CRISPR-Cas Genome Editing for Insect Pest Stress

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Global crop yield and food security are being threatened by phytophagous insects. Innovative methods are required to increase agricultural output while reducing reliance on hazardous synthetic insecticides. Using the revolutionary CRISPR-Cas technology to develop insect-resistant plants appears to be highly efficient at lowering production costs and increasing farm profitability. The genomes of a model insect, Drosophila melanogaster, and major phytophagous insect genera, viz. Spodoptera, Helicoverpa, Nilaparvata, Locusta, Tribolium, Agrotis, etc., were successfully edited by the CRISPR-Cas toolkits. However, this new method can alter an insect's DNA to either induce a gene drive or overcome an insect's tolerance to certain insecticides. The rapid progress in the methodologies of CRISPR technology and their diverse applications show a high promise in the development of insect-resistant plant varieties or other strategies for the sustainable management of insect pests to ensure food security.

CRISPR-Cas technology pest management plant stress resistance insect resistance

1. Introduction

Biotechnology performs a crucial role in controlling insect pests to protect crops. It improves yields in areas ranging from breeding for pest resistance to the genetically modified introgression of new genes [1]. The use of genome-editing techniques to create insect-resistant plants is still in its early stages. By manipulating the genes of both plants and insects, genome editing can be used to manage insect populations. The insect pests of crops can be controlled by inducing sterility in insect pests, interrupting pesticide resistance, or creating de novo resistance if adequate R-genes are lacking. Using CRISPR-Cas9 genome-editing technology, novel research is being done to modify insects to prevent them from feeding on and injuring plants and to modify plants to increase their efficacy in repelling insects. In this respect, the genome-editing platform has offered a new opportunity for generating designer plants, especially in circumstances where a targeted deletion is likely to produce elite and superior characteristics or to trigger a gene drive to selectively spread mutations contributing to the lethality of female insect populations.

2. CRISPR-Cas Genome Editing in Insects

In agriculture, CRISPR-Cas can be employed for crop protection through insect pest management. The genome editing of insects can be carried out successfully with a two-step technique involving the alteration of target DNA in insects and their eventual release into nature [2]. One of the earliest documented uses of the CRISPR-Cas system in insects was in *Drosophila* fruit flies, where effective modifications of the yellow gene were made [3].

The *BmBLOS2* gene was the focus of another reported successful application of this method in silkworms [4], which was followed by several successful applications. In a case study by Garczynski et al. [5], the codling moth genome was edited using CRISPR-Cas gene-editing technology in order to alter the viability and production of eggs by targeting a particular gene (*CpomOR1*). Worldwide, the codling moth is a significant pest to pome fruit. As a member of the pheromone receptor subfamily, the *CpomOR1* gene product is an odorant receptor. In the early-stage eggs of codling moths, single-guide RNAs (sgRNAs) were created to target the nucleotides of the *CpomOR1* gene. It was discovered that alterations, including insertions and deletions, were successfully introduced. By mating males with females who had *CpomOR1* gene alterations, the study tried to produce stable populations of edited codling moths by raising the young moths to adulthood. It was discovered that the modified females' fecundity and fertility were compromised, causing them to produce non-viable eggs. The result was the regulation of fruit pomes by the insects. However, it is still unclear exactly how *CpomOR1* affects the fertility and reproduction of codling moths. In another case, it was claimed that the migratory locust underwent a targeted heritable mutation as a result of the CRISPR-Cas technique. Locusts are dangerous agricultural pests that have an impact on a wide variety of crop plants. Their swarming behavior can result in very serious crop damage over large areas all at once, frequently leading to significant financial loss. The Li et al. [6] study involved the engineering of the guide-target RNA's sequence to prevent the odor receptor co-receptor gene from being expressed (*Orco*). *Orco* gene mutants were shown to have defective electrophysiological reactions to several odors, resulting in mutant locusts lacking their attraction to aggregation pheromones under crowding circumstances.

Although the transgenic Bt technology is well established and widely utilized, the development of insect resistance to Bt insecticidal proteins (ICPs) has become a significant concern. In order to avoid this, efforts are being made to build receptors in a way that will enable effective resistance management. By altering the *Helicoverpa armigera* genome, it is possible to successfully knock down the Cadherin receptors that are functionally connected to *Cry1Ac* toxin tolerance [7]. A base replacement in the encoding genes of the mid-intestinal receptor demonstrated how the genome of insects can change their resistance to insect pests. Modifying *Cry* protein binding receptors can be used to edit insect genomes to decrease plant vulnerability. Unique detoxifying enzymes produced by insects are crucial for resolving the chemical defense responses in many plant species. A possible alternative would be to focus on polyphagous bugs' detoxifying genes. Insect susceptibility resulted from targeting and deleting insecticidal detoxifying genes, such as gossypol-inducing cytochrome P450 [8]. The polyphagous insect *H. armigera's* susceptibility to phytotoxins was revealed with the CRISPR-Cas-mediated deletion of the *CYP6AE* gene cluster, which also made crops resistant to insects and showed the importance of these enzymes in the detoxification of several toxic phytochemicals [9]. The most long-lasting answer has consistently been this one.

The modification of target genes that can prevent chemical contact and mating pair recognition, which are crucial for efficient interactions between plants and insects, is another method to control insects using CRISPR-Cas. The

olfactory receptors *(ORs)* in insects are crucial for the identification of host plants and mating pair odorants. The *Or83b* gene mutation in Drosophila prevented the host from being detected [10]. Similarly to this, the CRISPR-Cas method's deletion of the *Orco* gene from *Spodopthera litura* affected its choice of a mating partner and host plant [2]. Implementing such technology would be a smart move to keep insects away from plants and prevent pest damage. In insects, female adults release pheromones that males pick up on. Males select mature females based on their pheromone cues. A CRISPR-Cas9-based odorant receptor 16 *(OR16)* knockout in *H. armigera* prevented males from detecting pheromone signals and prevented mating with immature females, which led to the dumping of infertile eggs and helped in controlling insects [11]. Another strategy for the control of insects is to use CRISPR-Cas9 to remove growth genes, such as the *abd-A (Abdominal A)* gene, from a variety of insects, including *Spodoptera litura* [12], *Spodoptera frugiperda* [13], and *Plutellaxy lostella* [14], which resulted in inabnormal gonads, disarmed prolegs, and the lack of body segment functions. The CRISPR-Cas9 technology was used to modify numerous other genes in a variety of insect pests. In *Drosophila melanogaster*, the *LUBEL*, *Scsa*, and *Kdr* genes were knocked out through CRISPR-Cas to limit normal growth and insecticide resistance [15]. Additionally, *Chitin synthase 1* and *nicotinic acetylcholine receptor α6* were replaced in order to limit insect population growth and insecticide resistance [16][17]. *Scsa* and *Kdr* genes were also knocked out for insecticide resistance [18][19]. In the case of *Spodopteraexigua*, the ryanodine receptor was substituted to control the insect population and its resistance to various insecticides [20], and the *CYP9A186 gene*, *a-6-nicotinic acetylcholine receptor (nAchR)*, and *P-glycoprotein* gene were knocked out to make the species susceptible to emamectin benzoate (EB) [21], and to increase its susceptibility to abamectin and emamectin benzoate [22]. Genome editing of the *SfABCC2* gene of *S. frugiperda* conferred resistance to the Cry1F toxin of *B. thuringiensis* [23] and two *ABC transporters* were differentially implicated in the toxicity of the two *Bacillus thuringiensis* Cry1 toxins of the invasive crop insect *S. frugiperda* [24]. Additionally, in *S. frugiperda*, the deletion of the *ABCB1* gene increased its susceptibility to emamectin benzoate, beta-cypermethrin, and chlorantraniliprole [25].To create resistance in *Helicoverpa armigera* to *cry2Aa* and *cry2Ab*, the *HaABCA2* gene was knocked out with CRISPR-Cas [26]. The *nAChR6* gene was knocked out in *Plutellaxy lostella* to render it resistant to spinosad [27]. *Dendrolimus punctatus* had the *DpWnt-1* gene knocked out, which caused defects in appendage development and anterior segmentation [28]. *Cinnabar and White* genes were altered to change the eye pigmentation in *Bemisia tabaci* and *Nilaparvata lugens* [29][30]. Malformations in embryonic development were caused by the CRISPR-Cas-9 disruption of the *White* and *paired* genes in *Ceratitis capitata* [31].

3. CRISPR-Cas-Mediated Gene Drive in Insect Pest Management

Genome editing using CRISPR-Cas creates a gene drive that is effective enough to propagate the changed genes across generations until they are released for mating. A gene drive is a technique for the rapid distribution of altered genes throughout an insect species' entire population. Gene drives based on CRISPR-Cas may cause sterility or mortality in targeted insect species due to gene disruption, which ultimately leads to a population collapse and even elimination due to severe recessive lethal changes [32]. A species will completely disappear as a result of this over the course of 15–20 generations. By selectively harming the X chromosome, the gene drive will

alter the male sex ratio. This causes the Y chromosome to be more common in the most viable sperms, resulting in a greater proportion of male progeny and a progressive decline in the number of females [32]. Therefore, releasing insect strains with undesirable features, including lethality, infertility, a biased sex ratio, insecticidal sensitivity, etc., is a successful method for insect pest control. For instance, it should be assumed that the Bt resistance management in *H. armigera* is a sustainable method since, in this case, gene deletion would only affect the species of *H. armigera* that is resistant to Bt toxins [33].

4. CRISPR-Cas Technology in Genome Editing of Crop Plants

Technologies such as CRISPR-Cas can improve plant quality to preserve crops and help them survive specific biotic and abiotic challenges [34][35]. Maintaining healthy plants is a part of the Integrated Pest Management Program because insects are drawn to unhealthy, diseased plants. Plants can be modified using CRISPR-Cas systems so that they produce or do not produce particular enzymes that can deter insect pests from coming into contact with the plant or can attract specific insect predators to feed on the bug species that are attacking the plant [36]. The process of genome editing is quickly increasing its potential and its chances for giving insect resistance traits to crop plants. The lack of a clearly defined source of resistance in the gene pool, however, has led to less research on altering plants for pest management. The goal of several efforts in order to alleviate this bottlenecking is to collect genes from uncharacterized crop plant accessions and wild relatives. However, due to poorly understood resistance characteristic genetics in uncharacterized accessions, significant advances could not be made [37]. On the other hand, a transgenic method was used to introduce insect resistance genes into crops from more remote origins, such as the Bt genes from bacterial sources. These transgenic plant species, however, encountered severe political, moral, and social opposition because of a lack of scientific understanding [38]. In this situation, the main challenge in modern agriculture is to develop an environmentally sound breeding strategy for crops that can accomplish two breeding objectives: the production of de novo tolerance in the absence of the proper R-genes and the tracking of the dynamics of pests by destroying insecticide resistance, killing, or inducing insect sterility. Any insect will choose to lay eggs on the host plant if the feed is available for its young. Plant volatile blends are combinations of volatiles that serve as cues for insects to select hosts and oviposition sites. Insects use their highly adaptable olfactory systems to detect suitable plants to serve as hosts by detecting volatile secondary chemicals in plants. According to the research done by Beale et al., altering volatile mixtures through genome editing can kill insects on host plants while making the plants resistant to them. When plants become infested with aphids, the sesquiterpene hydrocarbon (E)-β-farnesene (Eβf) is released, which reduces the populations of other hosts' ability to eat while luring *Diaeretiella rapae*, a parasitic wasp that has been shown to dominate the aphid population in transgenic plants [39]. The genetic engineering of plant volatile blends may be a different strategy for insect management. However, care should be made to ensure that the change does not have a negative impact on the species of beneficial insects.

It is also possible to enhance the host's immunity to pests by editing important plant immunity genes, such as genes regulating the target's interactions with insect effectors and resistance genes (R-genes). Although *S*-genes

make plants vulnerable to stress, R genes evaluate a plant's susceptibility to insect pests and diseases [40]. The editing of R and S genes for the development of insect resistance in plant species is emerging as a dependable method. Due to their growth, immunity, and behaviors that have been observed in rice, insects are known to be dependent on important chemical components contained in plants [41]. Genetic engineering in plants has been demonstrated in insect pest resistance by knocking off the *S*-genes ofthe plants. Tryptamine 5-hydroxylase encoding *CYP71A1* gene deletion using CRISPR-Cas caused tryptamine's conversion to serotonin in plants, which reduced plant hopper growth. Rice was altered by Lu et al. [41] using the CRISPR-Cas9 technology to make it resistant to the striped stem borer and the brown plant hopper *(Nilaparvat alugens) (Chilo suppressalis)*. The simultaneous deletion of two endogenous phytoene dehydrogenase (PDS) genes in *P. tomentosa* Carr., *PtoPDS1,* and *PtoPDS2* using the CRISPR-Cas9 technique resulted in the effective generation of endogenous gene mutations in the *Populus* [42][43]. By enhancing their endogenous defenses, CRISPR-Cas genome-editing techniques also made it possible to increase the population's resistance to insects. The golden promise barley variety's two beta-1-3 glucanase genes were altered with CRISPR-Cas9, which reduced the amount of calli that formed in sieve tubes. Therefore, the aphid *Rhopalosip humpadi* could not access the phloem sap, adversely affecting its growth and disrupting its predilection for particular hosts [44]. On the basis of a plant's outward appearance, insects can also recognize and target certain plants. It has been found that variations in plant color can influence an insect's host preferences. This was confirmed in red-leaf tobacco made by altering the anthocyanin pathway. By changing the color of the leaf, gene editing for insect pest tolerance in plants was demonstrated. This prevented the insect from recognizing the host plant. The red color of the leaves was the result of an excess of anthocyanin coloring. The *Helicoverpa armigera* and *Spodoptera litura* were discouraged by this color change [45]. This study demonstrated that CRISPR-based editing for pest management, where the insects are unable to recognize the host plant, may be resolved by altering the anthocyanin pathway. According to Li et al., the *GmCDPK38* mutant with the Hap3 deletion in soybeans showed significant resistance to common cutworms [46]. Additionally, the *GmUGT* gene deletions of 1bp and 33bp were made in soybeans to improve their resistance to *S. litura* and *H. armigera* [125.

5. Utilization of Crop Wild Relatives in Insect Resistance by CRISPR-Cas Technology

The insertion of foreign genes into plants is one of the key regulatory problems associated with transgenics that can be overcome with gene editing. The cultivated crops' forebears and close relatives, known as crop wild relatives (CWRs), are robust to biotic and abiotic stress but have low yields. After domesticating wild species and breeding plants, however, the cultivable germplasms and crops had large yields and could meet other human needs, but they could not withstand insect assault. Using CRISPR-Cas9 genome editing, we can effectively delete or modify the genes that cause insect susceptibility, or we can introduce unique features from CWRs to the cultivated species to create new cultivars that are insect-resistant [40].

Two steps can be taken to implement this. First, the de novo domestication of crops with insect-resistant wild cousins can be implemented. Gene-editing techniques can be used to alter the desired agronomic traits that are

caused by genes. There is evidence that the wild tomato *Solanum pimpinellifolium* is resistant to spider mites and other arthropod insect pests [47]. The multiplex CRISPR-Cas editing of six different genes in *S. pimpinellifolium* resulted in the production of a high-yielding tomato with insect and pest tolerance in a single generation [48]. This method, based on a plant's properties and molecular pathways, can be carefully applied to other CWRs. The de novo domestication of CWRs may, therefore, be a ground-breaking method for the development of crops with improved characteristics.

Second, using genes found in CWRs that are insect-resistant, the genome can alter the cultivated crops. By altering the genomes of cultivated crops to have the insect tolerance of wild species, the first study of variation in the sequences of individual insect-sensitive genes across vulnerable cultivated germplasms and resistant wild cousins using multiomics techniques may be accomplished [40]. The resistance genes can be successfully used for gene editing after being validated against related insects. This presents chances for resistance development in the gene pool of cultivated crops to control insect pests [49]. It has been suggested that commercially valuable crops can produce insect-resistant phenotypes utilizing CRISPR-Cas gene-editing-based sequence variation by using either over-expression or silencing techniques. However, this has not yet been demonstrated.

6. Limitations and Future Perspectives

Like other biotechnological techniques, genome-editing techniques specifically modify a gene through cellular and in vitro mechanisms. In the course of evolution, genome modification is beyond our control. However, when the genome is altered experimentally, it may primarily be for the benefit of humans. Its application to crop improvement should, likewise, be limited to breeding objectives that are both absolutely important and challenging to achieve within the confines of the current heterogeneity. Like any modern technology, there are still a number of legal questions about gene editing that the scientific community needs to address. In order to fully utilize this innovation potential for the advancement of global agriculture and the eradication of neophobia in society, it is essential to adopt a realistic viewpoint that is supported by the legislative bodies that uphold scientific norms. The CRISPR-Cas-based deliberate dissemination of genetic components into wild species of insects that alter the population's sex ratio or contribute to lethal mutations is a precise and environmentally sustainable method of battling pests. However, the emergence of insect resistance in response to a CRISPR-mediated gene drive could be a serious and ongoing problem at both the experimental and theoretical scales [50][51]. Multiplex gene editing, however, can overcome resistance [52]. Therefore, it is crucial to address insect resistance issues in order to reach an agreement on the ethics and science in favor of this technology.

Additionally, because engineered insect pests have the power to change the entire population or environment, the introduction of CRISPR-Cas-edited insects bearing gene drives into the ecosystem is linked to a number of biosafety concerns. Prior to their release, stringent risk assessments of non-target outcomes are also required. Unexpected post-release impacts on beneficial insects can have a negative influence on food chains and can alter the composition of communities [53]. Additionally, the disease can become worse due to the possibility of gene transfers between the target organisms and their non-target relatives. If the risks are appropriately managed in light of unanticipated environmental repercussions, gene-driven technology could prove effective in the targeted

extermination of insect pests, insect vectors for viruses, and alien insect species. Utilizing the terminator genes that permit the programmed life of modified insects and using tagged insects to monitor gene flow seem to be crucial steps in the safe use of gene drives in the context of risk management. Additionally, another option for the management of invasive pests is the use of robotic equipment and artificial intelligence to physically eliminate individual ests [54]. Robotics may not be as effective, though, when dealing with tiny insects, uneven terrain, and hidden eggs. Insect resistance to invasive pests has been successfully achieved via the CRISPR-Cas-based deletion of vulnerable genes. The fundamental problem associated with S gene deletions, which also add to the associated fitness penalty, is pleiotropic effects in the plant. However, it is possible to ensure insect resistance without affecting plant performance by altering the binding effector factor rather than the gene itself [55]. The CRISPR-Cas approach of creating insect resistance in crop species will, therefore, develop as a successful tool for supplying genetic traits in farmed varieties in a shorter amount of time. It is true that CRISPR-Cas-enabled genome-editing technology is a fast-evolving technique and, thus, the scope of its application in agriculture is expanding [56][57]. However, a thorough understanding of the gene and genome activities of the target species is required prior to its full adoption in the generation of insect pest resistance and plant protection. As Bt technology developed from recombinant DNA technology has revolutionized the management of insects in many economically important crops, including cotton, maize, soybean, and brinjal [58], the ease and multiplexing manner of CRISPR technology could also replace the currently used recombinant DNA technology for the insertion of R gene(s) in a faster manner.

7. Concluding Remarks

Despite being relatively young, the genome-editing techniques centered on CRISPR-Cas have already changed insects' functional genomics. With CRISPR-Cas, we can now quickly alter, remove, and add DNA almost anywhere we want in any crop or insect species to make plants immune to insect pests. Therefore, this technology needs to be enhanced in order to produce crop plants that are resistant to insect pests. Sincere and proactive measures in this regard are required in addition to protecting our crops from the significant output losses brought on by insect pest infestation. However, the fate of genome-modified products with CRISPR in crop enhancement projects will ultimately be determined by the worldwide legislative authorities. For novel crop cultivars, either product- or process-based regulation is followed by regulatory systems. The scope of the regulations implemented on CRISPR-based crops will have an effect on the cost of their production and will also dictate the pace at which they will reach commercial industries. The set of product-based legislation for crops created using CRISPR-Cas genome editing could be classified similarly to products created by classical mutagenesis, eliminating them from the restrictions imposed on products made via genetic modification. This would surely have an impact on the hopeful public perception of this technology and would help the majority of nations to adopt it. Many countries have given the green pass to CRISPR-edited products that carry no transgene(s). It is expected that CRISPR-Castechnology will lead to a new green revolution in agriculture if the timely deregulation of the adoption of CRISPR products and technological know-how is shared by open scientific practice.

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