genome editing in insects.

Initially, DNA endonucleases with custom-defined specificity, known as engineered nucleases, were created using proteins containing multiple zinc-finger binding domains. These domains were meticulously designed to identify specific target DNA sequences. They were then linked to a DNA endonuclease, such as Fok 1, resulting in what are known as zinc finger nucleases (ZFNs) (Kim *et al.* 1996). Bibikova *et al*. (2002) showcased the effectiveness of ZFNs in gene editing within *D. melanogaster*. However, the design complexities and considerable production expenses associated with crafting functional ZFNs present notable obstacles that limit their utilization.

Transcription activator-like effectors (TALEs) derived from the plant pathogen *Xanthomonas* represent an alternative programable DNA-binding protein system that has been harnessed for gene editing endeavors (Bogdanove and Voytas 2011). Crafting TALEs is comparably less intricate than devising zinc-finger-containing proteins. Nonetheless, employing the TALE system to generate precise TALE-endonucleases (TALENs) still necessitates substantial gene construction or synthesis efforts. Numerous reports, primarily of a technical nature, have highlighted the operational capabilities of TALENs in diverse insect species, along with the optimal conditions conducive to their functionality (Daimon *et al.* 2015).

CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins) constitutes an adaptive immune system present in bacteria and archaea. This system empowers these microorganisms to precisely identify and break down foreign intracellular DNA (Sorek *et al.* 2013). Cas 9 is a DNA endonuclease connected to the CRISPR/Cas system prevalent in *Streptococcus pyogenes*. The DNA sequence selectivity of Cas 9 is established through small linked RNAs (crRNA and tracrRNA), which are often amalgamated to form a solitary RNA known as a guide RNA or gRNA in laboratory settings (Sorek *et al.* 2013). In contrast to ZFNs and TALENs, the process of gene editing with Cas 9 doesn't involve the repetitive design and expression of new Cas 9 proteins. Rather, it entails generating concise, target-specific gRNAs that join with Cas 9 to confer the sought-after site precision. Despite being a relatively recent addition to the gene editing toolkit, Cas 9 is swiftly gaining traction among insect biologists. However, its predominant applications in insects thus far have been confined to technical investigations aimed at assessing the system's performance attributes (Gratz *et al.* 2014).

To address these challenges, innovative solutions are required. Genome editing techniques offer promising opportunities for effective insect control. The introduction emphasizes that genome editing involves precise modifications to an organism's DNA, enabling scientists to target and modify specific genes in insects. This technology has the potential to revolutionize insect control strategies by providing more precise and efficient methods for managing insect populations and mitigating the impact of insect-related problems.

Principles of genome editing techniques

It delves into the principles underlying genome editing techniques, providing a detailed understanding of CRISPR-Cas 9, TALENs, and ZFNs. It explains the fundamental components and mechanisms of each technique, including the use of guide RNA or DNA to target specific genomic sequences and the role of nucleases in introducing modifications.

CRISPR-Cas 9

CRISPR-Cas 9 is a revolutionary genome editing technology that has gained significant attention and revolutionized the field of genetic engineering. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a naturally occurring system found in bacteria and archaea that acts as an adaptive

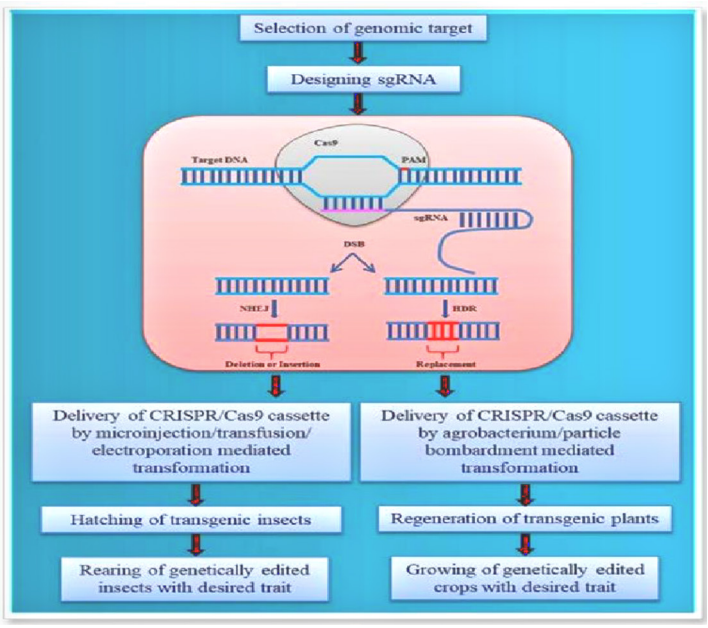

Fig. 1. Workflow of the CRISPR cas 9.

immune system, protecting them from viral infections. Cas 9 (CRISPR-associated protein 9) is an enzyme that plays a crucial role in the CRISPR system by cleaving DNA at specific target sites.

The CRISPR-Cas 9 system, adapted for genome editing, utilizes a guide RNA (gRNA) to target specific DNA sequences, directing the Cas 9 enzyme to induce double-stranded breaks. Cell repair mechanisms then introduce targeted genetic modifications or disrupt specific genes in a precise manner across various organisms, including insects. Numerous applications of CRISPR-Cas have demonstrated the ability to alter DNA sequences within insect or plant genomes (Wu *et al.* 2018). The Cas 9 protein derived from *Streptococcus pyogenes* (Sp) is presently the most commonly utilized source (Marraffini 2016). In this process, a Cas 9-protein bound to a single-guide RNA (sgRNA) targets and cleaves a specific DNA region adjacent to a protospacer adjacent motif (PAM). This action prompts the cellular DNA repair mechanism to generate a double-strand break (DSB). When a homologous repair template is absent, error-prone non-homologous end-joining (NHEJ) pathways come into play, leading to the formation of spontaneous insertions, deletions, or replacements at the DSB site. These alterations commonly lead to disruptions in gene functionality. Conversely, error-free homology-directed repair (HDR) mechanisms are triggered, facilitating precise gene modifications like knock-ins, knockouts, or mutations, provided suitable donor DNA templates resembling the sequence around the DSB site are accessible (Yin *et al.* 2017).

The NHEJ and HDR mechanisms have been effectively employed for genome editing in various insects and plants (Lu *et al.* 2018). Following the achievement of successful genome modification, the CRISPR-Cas construct is introduced into plants via methods such as Agrobacterium-mediated or particle bombardment-mediated transformation. Similarly, in insects, it's delivered into embryos through techniques like microinjection, transfusion, or electroporation-mediated transformation. These approaches facilitate the regeneration of transgenic species possessing the desired traits (Li and Scott 2016). The process of CRISPR-Cas genome editing in both plants and insects is succinctly depicted in the Fig. 1.

In the context of insect control, CRISPR-Cas9 has shown great promise. It offers a means to modify

Table 1. CRISPR-Cas genome editing in insects for insect pest management.

Insect	Target gene	Editing outcome
Drosophila melanogaster	Yellow	Knockout
		Knock-In
	Rosy	Knockout
	DSH3PX1	Knockout
	LUBEL	Knockout
	Chitin synthase 1	Substitution
	Nicotinic acetylcholine receptor α6	Substitution
	Scsa	Knockout
	Kdr	Knockout
	Ast	Knockout
	Eh	Knockout
	capa	Knockout
	Ccap	Knockout
	Crz	Knockout
	npf	Knockout
	Mip	Knockout
	mir-219	Knockout
	mir-315	Knockout
	white	Knockout
	Yellow and white	Knockout
	Yellow and rosy	Knockout
	Alk	Knockout
	TpnC	Knockout
	Wntless	Knockout
	Yellow, white and tan	Knock-In
	Act5C	Knockout
	lig4	Knockout
	mus308	Knockout
	Mod (mdg4)	Knockout
	Fdl	Knockout
	Chameau	Knock-In
	CG4221	Knock-In
	CG5961	Knock-In
	Clamp	Knockout
	Dα6	Knock-In
	Ebony	Knock-In
	wg	Knock-In
	wls	Knock-In
	Lis 1	Knock-In
	Se	Knock-In
	Ebony, yellow and vermilion	Knockout
	White and piwi	Knockout
	Salm	Knock-In
	Yellow, notch, bam, nos, ms (3) k81, and cid	Knockout
	Ms (3) k81	Knockout
	white	Knockout
	yellow	Knockout
	EGFP and mRFP	Knockout
	Ebony, yellow, wingless and wnt	Knockout
Drosophila subobscura	Yellow and white	Knockout
Drosophila suzukii	White (w)	Knockout
	DsRed (red fluorescence protein)	Knock-In
	White (w-)	Knockout
Spodoptera exigua	Seα6	Knockout
	Ryanodine receptor	Substitution
	CYP9A186 gene	Knockout
	P-glycoprotein gene	Knockout
	α-6-nicotinic acetylcholine receptor (nAchR)	Knockout
Spodoptera littoralis	Orco	Knockout
Spodoptera litura	Abdominal-A (slabd-A)	Knockout
	SlitPBP3	Knockout
	SlitBLOS2	Knockout
Spodoptera frugiperda	Sfabd-A	Indel
	BLOS2E93 TO	Knockout
	SfABCC2	Edit
	ABC transporters	Knockout
	ABCB1	Knockout
Helicoverpa armigera	nAchR	Knockout
	α-6-nicotinic acetylcholine receptor (nAchR)	Knockout
	HaCad	Knockout
	Cluster of nine P450 genes	Knockout
	CYP6AE	Knockout
	OR16	Knockout
	Tetraspanin	Knockout
	HaABCA2	Knockout
	White, ok, brown, and scarlet	Knockout
	NPC1b	Knockout
Helicoverpa punctatus	DpWnt-1	Knockout
Bemisia tabaci	White	Edit
Nilaparvata lugens	Cinnabar and white	Edit
	Nl-cn and Nl-w	Knockout
Ceratitis capitata	White eye (we) and paired gene (Ccprd)	Knockout
	eGFP_gRNA2, eGFP_gRNA2, 1 mM Scr7, and eGFP_gRNA2b–Cas9 complexes with ssODN_BFP donor templates	Knock-In

Table 1. Continued.

Insect	Target Gene	Editing outcome
Bactrocera dorsalis	White and transformer	Knockout
Anastrepha ludens	Astra-2	Knockout
Locusta migratoria	Orco	Knockout
	OfAgo1	Knockout
Cydia pomonella	CpomOR1	Knockout
Tetranychus urticae	Phytoene desaturase	Knockout
	PSST	Knockout
Leptinotarsa decemlineata	Vestigial gene (vest)	Knockout
Euschistus heros	Abnormal wing disc (awd), tyrosine hydroxylase (th), and yellow (yel)	Knockdown and knockout
Diaphorina Citri, Homalodisca vitripennis and Bemisia argentifolii	Thioredoxin and vermillion	Knockout
Diaphorina citri	ACP-TRX-2	Knockout
Mythimna separata	NPC1b	Knockout
Hyphantria cunea	Hcdsx	Knockout

the genomes of disease vectors, such as mosquitoes, rendering them unable to transmit diseases like malaria or dengue fever. By targeting genes involved in the transmission or replication of pathogens within the insect, researchers can potentially reduce the spread of these diseases (Table 1). CRISPR-Cas9 can also be used to engineer insects for agricultural pest management, allowing for the development of insect-resistant crops or the suppression of pest populations. However, the use of CRISPR-Cas 9 in insect control also raises ethical and regulatory considerations. The potential for gene drives, where engineered genes spread rapidly through populations, may have ecological implications and unintended consequences. Careful assessment and consideration of these ethical concerns and potential risks are essential to ensure the responsible and ethical use of CRISPR-Cas9 in insect control.

TALENs (transcription activator-like effector nucleases)

TALENs (transcription activator-like effector nucleases) are a class of engineered nucleases widely used in genome editing. TALENs offer precise and targeted modifications to the DNA sequences of organisms, including insects. They were developed based on naturally occurring DNA-binding proteins found in certain plant pathogenic bacteria.

TALENs, consisting of a DNA-binding domain from transcription activator-like effectors (TALEs) and a FokI endonuclease-derived nuclease domain, enable precise DNA targeting through modular repeat structures. Designed in pairs, TALENs induce double-stranded breaks, initiating repair via error-prone non-homologous end joining (NHEJ) or precise homology-directed repair (HDR) pathways for gene disruption or targeted modifications. TALENs have been employed to modify insect genomes for insect-resistant crops, gene function studies, and disease vector control, allowing precise gene editing. Their use raises ethical concerns, requiring careful evaluation of off-target effects and ecological impacts, with ongoing research focused on enhancing TALEN efficiency and specificity through improved delivery methods and design optimization.

ZFNs (Zinc finger nucleases)

ZFNs (zinc finger nucleases) are a class of engineered nucleases that have been widely used in genome editing. They provide a precise and targeted approach for modifying the DNA sequences of organisms, including insects. ZFNs are composed of two key components: Zinc finger proteins (ZFPs) and a DNA-cleaving domain. Zinc finger proteins are naturally occurring DNA-binding motifs that can be engineered to recognize specific DNA sequences. Each zinc finger module typically binds to a specific triplet of DNA bases. By combining multiple zinc finger modules, researchers can design custom ZFNs capable of recognizing and binding to specific DNA sequences of interest. The modularity of zinc finger proteins allows for flexibility in designing ZFNs to target different genomic sites.

The DNA-cleaving domain of ZFNs, derived from a restriction enzyme like FokI, induces double-stranded breaks at the target site. ZFNs, used in pairs, bind to each DNA strand, bringing FokI domains close for a break, repair occurs via error-prone NHEJ or precise HDR pathways, influencing gene disruption or targeted modifications.

ZFNs are utilized in insect control to develop resistant crops and study gene function in insects, showing potential for modifying disease vectors like mosquitoes to reduce disease transmission. Ethical considerations and careful evaluation of off-target effects are crucial for the responsible use of ZFNs in insect control, with ongoing research focused on improving specificity and efficiency.

Applications of genome editing in insect control

This topic focuses on the practical applications of genome editing in the field of insect control. It discusses how genome editing techniques can be employed to combat insect-borne diseases by modifying disease vectors to be resistant to pathogens or altering their ability to transmit diseases. The review paper also examines the potential of genome editing for developing insect-resistant crops, reducing pesticide use, and improving agricultural productivity. Furthermore, it explores the use of genome editing to target invasive species, including genetic modifications that render them unable to reproduce or impact their ability to survive and compete. This topic emphasizes the diverse applications of genome editing in insect control and its potential to revolutionize traditional pest management approaches.

Genetic modification for disease vector control

Genome editing techniques, such as CRISPR-Cas9, TALENs, and ZFNs, hold promise in combating vector-borne diseases like malaria, dengue, Zika and Lyme disease by precisely modifying disease-transmitting vectors to reduce pathogen transmission or suppress their populations. This targeted approach addresses significant public health challenges posed by these diseases worldwide. The objective is to disrupt or modify specific genes within the vectors, with the aim of achieving the following strategies.

Pathogen resistance: By modifying the genetic makeup of disease vectors, researchers can enhance their resistance to the pathogens they carry. For example, in the case of malaria, genetic modifications can be introduced to render mosquitoes resistant to the malaria parasite, Plasmodium. This strategy aims to reduce the transmission of the disease by interrupting the lifecycle of the pathogen within the vector.

Vector population suppression: Another approach is to engineer disease vectors with genetic modifications that suppress their population size. This can be achieved by disrupting genes essential for vector reproduction or survival. For instance, genes involved in fertility or development can be targeted to reduce vector population growth or impair their ability to transmit diseases.

Sterile insect technique: Genetic modification can also be utilized to implement the Sterile Insect Technique (SIT) for disease vector control. SIT involves the release of genetically modified sterile male insects into the wild population. These modified males compete with wild males for mating opportunities, reducing the overall reproductive success of the population. Over time, this can lead to a decline in the vector population, subsequently reducing disease transmission.

While the field of genetic modification for disease vector control is still evolving, ongoing research and advancements in genome editing techniques hold significant promise for developing innovative and effective strategies to combat vector-borne diseases and reduce their impact on human populations.

Precision agriculture and pest management

Precision agriculture and pest management are areas where genome editing techniques can contribute to more efficient and sustainable agricultural practices. By harnessing the power of genome editing, researchers can develop innovative strategies to control pests, increase crop yields, and reduce the environmental impact of agricultural practices.

Precision agriculture utilizes technology and data for site-specific optimization of farming processes,

with genome editing playing a key role in enhancing crop resistance to pests and diseases. By modifying specific genes, researchers can develop resilient crop varieties, reducing reliance on chemical pesticides and improving overall productivity.

Invasive species control

Invasive species pose a significant threat to ecosystems, biodiversity, and economic stability. These non-native species, when introduced into new environments, can outcompete native species, disrupt ecological balances, and cause harm to agricultural systems. Genome editing techniques offer potential solutions for invasive species control by providing tools to manage and mitigate their negative impacts.

Genome editing can be employed to target invasive species through various strategies:

Genetic modification: Genome editing tools such as CRISPR-Cas9, TALENs, and ZFNs allow precise modifications to the genomes of invasive species, enabling targeted alterations to genes affecting reproductive capacity, development, or survival. Disrupting genes responsible for fertility, for instance, can lead to reduced reproductive success and a decline in the invasive species population.

Gene drives: Genome editing techniques enable the engineering of gene drives in invasive species, promoting the rapid spread of modified genes that confer reproductive advantages or reduce population growth, potentially leading to the suppression or alteration of invasive species populations; however, careful consideration and rigorus risk assessment are essential due to potential ecological impacts.

Population suppression: Genome editing can also be used to develop population suppression strategies for invasive species. By targeting genes involved in key physiological processes, such as development, metabolism, or behavior, researchers can disrupt vital functions and reduce the fitness or survival of the invasive species. Population suppression strategies aim to reduce the abundance and impact of invasive species on ecosystems.

Limitations and future perspectives

Genome-editing techniques, akin to other biotechnological methods, specifically target genetic modifications through cellular and *in vitro* mechanisms. While genetic alterations naturally occur in evolution, deliberate experimental changes are made for human interests, especially in enhancing crops. The legal and ethical implications of gene editing persist within the scientific community, emphasizing the need for a practical standpoint supported by legislative bodies to unlock its potential for global agriculture. Deliberate distribution of genetic components through CRISPR-Cas for pest management emerges as a precise and ecologically responsible strategy. Nonetheless, the rise of insect resistance triggered by a CRISPR-mediated gene drive could pose a significant and persistent challenge on both experimental and theoretical fronts (Unckless *et al.* 2017). On the other hand, multiplex gene editing has the potential to surmount resistance (Marshall *et al.* 2017). Hence, it becomes imperative to tackle challenges related to insect resistance, aiming for a consensus that aligns ethics and science in support of this technology. The introduction of CRISPR-Cas-edited insects with gene drives raises biosafety concerns due to their potential to impact entire ecosystems. Conducting through risk assessments for unintended consequences on non-target entities, particularly beneficial insects, is essential to prevent disruptions in food chains and adverse changes in community composition (Akumo *et al.* 2013). Moreover, there is a potential for diseases to exacerbate due to the potential gene transfers between target organisms and their non-target counterparts. If these risks are adeptly handled considering unforeseen environmental impacts, gene-driven technology holds promise for precisely eliminating insect pests, insect vectors for viruses, and foreign insect species. Incorporating terminator genes that regulate the lifespan of modified insects, along with using tagged insects to track gene flow, emerges as vital measures for the biosafe application of gene drives within the scope of risk management. Furthermore, an alternative strategy for combating invasive pests involves deploying robotic equipment and artificial intelligence to physically eradicate individual pests (Young 2017). However, robotics might encounter limitations when dealing with small insects, uneven

terrains, and concealed eggs. On the other hand, combatting invasive pests through the CRISPR Cas-based deletion of susceptible genes has demonstrated successful insect resistance. Yet, S gene deletions, while addressing vulnerabilities and imposing a related fitness cost, can lead to pleiotropic effects within the plant. Nonetheless, it's achievable to confer insect resistance while preserving plant performance by modifying the binding effector factor instead of the gene itself (Li *et al.* 2012). Consequently, the CRISPR-Cas method for establishing insect resistance in crop varieties will evolve into an effective tool for swiftly imparting genetic traits to cultivated strains within a reduced timeframe. Indeed, the rapid evolution of CRISPR-Cas-enabled genome editing technology underscores its dynamic nature, leading to an expanding scope of applications in agriculture (Paul *et al.* 2022). Nonetheless, a comprehensive comprehension of the genetic and genomic workings of the specific target species is imperative before fully embracing its use for developing resistance against insect pests and safeguarding plants. The utilization of Bt technology, originating from advancements in recombinant DNA techniques, has brought about a revolution in insect management across various economically significant crops, such as cotton, maize, soybean, and brinjal (Islam and Molla 2021). The user-friendly and versatile nature of CRISPR technology could potentially supplant the present recombinant DNA approach for faster insertion of R genes, offering a more streamlined and efficient process.

CONCLUSION

Genome editing technologies have revolutionized biology, offering solutions for healthcare, agriculture, and the environment. CRISPR-Cas 9, TALENs and ZFNs enable precise genetic modifications, benefiting disease treatment, crop traits and ecosystem management. Responsible use is vital, risk assessment, ethics, and safety evaluations guide applications. Environmental impact and unintended consequences need through evaluation. Regulations ensure safe deployment, with public engagement building trust. Global collaboration and standards promote ethical genome editing. The future promises transformative advancements in health, agriculture, and environment. Safe, ethical governance unlocks genome editing's potential for global benefits.

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