Genome Editing for Resistance to Insect Pests: An Emerging Tool for Crop Improvement

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ABSTRACT: Plants are challenged incessantly by several biotic and abiotic stresses during their entire growth period. As with other biotic stress factors, insect pests have also posed serious concerns related to yield losses due to which agricultural productivity is at stake. In plants, trait modification for crop improvement was initiated with breeding approaches followed by genetic engineering. However, stringent regulatory policies for risk assessment and lack of social acceptance for genetically modified crops worldwide have incited researchers toward alternate strategies. Genome engineering or genome editing has emerged as a new breeding technique with the ability to edit the genomes of plants, animals, microbes, and human beings. Several gene editing strategies are being executed with continuous emergence of variants. The scientific community has unraveled the utility of various editing tools from endonucleases to CRISPR/Cas in several aspects related to plant growth, development, and mitigation of stresses. The categorical focus on the development of tools and techniques including designing of binary vectors to facilitate ease in genome engineering are being pursued. Through this Review, we embark upon the conglomeration of various genome editing strategies that can be and are being used to design the genomes of plants, animals, microbes, and human beings. Several gene editing strategies are being executed with continuous emergence of variants. The scientific community has unraveled the utility of various editing tools from endonucleases to CRISPR/Cas in several aspects related to plant growth, development, and mitigation of stresses. The categorical focus on the development of tools and techniques including designing of binary vectors to facilitate ease in genome engineering are being pursued. Through this Review, we embark upon the conglomeration of various genome editing strategies that can be and are being used to design insect pest resistance in plants. Case studies and novel crop-based approaches that reiterate the successful use of these tools in insects as well as in plants are highlighted. Further, the Review also provides implications for the requirement of a specific regulatory framework and risk assessment of the edited crops. Genome editing toward insect pest management is here to stay, provided uncompromising efforts are made toward the identification of amiable target genes.

1. INTRODUCTION

Improvement in crop productivity is an important concern to be addressed in the given scenario of accelerating population and climatic fluctuations. Agriculture in the 21st century is bogged down by incessant challenges from several biotic and abiotic stresses, insect pests being one of the foremost. Insects are responsible for the reduction in potential yields resulting in stagnated productivity. Most of the economically important crops suffer a wide range of yield losses despite operating control measures. Farmers have however adopted chemical pesticides for the control of insect pests, the widespread use of which can be deleterious to mankind and the environment. These problems have motivated researchers globally to develop novel and environmentally friendly insect control strategies. Hence, the prime focus of modern agriculture has been to achieve enhanced yields with the existing land and resources for global food security and agricultural sustainability.1

With the advent of genetic engineering and plant biotechnology, pest management has reached new horizons. Genetic modification of a range of crop plants with several insect resistance genes (*Bacillus thuringiensis* Bt-ICPs) and continued implementation of Bt gene introgressed-crops have shown trust worthy impact on productivity and sustainability of the technology. However, development of resistance in the insect pests to the Cry toxins has been a major apprehension.2 Mutation(s) in the genes encoding for receptor molecules distracts interaction between the insect and the toxin, resulting in the emergence of the resistant insect-pest population. To deliver extended efficacy against insect pests and avert resistance, chimeric toxins developed by domain swapping of toxins and usage of combinatorial ICPs are also being exploited.2 Alternatively, other strategies like RNA interference (RNAi) aimed at silencing of selected genes involved in insect feeding resistance genes (*Bacillus thuringiensis* Bt-ICPs) and continued implementation of Bt gene introgressed-crops have shown trust worthy impact on productivity and sustainability of the technology. However, development of resistance in the insect pests to the Cry toxins has been a major apprehension.2 Mutation(s) in the genes encoding for receptor molecules distracts interaction between the insect and the toxin, resulting in the emergence of the resistant insect-pest population. To deliver extended efficacy against insect pests and avert resistance, chimeric toxins developed by domain swapping of toxins and usage of combinatorial ICPs are also being exploited.2

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and development have also been devised and assessed for incorporation in crop improvement programs.

Cross-breeding of susceptible and resistant germplasm for mitigating insect pests would be another sustainable approach. However, lack of resistant gene pools along with incompatibility in many crop species has minimized the scope of breeding for insect resistance. In the recent past, advent of genomics and sequencing of plant genomes have opened new avenues for their exploitation in crop improvement programs. Digging deeper into the genomes and other levels of cellular functions have revealed the importance of sequence variation in the ability of plants to exhibit particular traits. This led to the inception of the next level of biotechnological intervention called “Genome engineering” or “Genome editing”. This fascinating tool has emerged as an imperative contribution that allows modifications in the genome by adding/editing/deleting particular stretch of DNA sequences, thereby providing opportunities for utility in plants, animals, and humans. In the present scenario of constricted agricultural fields and increased load of insect pests on crop plants, genome editing will serve as a potential tool to combat insect pests, and hence, it has been described as the “new breeding technology”.

Considering the success of genome editing in plants and aptness to combat biotic stress factors, this Review focuses on key strategies available for precise editing for insect management in plants. The novelty of the Review lies in the presentation of pioneering studies that focused on the utilization of genome editing for insect management not only in plants to deter insects but also in target pests for reduced ability to attack plants. Further, the utility of crop wild relatives, which are endowed with several resistance traits possessing sequence polymorphisms and acting as a reference for editing cultivated counterparts will also be discussed. Emerging innovative advancements that play vital roles in editing as well as plant transformation techniques for successful utilization of the technology will also be showcased in the Review. Knock down of key regulators through genome editing would serve as a futuristic approach to combat insect pests.

2. GENOME EDITING TOOLS AND STRATEGIES

Genome editing or gene editing is one of the most imperative tools in modern biology and comprises technologies that enable scientists to change/edit an organism’s DNA. The last two decades have witnessed the rise in the concept of genome editing with interesting and constant modifications for the betterment of technology. These technologies rely on nucleases to cut specific genomic target sequences. Double-stranded breaks (DSBs) promote DNA repair through a nonhomologous end-joining (NHEJ) mechanism, resulting in the introduction of small insertion and deletion (indel) at specific sites. When DSB followed by indel occurs in a coding region causing a frame shift, it leads to gene inactivation and creation of a mutation. This process can be used to generate direct modifications in plants and is a potential tool for targeted genome editing. A number of engineered nucleases such as homing endonucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), oligonucleotide-directed mutagenesis (ODM), and CRISPR (clustered regularly interspaced short palindromic repeats) nucleases have been identified that make targeted breaks to activate gene conversion.

Homing endonucleases (HEs) or meganucleases are enzymes that introduce site-specific DNA breaks and are encoded by homing endonuclease genes (HEGs). HEs have been utilized for modification of maize for production of male-sterile plants by knocking out MS26 (cytochrome P450-like gene) and in cotton for production of herbicide resistance. However, HEs have not attained widespread adoption as they lack modular DNA-binding domains.

Zinc fingers (ZF) are DNA-binding domain transcription factors found in eukaryotic genomes. A nonspecific DNA-cleave domain of the restriction endonuclease FokI is used in ZFNs for editing. They have been successfully utilized to modify different traits like herbicide tolerance in Zea mays and improvement of seed oil composition in Brassica napus. Zinc-finger nickases (ZFN nickases) are specific and advanced when compared with ZFN-mediated genome editing.

TALE nucleases have been yet another option for programmable engineering. TALEs (transcription activator-like effectors) are naturally occurring bacterial DNA binding proteins from Xanthomonas that infect various plant species through their type III secretion system. They are localized in the plant cell nucleus and activate the expression of host plant genes by binding to promoter sequences that induce bacterial infection. TALEs can be custom designed to develop TALENS (transcription activator-like effector nucleases) by addition of nuclease domain of FokI restriction endonuclease. TALENs can be used as a precise method to edit plant genomes and facilitate targeted gene editing by homologous recombination of chromosomal DNA with the desired exogenous DNA. The utility of TALENs in crop improvement programs for diverse traits in crops like sugar cane, maize, soybean, tomato, rice, wheat, and barley has been reported. TALEN-based method usually requires two proteins, but recent improvement has made it possible to use a single protein, called “compact TALEN” (cTALEN). TALENs with a mitochondrial localization signal (mitoTALENs) (Table 1) can also be used to edit mitochondrial DNA.

Oligonucleotide-directed mutagenesis (ODM) is yet another unique gene editing tool to generate targeted mutagenesis. ODM uses specific synthetic oligonucleotides (10 to 100 bps long) that share homology with a target sequence in the genome, except that it contains a single base pair modification toward achieving site-directed editing of gene/sequence of interest. Oligonucleotides with a single mismatch bind to homologous sequences in the genome, which is recognized by DNA repair enzymes of the cell that introduce an altered nucleotide using their repair mechanism. This generates the target single nucleotide base editing in the plant genome for a novel trait and has been demonstrated in Arabidopsis thaliana, Nicotiana tabacum, Oryza sativa, Zea mays, and Brassica napus for herbicide resistance.

The latest revolutionary technology for genome editing is based on the RNA-guided engineered nucleases called CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9, which hold great promise because of their specificity, simplicity, efficiency, and versatility by addressing key challenges posed by other genome editing tools. CRISPR systems have evolved as an adaptive immune system for Archaea and bacteria and are broadly classified into class I and II based on the type of effector molecules. They are further grouped into six distinct types (I–VI) according to the architecture of CRISPR/Cas (CRISPR-associated) loci and type of Cas proteins with crRNA for CRISPR interference. Type II CRISPR system derived from Streptococcus pyogenes is the most widely used genome editing tool that uses a distinct DNA endonuclease, Cas9 to recognize dsDNA substrates and
Table 1. Various Modifications in Genome Editing Tools and Their Key Features

| Editing Technique | Variant/Advancement | Utility in Plants | Reference |
|-------------------|---------------------|-------------------|-----------|
| TALENs            | N-terminal wild-type I-TevI fused N or C terminal with a TALE DNA-binding scaffold. | Design was validated in tobacco protoplasts. | 8 |
|                  | TALEN target site (TTTS) was selected in the noncoding phospholipidosis IF gene. | TALEN design confirmed activity on par with conventional Fold TALEN. | 9 |
|                  | TALEN design confirmed activity on par with conventional Fold TALEN. | TALEN in rice and maize showed high sequence specificity. | 5 |
|                  | Lent mini-TALEN (mini-TALEN) is a commercially available vector that can be used to target multiple genes simultaneously in plants. | They can replicate a transcript seed in plants. | 5 |
|                  | Lenti-CRISPR is a commercially available vector that can be used to target multiple genes simultaneously in plants. | They can replicate a transcript seed in plants. | 11, 12 |
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2.1. Variants of CRISPR-Cas9

Recently, variations in CRISPR system are being developed and validated to mitigate the limitations posed by the conventional CRISPR-Cas9 editing tool. Cas9 enzymes isolated from various other bacterial species have distinct and expanded PAM sequences that can increase on-target specificity. An endonuclease Sacs9 from Staphylococcus aureus, Nmecas9 from Neisseria meningitides, and Cas12a enzymes (Cpf1) from Prevotella and Francisella with distinct PAM specificities have been identified to possess better features than that of SpCas9. In the SpCas9 system, the PAM sequence is NGG, and it creates DSBs proximal to the recognition site, three nucleotides away, which creates blunt ends. In contrast, TTTS acts as a PAM and creates DSBs distal from the recognition site, resulting in S’ sticky ends with 4–5 nucleotides overhang in Cpf1 nuclease. In fact, the Cpf1 editing system unlocks new possibilities for plant genome engineering with high specificity as compared to SpCas9.

Researchers carried out genome editing experiments in three different model plants, namely, tobacco (N. benthamiana), tomato, and Arabidopsis thaliana. Absence of off-target mutations either in related sequences or other sites in the genome showed Cpf1 as a viable alternative to SpCas9 for plant genome engineering. In addition to Cpf1, approximately 53 other CRISPR/Cas members have been characterized among which the Cr22 nuclease isolated from Leptotrichia shahii can target single-stranded RNA with its dual nuclease activity. Similarly, mito-TALENs and mito-CRISPRs were developed to reduce mtDNA copy number in both human cells and zebrafish. Researchers have edited mtDNA using a knock-in strategy by exogenous single-stranded DNA with a short homologous arm which targeted loci accurately.

2.2. Chimeric Fusion Technology: Advancements in Cas9-Based Editing

In tandem with the fascinating variations being made for effective utilization of CRISPR/Cas9 system across several systems (Table 1), prime editing, a unique protein fusion technology has been developed to improve homology directed repair (HDR). A distinct protein called prime-editor, a fusion of Cas9 Nickase (cleaves single stranded DNA instead of double stranded) and reverse transcriptase proteins, which mutually forms a complex with prime-editing guide RNA (pegRNA), is directed to the target site and thus acts as a carrier with a distinct nuclease domain HNH (histidine asparagine histidine) or RuvC (resolvase enzyme domain). A crRNA (crispr RNA)/gRNA (guide RNA) is used by Cas9 to incorporate an effector complex and guide the cas9 complex to the invading DNA to generate DSBs. A novel type of RNA called as “trans-activating crRNA” (tracrRNA) is essential for processing of crRNAs and cleavage of Cas9 nuclease complex. TracrRNA is of 25 bases with perfectly paired complementarity to CRISPR repeats. Both crRNA and tracrRNA collectively hybridize because of complementarity and are processed by RNaseIII into mature products. These mature RNA products form a complex (called gRNA) that guides the Cas nuclease to recognize and cleave at a specific target region just adjacent to a recognition sequence called as protospacer adjacent motif (PAM), consisting of 2–6 base pairs at 3’ end. The CRISPR/Cas9 system has been demonstrated to create a simple RNA-programmable method to mediate genome editing in plant cells and can be used to generate gene knockouts (via insertion/deletion) or knockins (via HDR). However, a major limitation of the CRISPR-Cas9 system has been the generation of off-target cleavage sites because of which variants of the CRISPR/Cas9 system are being developed (Table 1).
of the desired edit. PegRNA consists of a specific sequence for binding with 3′ end of the nicked DNA strand, which then acts as a guide sequence for reverse transcriptase that mediates transfer of genetic information. The cell’s DNA repair machinery removes single-stranded DNA flaps generated during the process by a series of DNA cleavage and ligation steps. Prime-editing system provides advantages over traditional CRISPR-Cas9-based gene editing as off-target instances are speculated to be far fewer when compared to off-target sites generated by Cas9. The latest addition to reiterate the usefulness of prime editing has been provided by studies in rice and wheat after successful optimization of codons, promoter, and editing conditions. The study, a proof of concept in rice and wheat protoplasts, has demonstrated the success of plant prime editors in point mutations, insertions, and deletions.

Advanced strategies for further improvement of HDR in plants are being continuously worked upon. Accordingly, a novel approach fusing Cas9 with VirD2 protein of *Agrobacterium tumefaciens* has been developed for genome editing in rice. This strategy was formulated considering the function of VirD2 relaxase to bind and facilitate proper integration of repair templates of various lengths at the target site (Table 1).

In the last couple of years, Cas nuclease-based genome editing has been dominating the field of genome editing and overtaking the previously identified techniques. Scientists have contributed enormously toward improvement of Cas9 nucleases as well as intricate details about plant transformation aspects. Efforts globally are underway for efficient design of vectors and cloning for plant transformation. Amenable transformation methodologies based on the plant species under study are being used, which include biolistics, protoplast-based, and floral dip transformations. However, *Agrobacterium tumefaciens* has proven to be the most successful transformation strategy being followed for genome editing. Response to transformation and successful realization of regenerated plants has always been a problem for biotechnologists. Not all plant species respond similarly to tissue culture because there exists a setback in crop improvement programs. The scientific community addressed this concern with the development of nontissue culture-based *in planta* transformation methodologies. These strategies have the potential to be used as alternatives to regeneration-based methodologies as they are less cumbersome and more time efficient. Recently, genome editing in transgenic plants was demonstrated using meristems that produce shoots with targeted DNA modifications that could be transmitted to the next generation. This method was developed for gene-edited plants through *de novo* meristem induction that avoids time-consuming tissue culture regeneration methods. Exhaustive research in the development and utilization of gene editing techniques has led to the emergence of newer variants by the day leading to the conception of fresh avenues for precision genome engineering in plants.

### 2.3. Supremacy of CRISPR-Based Gene Editing over RNA Interference

Both RNA interference (RNAi) and CRISPR-based genome editing strategies are being exploited in plants by various research groups globally for the demonstration of their utility. The primary difference is that RNAi reduces gene expression (knockdown) at the mRNA level, while CRISPR completely silences the gene at DNA level (knockout) permanently. Inefficient knockdown of target genes by RNAi leads to leaky expression of target mRNA thereby producing off-target effects where additional genes are uninun-
tionally affected, owing to poor reproducibility. However, CRISPR-based tools produced increased sensitivity and reduced off-target effects in the edited genotypes. The major difference between CRISPR and RNAi is that CRISPR results in complete loss-of-function effects in most cases, which is in contrast to RNAi that generally causes a hypomorphic effect causing partial loss of gene function. Thus, CRISPR generates stronger and consistent phenotypes in comparison to RNAi.

3. GENOME EDITING IN CROPS FOR MANAGEMENT OF INSECT PESTS

Biotechnological interventions in the management of insect pests to protect crop yield have been enormous. They range from breeding for insect resistance to transgenic introgression of novel genes. The utility of genome editing tools for the development of insect-resistant plants has conversely been scarce. A fascinating aspect of insect management through gene editing has the advantage of modifying both insects and plants (Figure 1). While innovative research is being carried out to modify the insects to avert their efficacy to attack, plant modifications are being carried out to increase their efficiency to deter insects. We have comprehended scientific efforts being made and those that can be envisaged toward genome editing of plants for insect management.

3.1. Genome Editing in Insects as an Approach to Modify and Mitigate Pest Population. Genome editing in insects can be successfully employed in a two-step strategy involving the modification of target insects, and their subsequent release into the natural environment. Although transgenic Bt technology has been well proven and exploited globally, resistance development against Bt insecticidal proteins (ICPs) has been the major fear. To evade this, efforts are being made to engineer receptors such that resistance management can be efficacious. Knockdown of cadherin receptors that are genetically linked to Cry1Ac toxin resistance is an evidence for successful genome editing in H. armigera. This strategy can also be adapted to modify target sites in midgut receptors responsible for resistance development against BtICPs or insecticidal proteins (Figure 1). Insects possess specialized detoxification enzymes responsible to overcome chemical defense response in various plant species. A strategy to target detoxification genes in polyphagous pests can be a promising option. Knockdown of insecticidal detoxification genes like gossypol-inducing cytochrome P450 resulted in insect susceptibility. CRISPR/Cas9 knockdown of CYP6AE gene cluster in the polyphagous pest H. armigera proved the role of these enzymes in detoxification of various toxic phytochemicals.

Another level of insect management using genome editing is the ability to target genes that could disrupt chemical communication and mating partner identification. These two are among the very many factors that establish successful plant—pest interactions. In insects, olfactory receptors (ORs) are important for the recognition of host plant as well as mating partner odorant. In the case of Drosophila, a mutation in Or83b gene disrupted the selection of the egg-laying site (host) and impaired olfactory detection capacity. Similarly, knock out of the Orco (olfactory receptor coreceptor) gene in Spodoptera litura through CRISPR/Cas9 disruption in the mating partner selection and loss of identity toward host plants leaving them anosmia. Adoption of such technologies will be a potential option to keep insect pests away from the crops and prevent insect damage.

In insects, female adults release pheromones and convey males, the status of their maturity prior to mating. Males access the pheromone signals and select mature females. CRISPR/Cas9-based knock out of odorant receptor 16 (OR16) in H. armigera made males unable to receive pheromone signals from mature females, thus resulting in mating with immature females that subsequently led to dumping of sterile eggs. Knock out of OR16 receptor in lepidopteran pests can therefore be a novel and effective strategy to regulate mating time for pest management in agricultural crops.

Another approach to target insects for pest management is by knocking out developmental genes such as abd-A (Abdominal-A) gene, a transcriptional factor involved in downstream regulation of various target genes that are extensively involved in development. Loss of function mutants through CRISPR/Cas9 resulted in the generation of abd-A mutant phenotypes in various agricultural pests like Spodoptera litura, Spodoptera frugiperda, and Plutella xylostella. Insects thus produced showed deformity in body segments, disarmed prolegs, anomalous gonads, and embryonic lethality indicating the success of gene editing tools (Table 2).

Table 2. Genome Editing in Insects for Pest Management

| trait                        | target insect          | target gene                          | references |
|------------------------------|------------------------|--------------------------------------|------------|
| body segmentation            | Spodoptera litura      | Slab-A (S. litura abdominal-A)        | 16         |
|                              | Spodoptera frugiperda  | Sfabd-A (indel mutations)             | 22         |
|                              | Plutella xylostella    | abdominal-A (gene knockout)           | 16         |
| mating time and partner      | Helicoverpa armigera   | OR16 (odorant receptor 16)            | 16         |
|                              | Spodoptera littoralis  | Orco (olfactory receptor coreceptor)  | 21         |
| regulation of detoxification | Helicoverpa armigera   | CYP6AE gene cluster (gene knockdown)  | 19         |

3.2. CRISPR-Based Gene Drive in Insects for Crop Protection. CRISPR editing creates a “gene drive” that is powerful enough for transgenerational spread of edited genes. In sexual reproduction, when one set of chromosomes contain a gene drive, it dominates over the other partner’s genetic makeup and drives the gene flow to be inherited with greater frequencies than those predicted by Mendelian inheritance. This “super Mendelian inheritance” allows passage of such genes from one generation to another even when they reduce the fitness of the particular organism. This would result in the complete disappearance of a particular species within 15–20 generations even if few individuals with CRISPR-based gene drive are released into the environment for mating. This leads to the creation of a rigid control over ecosystems by humans, which would disturb the ecological balance in nature in the absence of a particular species. In this direction, utilization of genome editing tools to target non Mendelian genes and genes that do not undergo gene drive would be an environmentally friendly approach. For instance, management of Bt resistance in H. armigera can be considered to be a sustainable approach when compared with other reported studies, since gene knock down in this case would only affect resistant populations of H. armigera against Bt toxins. Management of resistance in insect populations by using knock out studies would be more...
encouraging than those aimed to target traits related to reproduction and development in insects, which would lead to ecological havoc.

3.3. Targeting Plant Genes through Genome Editing for Insect Management. Genome editing in plants has been successful against several fungal, bacterial, and viral diseases in different agricultural crops. However, editing plants for insect pest management has been less exploited. Through this Review, we discuss the possibilities of targets that can be engineered for sustainable insect management (Figure 1). Most polyphagous pests recognize host plants using the plant’s own volatile blends, visual appearance, gustatory clues, oviposition sites, and their interactions are coevolved. Any insect prefers to lay eggs on a host plant to confirm the availability of preferred feed for its young ones. Plant volatile blends are a mixture of volatiles, of which only a few are recognized by insects as clues for host selection and oviposition site detection. Studies have demonstrated that changes in volatile blends retract insects from host plants. In plants, aphid infestation leads to release of a sesquiterpene hydrocarbon (E)-β-farnesene (Eβ) which retracts feeding by other host populations and attracts a parasitic wasp Diaeretiella rape that controls aphid population as seen in transgenic plants. Alteration of plant volatile blends through genome editing can be an alternative strategy in pest management. However, care should be taken such that the modification will not lead to deleterious effects toward beneficial insect population.

Visual appearance of plants plays a prominent role in the ability of insects to recognize and attack host plants. Alteration in plant pigmentation has been found to modify insect host preferences. This phenomenon was reported in red leaf tobacco, a transgenic that was developed by the modification of anthocyanin pathway. The study demonstrated that overproduction of anthocyanin pigment resulted in the red coloration of leaves in the transgenic tobacco plant. This change in leaf color proved to be acting as a deterrent to the herbivores, Spodoptera litura and Helicotheca armigera confirming the importance of leaf color and appearance on host recognition in insects. This strategy has been effectively utilized in the area of genome editing for biotic stress resistance. Taking these leads, alteration of the flavonoid pathway for resistance toward mosaic virus in soybean was successfully demonstrated. These studies demonstrate that engineering the anthocyanin pathway is a possible approach for CRISPR-based editing for pest management.

Plants have devised a plethora of strategies in response to the attack of biotic stress factors. While the resistance genes (R genes) decide the ability of plants to resist pests/diseases, susceptible genes (S genes) make them succumb to the stress. Editing of susceptible genes for the development of insect resistant plants is emerging as a reliable strategy (Figure 1). Insects rely on important chemical components from plants for their development, immunity, and behavior. This has been efficiently demonstrated in rice. CRISPR/Cas9-based knock down of CYP71A1 gene encoding tryptamine 5- hydroxylase which catalyzes the conversion of tryptamine to serotonin resulted in reduced growth in plant hopper. The study was hypothesized on the fact that serotonin, a neurotransmitter from plants is essential for larval immunity and behavior.

4. EXPLOITATION OF CROP WILD RELATIVES FOR ENGINEERING RESISTANCE TO INSECTS

Insertion of foreign genes into plants has been one of the major regulatory concerns in the case of transgenics, which can be overcome by genome editing. In continuation with the various strategies outlined in this Review, the use of crop wild relatives (CWRs) is another lucrative option. CWRs are ancestors or close relatives of cultivated crops and are resilient to various biotic and abiotic stress factors. The surge in the advancement of genomics and its usefulness in crop improvement programs have enabled scientists to acquire deeper molecular insights into their resistance response.

Studies on CWRs have implicated the loss of resilience traits to have occurred during the crop domestication process (made selection), also referred to as “domestication syndrome” according to which crops were developed to fulfill human needs and lacked the ability to combat environmental fluctuations. In line with this concept, the majority of cultivated germplasm were unable to manage the insect attack. Though breeding for insect resistance using CWRs can be a viable strategy to increase genetic diversity of the primary gene pool, it has not been successful in most of the crops because of biological barriers, linkage drag, cross-incompatibility, and sterile embryo production.

Advanced genetic engineering tools like transgenesis and genome editing provided opportunities to introduce novel traits into cultivated species from the CWRs. Another important aspect is identification of genetic variation which is responsible for insect resistance. Comparative transcriptome and proteome analysis in response to insect feeding is an effective strategy to identify sequence and expression level polymorphism, where introgression is not feasible. The aforementioned advancement in modern biotechnology tools opened two horizons (Figure 2);

Figure 2. An overview of utility of crop wild relatives for combating insect pests. (A) De novo domestication: CRISPR-mediated modification of resistant wild relatives for their domestication. (B) Gene editing for insect resistance: utilization of natural variation for improvement of susceptible cultivars for resistance against insect pest(s) through CRISPR.

first, de novo domestication of crop wild relative that possesses insect resistance and second, genome editing of cultivated crops utilizing insect resistant genes present in CWRs.

4.1. De Novo Domestication of CWRs for Insect Resistance. Wild relatives are bestowed with a large number of resistance traits but lack agronomic traits like high yield, fruit/grain size, preferable plant structure, and so on. Knowledge
available from breeding and genetics aid in the identification of genes responsible for desired agronomic traits that can be exploited by genome editing tools. Wild tomato *Solanum pimpinellifolium* is reported to be resistant to a wide range of arthropod pests including spider mites. Multiplex CRISPR-Cas9 editing of six different genes in *S. pimpinellifolium* in a single generation produced a high yielding tomato, restored with other resilience traits present in wild tomato. This strategy can be meticulously extended to other CWRs based on the traits and molecular mechanisms. *De novo* domestication of CWRs can therefore be a revolutionary strategy for the development of crops with improved traits (Figure 2A).

4.2. Genome Editing of Cultivated Germplasm Using Genomic Resources from CWRs for Insect Management.

Understanding natural variations in CWRs linked to insect resistance traits and deciphering the underlying molecular mechanism is a pertinent endeavor for the successful use of CWRs in improvement programs. CWRs are known to be bestowed with resistance against various insect pests due to antixenosis and antibiosis. Editing of genes in the cultivated crops based on the respective variation in CWRs would be a tangible approach to introduce variability (Figure 2B). This can be feasible by initial assessment of variation in the sequences of relevant insect responsive genes between susceptible cultivated germplasm and the resistant wild relative using multionic strategies. Upon validation of resistance genes against the relevant pest, they can be successfully utilized for genome editing. These approaches provide opportunities to enrich the resistance in the cultivated gene pool to combat insect pests. The recent past has witnessed profound research activities in this area which can be a platform for genome editing for insect pest management. Use of sequence variation for the development of a resistant phenotype has been demonstrated using either overexpression or silencing strategies in economically important crops. However, resistance through genome-editing-based sequence variation has not yet been proved.

5. REGULATORY ASSESSMENT OF PLANTS DEVELOPED USING GENOME EDITING TECHNOLOGIES (GETS)

Genetic engineering through transgenic technology has revolutionized trait modifications in plants. Transgenics are produced by the transfer of alien genes and T-DNA integration is a random process, possibly leading to positional or unintended effects along with the insertion of a vector backbone. These proved to be major concerns pertaining to humans, animals, and the environment. This paved the way for the emergence of regulatory rules outlined by various countries regarding the biosafety assessment of transgenics. Monitoring of transgenics with stringent risk assessment has become an essential component before their entry into farmer’s fields. Despite rigorous safety assessment of transgenics, their acceptance and popularization have been varying across countries. This has resulted in a serious impediment in the speed at which the technology can be used in crop improvement programs. The past decade has seen the development and propagation of highly precise and efficient strategies for improvement of crop traits through genome editing. Based on the level of genome modification, the edited plants can be congregated as Group I, containing single or few base modification/deletion/insertion, leading to less complexity in phenotype/genotype. Group II consists of edits of several base pairs leading to certain level of complexity in phenotype/genotype. Group III consists of insertion of a foreign gene creating a new trait attribute like a new metabolic pathway, new protein, or RNA. Products of GEd group I are to be assessed for their phenotypic equivalence analysis, and those of group II are to be assessed for trait efficacy through confined/contained field trials along with phenotypic equivalence analysis. However, products of group III consist of a complete gene inserted into the genome and thus need to undergo biosafety regulations as that of GMOs. Moreover, the engineered nucleases, gRNAs, and other molecules incorporated into the plant genome will remain in the plant only in T0 generation and are segregated in advanced generations. Hence, conscious and considerate efforts toward formulating guidelines for biosafety/risk assessment of genome-edited plants are required. These facts have enabled the United States to accept the genome edited plants as nontransgenics, whereas the European Union still considers them as transgenics. Regulatory guidelines need to be framed with all these aspects in mind such that there is no cessation in the research and commercial utility of genome editing in crop improvement programs.

6. FUTURE PERSPECTIVES

Genome editing is steadily and consistently emerging as a tool of choice by laboratories globally for elucidation of gene function as well as its translational utility. They are being harnessed in diverse crop improvement programs toward mitigation of biotic as well as abiotic stresses. The editing strategies have evolved as beneficial tools because of their high specificity, efficiency, cost, and time effectiveness. Despite success in economically important crops to combat pathogens, their use in insect management has not been exploited to the fullest. Notwithstanding the intellectual exercise toward designing of strategies for insect pest resistance in both insects and plants, the success has been meager. Though editing insects is an intriguing option, it requires caution in the selection of traits that need to be environmentally friendly so that the food chain is not affected. The major setback has been the lack of availability of target genes in contrary to other stresses. Therefore, emphasis needs to be given by scientists globally to the identification of resistance sources which can form a platform for insect management. Various innovative targets are being envisaged on the basis of their suitability to reduce off-target effects on target insects. Extensive phenotyping and enrichment of the resistance gene pool is an important hint that needs to be addressed in a path breaking manner. This requires assessment of the available germplasm including wild relatives of specific crops for insect response and identification of stress responsive genes using multi omics approaches. Multiplex editing of such identified resistance genes using high-throughput transformation techniques form the nitty gritty of insect management using genome editing. With such studies already in vogue, targeted mutations leading to the conversion of susceptible plants to those that are able to manage their respective pests is not distant in space or time. These aspects together with regulatory considerations on the gene edited crops could lead to the success of the technology not only toward scientific progress but also societal acceptance.

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