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Review

CRISPR-Cas Genome Editing for Insect Pest Stress Management in Crop Plants

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Abstract: 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 both 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. This new method, however, has the ability to alter an insect's DNA in order 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. This paper reviewed and critically discussed the use of CRISPR-Cas genome-editing technology in long-term insect pest management. The emphasis of this review was on the prospective uses of the CRISPR-Cas system for insect stress management in crop production through the creation of genome-edited crop plants or insects. The potential and the difficulties of using CRISPR-Cas technology to reduce pest stress in crop plants were critically examined and discussed.

Keywords: CRISPR-Cas technology; pest management; plant stress resistance; insect resistance

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1. Introduction

Insects are the primary biotic stressors that pose a severe threat to crop loss globally due to their direct feeding behavior on crops and their ability to spread plant diseases [1]. It is estimated that one-fourth of crops is destroyed annually by insects [2]. New techniques for the management of phytophagous insect pests would contribute to the protection of crops and, thus, raise the yield of crops. The major insects that cause significant decreases in crop production are sap-sucking and chewing pests [3]. Recent advances in the molecular foundation of the interactions between insects, plants, and biotechnological techniques, such as genome editing, offer solutions to these problems [4]. Recently, it was discovered that designed nucleases have an enormous potential for genome editing in both plants and insects [5,6]. The application of genome-editing methods has dramatically grown over time. At the moment, CRISPR-Cas is the most widely used method of genome editing [7,8]. Genome editing using the CRISPR-Cas system has proven to be successful in creating a variety of agronomic traits, including a long-lasting resistance to insect pests [5].

Genome editing, or gene editing, is a method of genetic manipulation that involves inserting, deleting, labeling, changing, or substituting DNA into the genome of a living being

in order to generate a desired attribute [9]. The four main categories of sequence-specific nucleases in gene editing to date are mega nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas) [10,11]. The newest and most sophisticated genome-editing technology is that of the CRISPR-Cas RNA-guided nucleases that were modified from bacterial innate immune mechanisms and derived from Type II CRISPR-Cas9 mechanisms [12]. In order to secure food supplies for the world's expanding population and to fulfill Sustainable Development Goal 2 (zero hunger), contemporary agriculture practices using stress-resistant crops and genetically modified crops are given emphasis. The use of synthetic chemical insecticides to control insect pests during crop production is expensive and hazardous to humans and to the environment. Additionally, it has a bad impact on biodiversity and unintended insects. We have already observed the application of numerous insect resistance genes in genetically modified crops, such as *Bacillus thuringiensis* (Bt)-insecticidal crystal proteins (ICPs), which has had a significant influence on productivity and sustainability [13]. CRISPR-Cas gene editing is emerging as an effective tool for the development of insect-resistant plants to promote sustainable agriculture. The process of developing resistance against insects with this promising tool includes changing the effect or target interactions, knocking out host-susceptible genes, uncoupling the antagonistic action of defense hormones, and so on [14]. CRISPR-Cas gene editing has been effectively used over the past ten years to create insect-resistant plants and to modify a number of insects. This approach has demonstrated great promise for increasing crop output through the sustainable management of insect pests. Insect pest control in agriculture could benefit from the development of genome-edited agricultural plants and insects. This review aimed to summarize the recent developments in the use of CRISPR-Cas in the generation of insect-resistant plants as well as in the application of this revolutionary technology to the modification of the genomes of phytophagous insects. We also discussed the difficulties and the potential use of the CRISPR-Cas toolbox for the sustainable management of insect pests.

2. CRISPR-Cas System and Its Mechanism of Genome Editing

CRISPR is the acronym for clustered regularly interspaced short palindromic repeats, and Cas is the CRISPR-associated protein. It is a natural defense mechanism found in the genomes of prokaryotic organisms such as bacteria (45%) and archaea (84%) [15]. It is a gene-editing technology that targets a specific DNA section, makes a precise cut at the target site, and makes the gene nonfunctional or replaces one version of the gene with another. It was invented by Jennifer Doudna, Emmanuelle Charpentier, and Fang Zhang in 2012, and the former two scientists were awarded the Nobel Prize in Chemistry in 2020 [16]. This is the most current technology for gene editing. This CRISPR-Cas9 editing technology is categorized into type I, II, and III, and, in the type II systems, the Cas9 nuclease requires DNA that matches a single RNA guide (sgRNA) [17]. The two primary components of the CRISPR-Cas system are the guide RNA and the Cas protein. The Cas9 protein, a nuclease enzyme that is commonly referred to as molecular scissors, is responsible for cutting the DNA. Guide-RNAs are molecules that direct Cas9 to the chosen spot in the genome, where it will remove the existing sequence and replace it with the new one [18]. It has recently become a very effective, fast, and speedy genome-editing tool [19,20]. This CRISPR-Cas technology can now be used to edit multiple genes at a time or even a single base, also known as epigenetic editing.

Several CRISPR-Cas applications have been shown to change the DNA sequences of insect or plant genomes [21–23]. *Streptococcus pyogenes* (Sp) is the source of the Cas9 protein that is currently most frequently employed [24]. In this procedure, a Cas9-protein-associated single-guide RNA (sgRNA) cleaves a particular target DNA region next to a protospacer adjacent motif (PAM), triggering the cellular DNA's repair system to create a double-strand break (DSB). Without the homologous repair template, error-prone non-homologous end-joining (NHEJ) pathways are activated, resulting in spontaneous

insertions/deletions or even replacements at the DSB site, which typically disrupt gene function. On the other hand, error-free homology-directed repair (HDR) mechanisms are activated, leading to mutations that undertake precise gene alterations, including knock-ins, knockouts, or mutations, if donor DNA templates are available that are similar to the sequence surrounding the DSB site [25]. The NHEJ and HDR mechanisms have currently been successfully co-opted for genome editing in a variety of insects and plants [14,22,26]. After successful genome modification, the CRISPR-Cas construct is delivered into plant cells through *Agrobacterium*-mediated or particle-bombardment-mediated transformation methods and into the embryos of insects through microinjection, transfusion, or electroporation-mediated transformation methods for the regeneration of transgenic species with desired characteristics [14,22,27]. The workflow of CRISPR-Cas genome editing in plants and insects is briefly illustrated in Figure 1.

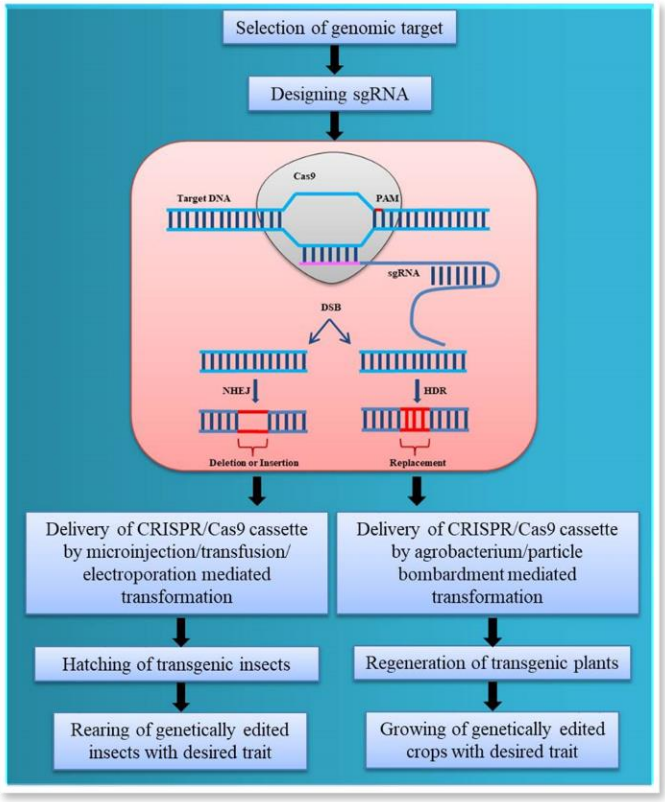

Figure 1. Workflow for CRISPR-Cas9-based genome editing in insects and plants for insect resistance.

3. CRISPR-Cas Genome Editing in Agriculture for the Management of Insect Pests

Biotechnology performs a crucial role in the control of insect pests to protect crops and improves yields in areas ranging from breeding for pest resistance to the genetically modified introgression of new genes [28]. 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 [22,29]. 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.

The agricultural biotechnology sector has been threatened with the problem of insect resistance to the Bt trait; thus, biotech companies are searching for a novel, economically viable, and environmentally responsible solution to the problem. In the biotech sector, CRISPR-Cas9 gene editing has emerged as the leading method for the control of insect pests [30]. In order to successfully alter a gene's function, genome-editing technology actually leverages the cell's own internal processes. Genome editing makes sure that the DNA sequence of a specific target genome is altered via the addition, deletion, and/or substitution of DNA bases [31].

3.1. 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 witha two-step technique involving the alteration of target DNA in insects and their eventual release into nature [32]. 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 [33].

The *BmBLOS2* gene was the focus of another reported successful application of this method in silkworms [34], which was followed by several successful applications. In a case study by Garczynski et al. [35], 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. [36] 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 [37]. 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 [38]. 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 [39]. 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 [40]. Similar 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 [32]. Implementing such technology would be a smart move to keep insects away from plants and to 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 [41]. 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* [42], *Spodoptera frugiperda* [21], and *Plutellaxy lostella* [43], which resulted 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 [44]. Additionally, *Chitin synthase 1* and *nicotinic acetylcholine receptor α6* were replaced in order to limit insect population growth and insecticide resistance [45,46]. *Scsa* and *Kdr* genes were also knocked out for insecticide resistance [47,48]. In the case of *Spodopteraexigua*, the ryanodine receptor was substituted to control the insect population and its resistance to various insecticides [49], 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) [50], and to increase its susceptibility to abamectin and emamectin benzoate [51]. Genome editing of the *SfABCC2* gene of *S. frugiperda* conferred resistance to the Cry1F toxin of *B. thuringiensis* [52] and two *ABC transporters* were differentially implicated in the toxicity of the two *Bacillus thuringiensis* Cry1 toxins of the invasive crop insect *S. frugiperda* [53]. Additionally, in *S. frugiperda*, the deletion of the *ABCB1* gene increased its susceptibility to emamectin benzoate, beta-cypermethrin, and chlorantraniliprole [54].To create resistance in *Helicoverpa armigera* to *cry2Aa* and *cry2Ab*, the *HaABCA2* gene was knocked out with CRISPR-Cas [55]. The *nAChR6* gene was knocked out in *Plutellaxy lostella* to render it resistant to spinosad [56]. *Dendrolimus punctatus* had the *DpWnt-1* gene knocked out, which caused defects in appendage development and anterior segmentation [57]. *Cinnabar and White* genes were altered to change the eye pigmentation in *Bemisia tabaci* and *Nilaparvata lugens* [58,59]. Malformations in embryonic development were caused by the CRISPR-Cas-9 disruption of the *White* and *paired* genes in *Ceratitis capitata* [60] (Table 1).

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

Name of Insect	Target Gene	Editing	Outcome
Drosophila melanogaster	*Yellow*	Knockout, Knock-In	Generated designer flies [33]
	Rosy and *DSH3PX1*	Knockout, Knock-In	Executed efficient and complex genomic manipulations [33]
	LUBEL	Knockout	Reduced survival rate [44]
	Chitin synthase 1	Substitution	Controlled insect population and resistance to various insecticides [45]
	Nicotinic acetylcholine receptor α6	Substitution	Controlled insect population and resistance to various insecticides [46]
	Scsa	Knockout	Reduced normal growth [47]
	Kdr	Knockout	Reduce insecticide resistance [48]
	Ast, *Eh*, *capa*, *Ccap*, *Crz*, *npf*, *Mip*, *mir-219*, *mir-315*, and *white*	Knockout	Targeted mutagenesis [61]
	Yellow	Knockout	Effectively targeted mutagenesis [62]
	Yellow and white	Knockout	Highly efficient and varied genome-editing efficiencies [63]
	Yellow and rosy	Knockout, Knock-In	First report using the CRISPR/Cas9 system to mediate efficient genome engineering in Drosophila [64]
	Alk	Knockout	Establishing mutations [65]
	TpnC	Knockout	Confirmed that the myofibril assembly wasrelated to TpnC gene [66]
	Wntless	Knockout	Amplified the cleavage Efficiency [67]
	Yellow, *white*, *and tan*	Knock-In	Attaining single or multiple allelic substitutions [68]
	Act5C, *lig4*, and *mus308*	Knockout, Knock-In	Genome editing in *Drosophila* S2 cells [69]
	Mod(mdg4)	Knockout	Validation of a functional gene involved in trans-splicing that influenced the development in flies [70]
	Fdl	Knockout	Analyzing or manipulating protein glycosylation pathways [71]
	Chameau, *CG4221*, and *CG5961 mRNA*	Knock-In	A problem associated with "ends-in" recombination was resolved [72]
	Clamp	Knockout	The expression of a sex-specific gene was regulated [73]
	Dα6	Knock-In	Resistance to spinosad [46]
	Yellow	Knock-In	Heterozygous recessive mutation was converted to homozygous loss of function [74]
	Ebony, *yellow*, *wg*, *wls*, *Lis1*, *and Se*	Knockout, Knock-In	Non-transgenic individuals exhibited less efficient knock-in than transgenic individuals did [75]
	Ebony, *yellow*, and *white*	Knockout, Knock-In	Enhanced efficiency of gene targeting [76]

Table 1. *Cont.*

Name of Insect	Target Gene	Editing	Outcome
	Ebony, *yellow*, and *vermilion*	Knockout, Knock-In	Donor template and sgRNA plasmids were injected into Cas9 transgenic embryos in Drosophila [77]
	White and *piwi*	Knockout, Knock-In	Prevented off-target effects during the generation of indel mutants [77]
	Salm	Knock-In	Flexible modification of fly genome [78]
	Yellow, *notch*, *bam*, *nos*, *ms(3)k81*, *and cid*	Knockout	Temporally and spatially inhibited gene expression [79]
	Ms(3)k81, *white*, and *yellow*	Knockout, Knock-In	CRISPR-mediated genome editing was shown in Drosophila [80]
	EGFP and *mRFP*	Knockout	Induction of mutations [81]
	Ebony, *yellow*, *wingless*, and *wnt*	Knockout, Knock-In	Different patterns of expression [82]
Drosophila subobscura	*Yellow* and *white*	Knockout	Gene functions were analyzed in a non-model Drosophila species [83]
Drosophila suzukii	*White (w)* and *sex lethal (Sxl)*	Knockout	Controlled insect population and resistance to various insecticides [27]
	DsRed (red fluorescence protein)	Knock-In	Studied sexing and monitoring [84]
	White (w-)	Knockout	Absence of mating and copulation failure [85]
Spodoptera exigua	*Seα6*	Knockout	Resistance to insecticides [23]
	Ryanodine receptor	Substitution	Controlled insect population and resistance to various insecticides [49]
	CYP9A186 gene	Knockout	Susceptibility to emamectin benzoate (EB) [50]
	P-glycoprotein gene	Knockout	Susceptibility to abamectin and emamectin benzoate [51]
	a-6-nicotinic acetylcholine receptor (nAchR)	Knockout	Resistance to spinosyn insecticides [23,65,66]
Spodoptera littoralis	*Orco*	Knockout	Reduced survival rate [32]
Spodoptera litura	*Abdominal-A (slabd-A)*	Knockout	Defected body segmentation and pigmentation [42]
	SlitPBP3	Knockout	Destroyed pest insect mating [86]
	SlitBLOS2	Knockout	Coloration of the integuments, a marker gene for functional studies and pest control strategies [87]
Spodoptera frugiperda	*Sfabd-A*	Indel	Defected body segmentation [21]
	BLOS2E93 TO	Knockout	Developed mutants [88]
	SfABCC2	Edit	Resistance to Cry1F toxin of *Bacillus thuringiensis* [52]
	ABC transporters		Toxicity of two *Bacillus thuringiensis* Cry1 toxins to the pest [53]
	ABCB1	Knockout	Susceptibility to emamectinbenzoate, beta-cypermethrin and chlorantraniliprole [54]

Table 1. *Cont.*

Name of Insect	Target Gene	Editing	Outcome
Helicoverpa armigera	*nAchR*	Knockout	Resistance to insecticides [23]
	a-6-nicotinic acetylcholine receptor (nAchR)	Knockout	Resistance to spinosyn [23,68,69]
	HaCad	Knockout	Resistance to Bttoxin Cry1Ac [37]
	Cluster of nine P450 genes	Knockout	Identification of the key players in the insecticide metabolism [39]
	CYP6AE	Knockout	Regulation of detoxification enzymes [39]
	OR16	Knockout	Destroyed pest insect mating [41]
	Tetraspanin	Knockout	Resistance to Bt toxin cry1Ac [89]
	HaABCA2	Knockout	Resistance to cry2Aa and cry2Ab [55]
	White, *ok*, *brown*, and *scarlet*	Knockout	Differential distribution of eye pigments that are helpful in elucidation of biosynthetic pathway [90]
	NPC1b	Knockout	NPC1b wasvital for growth and dietary cholesterol uptake [91]
Helicoverpapunctigera	*HaABCA2*	Deletion	Resistance to both cry2Aa and cry2Ab [55]
Plutellaxy lostella	*Abdominal-A*	Knockout	Defected body segmentation [43]
	Pxabd-A	Knockout	Providing novel ideas for pest management [43].
	PxCHS1	Knockout	Described the resistance management strategies of major agricultural pests [45]
	PxABCC2 *PxABCC3*	Knockout	Resistance to cry1 Ac protoxin [92]
	nAChRα6	Knockout	Resistance to spinosad [56]
	LW	Knockout	Weaker phototaxis and reduced locomotion [93]
	Pxdsx	Knockout	Altered expression of sex-biased genes [94]
Dendrolimus punctatus	*DpWnt-1*	Knockout	Defected anterior segmentation and appendage development [57]
Bemisia tabaci	*White*	Edit	Altered eye pigmentation [58]
Nilaparvata lugens	*Cinnabar* and *white*	Edit	Altered eye pigmentation [59]
	Nl-cn and *Nl-w*	Knockout	Paved a path for gene-function interrogation [59]
Ceratitis capitata	*White eye (we)* and *paired gene (Ccprd)*	Knockout	Embryonic developmental malformations [60]
	eGFP_gRNA2, *eGFP_gRNA2, 1 mM Scr7*, and *eGFP_gRNA2b–Cas9 complexes with ssODN_BFP donor templates*	Knock-In	Conversion of eGFP to BFP [95]
Bactrocera dorsalis	*White and transformer*	Knockout	Various phenotypic effects [96]
Anastrepha ludens	*Astra-2*	Knockout	The mutation caused sterility [97]

Table 1. *Cont.*

Name of Insect	Target Gene	Editing	Outcome
Locust amigratoria	*Orco*	Knockout	Generated lossoffunction for the management of insect pests [36]
	OfAgo1	Knockout	Cuticle disruption [98]
Cydia pomonella	*CpomOR1*	Knockout, Knock-In	Affected egg production and viability [35]
Tetranychus urticae	*Phytoene desaturase*	Knockout	Functional studies [99]
	PSST	Knockout	Pyridaben resistance [100]
Leptinotarsa decemlineata	*Vestigial gene (vest)*	Knockout	Functionally characterized vest gene and mutagenesis [101]
Euschistus heros	*Abnormal wing disc (awd)*, *tyrosine hydroxylase (th)*, and *yellow (yel)*	Knockdown and knockout	Managing insect pests [102]
Diaphorina Citri, *Homalodisca vitripennis*, and *Bemisia argentifolii*	*Thioredoxin* and *vermillion*	Knockout	Protected food crops from different pathogens and insect vectors [103]
Diaphorina citri	*ACP-TRX-2*	Knockout	Innovative breakthrough in gene editing [104]
Mythimna separata	*NPC1b*	Knockout	Hampered nutrient absorption [105]
Hyphantriacunea	*Hcdsx*	Knockout	Sex-specific sterility [106]
Agrotis ipsilon	*AiTH*	Knockout	Narrowing in the eggshell [107]
Danaus plexippus	*Clk*	Knockout	Defined the role of the clk gene in the control of migration behavior [108]
Bombyx mori	*BmBLOS2*	Knockout	Generated designer flies [34]
	BmOrco	Knockout	Impaired olfactory sensitivity [109]
Tribolium castaneum	*EGFP*	Knockout, Knock-In	Controlled insect pests and created resistance to insecticides [110]
	Tribolium E-cadherin	Knockout	Knockout study [110]
Gryllus bimaculatus	*Dop1*	Knockout	Destroyed appetitive reinforcement [111]
Rhopalosiphum padi	*ß-1-3glucanase*	Knockout	Reduced callose deposition in maize [112]
Ostrinia furnacalis	*ABCC2*	Knockout	Resistance to *Btcry1Fa* toxin [113,114]

3.2. 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 [115]. 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 [115]. 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 [89].

3.3. 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 [6,113]. 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 [116]. 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 [117]. 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 [118]. 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 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 [119]. 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 [120]. The editing of R and S genes for the development of insect resistance in plant species is emerging as a dependable method. Due totheir growth, immunity, and behaviors that have been observed in rice, insects are known to be dependent on important chemical components contained in plants [22]. 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 alteredby Lu et al. [22] 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* [121,122]. 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 withCRISPR-Cas9, which reduced the amount of callus that formed in sieve tubes. Therefore, the aphid *Rhopalosip humpadi* could not access the phloem sap, which adversely affected its growth as well as disrupted its predilection for particular hosts [63]. 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 [123]. 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 [124]. Additionally, the *GmUGT* gene deletions of 1bp and 33bp were made in soybeans to improve their resistance to *S. litura* and *H. armigera* [125] (Table 2).

Table 2. CRISPR-Cas genome editing in crops for insect pest management.

Name of Crops	Target Gene	Editing	Outcome
Barley	*Beta-1-3 glucanase*	Alteration	Resistance to aphid infestation [112]
Paulownia tomentosa	*PtoPDS1* and *PtoPDS2*	Deletion	Enhanced endogenous defenses and increased resistance to insects [121,122].
Tobacco	Anthocyanin pathway	Alteration	Discouraged insect attack [123]
Soybean	*GmCDPK38*	Knockout	Resistance to common cutworm [124]
	GmUGT	1bp and 33bp deletion	Enhanced resistance to *Helicoverpaarmigera* and *Spodopteralitura* [125]
	Cry 8 like		Resistance to Coleopteran-*Holtrichiapanallele* [126]
Solanum pimpinelli-folium	Six different genes	Editing	Resistance to insect pests [127].

Table 2. *Cont.*

Name of Crops	Target Gene	Editing	Outcome
Rice	*OsCYP71A1*	Deletion	Resistance to the striped stem borer and the brown plant hopper [22].
	Cry2A	Transgene	Resistance to leaf folder [128]
	Mannose-specific GNA	Transgene	Resistance toBPH *(Nilaparvata lugens)* and hemipteran pest [127]
	Cry1AC + ASAL	Transgene	Resistance to yellow stem borer, leaf folder, and BPH [129]
	Cry1Ab + vip3A	Transgene	Resistance toAsian stem borer and rice leaf folder [130]
	Cry2AX1	Transgene	Resistance to rice leaf folder [131]
	Cry2Aa + cry1Ca	Transgene	Resistance to *Chilo suppressalis* [132]
	Cry2AX1 (cry2Aa + cry2Ac)	Transgene	Resistance to Lepidopteran pest [133]
Cotton	*Cry2AX1*	Transgene	Resistance to *H. armigera* [134]
	Cry2Ab, *cry1F*, and *cry1AC*	Transgene	Resistance to Lepidopteran pest *H. armigera*, and *S. litura* [135]
	Cry1AC and *cry2Ab*	Transgene	Resistance to *S. litura* [136]
	Cry2AX	Transgene	Resistance to *H. armigera* [137]
	Cry1Aa	Transgene	Resistance to *Anthamous grandis* [138]
	Cry1Ab	Transgene	Resistance to *Heliothis* [139]
	Cry1Ab+ NptII	Transgene	Resistance to *H. armigera* [140]
	Vip3AcAaa (vip3Aa1 + vip3Ac1)	Transgene	Resistance to Lepidopteran [141]
	Vip3A + cry1Ab	Transgene	Resistance to *Heliothis. zea and H. virescens* [142]
	Insect gut-binding *lectin* from *Sclerotium rolfsii*	Transgene	Resistance to chewing and sucking pest [3]
	Cry51Aa2	Transgene	Resistance to *Lygus* species [143]
	Cry1Be + cry1Fa	Transgene	Resistance to *S. litura* and *O. nubialis* [144]
Maize	*Cry1Ab/cry2Aj*	Transgene	Resistance to *S. exigua* and *Harmonia axyridis* [145]
Mustard (*Brassica juncea*)	*Lectin protease protein (lentil lectin-LL CPPI)*	Transgene	Resistance toaphid [146]
	Colocasia esculenta tuber agglutinin (CEA) + GNA	Transgene	Resistance tomustard aphid *(Lipaphis erysimi)* [147]

Table 2. *Cont.*

Name of Crops	Target Gene	Editing	Outcome
Sugarcane	*Cry2Aa+ cry1Ca Cry1Ab + cry1Ac*	Transgene	Resistance to shoot borer [148]
	Vip3A	Transgene	Resistance to sugarcane stem borer *(Chilo infuscatellus)* [149]
Potato	*Hv1a/GNA*	Transgene	Resistance to peach potato aphids and grain aphids [150]
	Galanthus nivalis agglutinin (GNA)	Transgene	Resistance to aphids [151]
Wheat	*Pinelliapedatisecta agglutinin (PPA)*	Transgene	Resistance to aphids [152]
Cowpea	*Arcl* on *APA* locus of *Phaeselous vulgaris*	Transgene	Resistance to bruchids [153]
	Vip3Ba1	Transgene	Resistance to legume pod borer *(Maruca vitrata)* [154]
Pigeon pea	*Cry2Aa*	Transgene	Resistance to *H. armigera* [155]
	Cry2Aa	Transgene	Resistan topod borer—*H. armigera* [156]
	Cry1AC, cry2Aa	Transgene	Resistance to *H. armigera* [157]
Chickpea	*CryIIAa*	Transgene	Resistance to pod borer [158]
Tomato	*Cry1Ac*	Transgene	Resistance to *Tuta Absoluta*—tomato leaf miner [159]
	Remusatia vivipara (rvl 1) and Sclerotium-rolfsii(srl 1)	Transgene	Resistance to root knot nematode *(Meloidogyne incognita)* [160]
	Cry1Ab	Transgene	Resistance to *T. absoluta* [161]
Castor	*Cry1AC*	Transgene	Resistance to Lepidoteran—*Achaea Janata* and *S. litura* [162]
Sweet potato	*Cry1Aa*	Transgene	Resistance to *S. litura* [163]

3.4. 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 [120].

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 [164]. 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 [165]. 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 [120]. 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 [166]. 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.

4. 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's 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 [167,168]. Multiplex gene editing, however, can overcome resistance [169]. 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 [118]. 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, andalien 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 biosafe 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 pests [170]. 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 [171]. 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 [172,173]. 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 [174], 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.

5. 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 the CRISPR-Cas technology 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 an open scientific practice.

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