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Review on: CRISPR-Cas 9: A Novel Genome Editing Tool for Insect Pest Management

Rehana Akbar[1*], Sajad Mohi-ud-din[2], Summira Rafiq[1], Raheeba Tun Nisa[3], Mohmmad Asif Sheikh[1], Amreena Akhter[4], Iram Iqbal[3] and Ulfat Jan[1]

[1]Division of Entomology,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (J&K), India.
[2]Head, KVK, Srinagar,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (J&K), India.
[3]Division of Plant Pathology,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (J&K), India.
[4]Faculty of Forestry,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (J&K), India.

(Corresponding author: Rehana Akbar)*
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ABSTRACT: Insect pests pose a severe danger to crop productivity, with insects destroying over 40% of crops globally each year. Pesticides used indiscriminately resulted in insect resistance, a reduction in beneficial insect populations, and a slew of other negative consequences for humans and the environment. In addition, the development of transgenic crops has raised ethical concerns, prompting researchers to consider other approaches. The recent advancement of gene-editing technologies such as the CRISPR-CAS9 system has opened up new possibilities for the creation of novel pest control strategy. This review will provide knowledge about efficiency of CRISPR/CAS9 and also some research ideas for CRISPR/CAS9- based integrated pest management. Due to its specificity and trouble-free handling it has been used to a large range of organisms for various research purposes. CRISPR is a technique for editing parts of the genome by removing or modifying DNA. The CRISPR Cas technique functions through a ribonucleoprotein complex, in which Cas9's target recognition lobe interacts with homologous sgRNA to direct specific binding to target DNA, and the excision lobe breaks the DNA. Genome editing's potential has opened up a world of possibilities for designing various features in plants and insects. Genome editing is used to develop pest resistant crops by changing the effector–target relationship and knocking out host susceptibility genes, whereas in insects genome editing is done by knocking out genes responsible for insecticide resistance.

Keywords: Insect Pest, Susceptible Genes, Management, Genome Editing.

INTRODUCTION

Agriculture faces significant problems in the twenty-first century, including the requirement to deliver high-quality agricultural goods to support rapidly growing worldwide human populations while avoiding environmental impact. Insect pests are the most common biotic stressor, causing significant crop losses worldwide through direct feeding and disease spread (Douglas, 2018). Insect pest species damage agricultural commodities, reducing producer output and profitability and thus affecting product quality, so the development of novel and effective insect pest control tactics remains a research challenge for farmers and the scientific community. Farmers, on the other hand, have developed chemical pesticides for the control of insect pests, which, if widely used, could be harmful to humans and the environment. These issues have prompted scientists all across the world to design fresh, ecologically friendly insect control technologies. As a result, the primary goal of contemporary agriculture has been to increase yields with limited land and resources in order to ensure global food security and agricultural sustainability. Pest management has expanded to new heights with the introduction of genetic engineering and plant biotechnology. The continuous use of Bt gene introgressed crops and genetic alteration of a variety of crop plants with various insect resistance genes, such as Bt genes, has proven a positive impact on productivity and the technology's sustainability. However, the development of insect tolerance to cry poisons is a big concern. Another sustainable strategy would be the production of insect pest resistant cultivars through cross breeding. However, the lack of a resistant gene pool, as well as incompatibility issues in many crops, has limited the scope of insect resistance breeding. These issues prompted the development of genome editing, the next level of biotechnological intervention. This intriguing technology has emerged as a critical addition since it enables for genome changes by adding, editing, or removing certain DNA sequences, allowing for applications in plants, animals, and humans. Genome editing will act as a viable technique to battle insect pests in the current environment of constrained agricultural lands and rising insect pest burden on crop plants. There are four families of genome editing tools *i.e* ZFN, TALEN, hoeming endoneucleases and CRISPR-Cas9. Because of its great specificity and applicability, CRISPR Cas9 is being researched as a promising technique for genome editing. Cas9-mediated genome editing and its application in insect resistance in plants are discussed in this review.

CRISPR CAS9: A NOVEL APPROACH FOR IMPROVING PLANT RESISTANCE TO INSECT PEST

CRISPR: (clustered regularly interspaced palindromic repeats) is a group of DNA sequences found in prokaryotic species' genomes. These sequences are acquired by prokaryotes from bacteriophages that have previously infected them.. They used these sequences to detect and destroy the DNA of the same bacteriophage upon its reinfection. As a result, these sequences serve an important role in prokaryotes' anti-phage defence system and provide a sort of acquired immunity. CRISPR Cas9 consists of a cassette of spacers and repeats, Cas genes that produces cas9 and leader sequence transcribes tacrRNA and CrRNA. The crispr requires a short synthetic gRNA sequence that bind to target DNA and cas9 nuclease enzyme that cleaves 3-4 bases after PAM (Jinek *et al*., 2012). RuvC and HNH are two scissors that make up Cas9 nuclease. It has been frequently used in plant and animal genome editing using the cleavage process. Steps involved in implementation of CRISPR (Fig. 1).

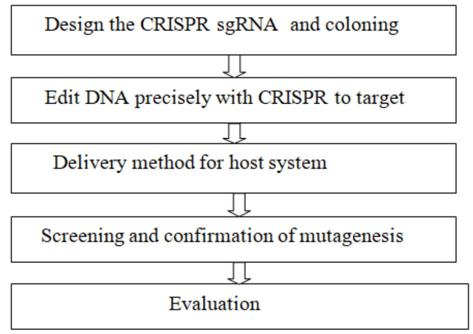

Fig. 1.

The ability to modify both insects and plants is an interesting element of pest management through gene editing.

Editing of Susceptible Genes in Plants for Insect Pest Management. Plant genome editing for pest management include knocking out sensitive genes, which favors the insect and improves their resistance, modifying plant volatile mixes, and changing the colour of the leaves, which makes plants unappealing to pests. So far, the CRISPR Cas9 gene editing technology has been used in over 20 crop species (Ricroch *et al*., 2017) for a variety of features such as yield improvement and biotic and abiotic stress management. Various published articles are regarded as proof-of-concept research since they narrate the use of the CRISPR Cas9 approach to knock out specific genes involved in abiotic or biotic stress tolerance. Rice genome shows an abundance of potential PAM sites (Xie and Yang 2013). In the near future, CRISPR technology could be used to target any trait of interest in the rice genome. Lu *et al*. (2018) investigated on resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. They successfully edited the rice plant by targeted CY71A1 gene which is susceptible gene in rice plant favours Brown plant hoppers. As this gene helps in conversion of trptamine to serotonin which is essential for insect immunity and behaviour. Targeting of this gene by CRISPR Cas9 system showed reduced feeding, survival and growth of Brown plant hopper.

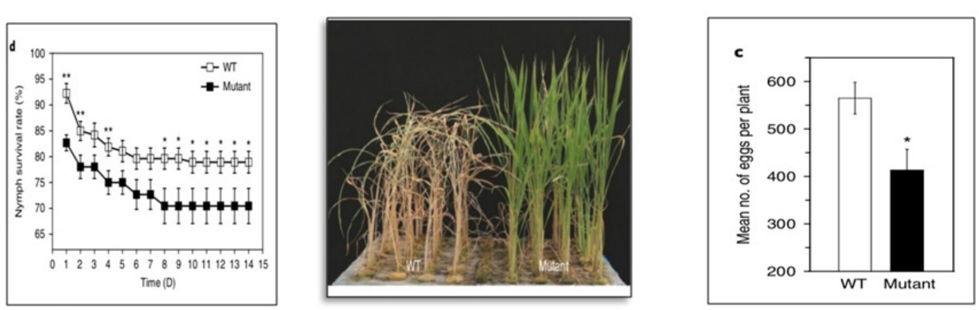

Fig. 2.

Callose deposition in sieve tubes was reduced after CRISPR Cas9 caused mutations in two -1-3 glucanase genes of the golden promise cultivar of barley. Thus the aphid *Rhopalosiphum padi* failed to access the phloem sap and it negatively affected the aphid and diminished the host preference in barely (Kim *et al*., 2020).

CRISPR Genome editing in insects to modify and mitigate pest population

The pest control by genome editing in insects is done to eliminate pesticide or other toxin resistance by knockout or editing of genes responsible for producing enzymes that detoxify the toxins inside the insect body. Crops that have been genetically modified to produce insecticidal proteins from the bacterium *Bacillus thuringiensis* have revolutionised pest control and also reduced inorganic pesticidal use that led protection of natural enemies and thereby enhanced biological control. However swift development of resistance to BT toxins by insect pests reduced Bt crops efficacy. Some researches found that midgut receptors present in lepidopteran insects are genetically linked with Cry toxin resistance. Wang *et al*. (2016) reported that a cadherin receptor is involved in cry1Ac resistance in *Helicoverpa armigera*. The disruption of cadherin gene HaCad by CRISPR-Cas9 system, the mutant individuals showed 549-fold higher resistance to Cry1Ac compared with control strain. Insecticide resistance is a unique example of rapid adaptive evolution that has revealed a wealth of information about genetic variation. Insects have detoxifying enzymes that help them overcome chemical defences. The CRISPR Cas9 strategy targets detoxification genes in insects. The CRISPR Cas9 system has recently been utilised to examine the mechanism underlying spinosad resistance. A site-specific mutation (G275E) into the nicotinic acetylcholine receptor (nAChR) Dα6 subunit and although the spinosad insecticide resistance level of flies with the G275E mutation (66-fold higher than non mutated flies) was lower than that of individuals with a Dα6- null mutation(311- fold higher than non-mutated flies), it sufficiently demonstrated that the G275E mutation is directly related to spinosad resistance (Zimmer *et al*., 2016).

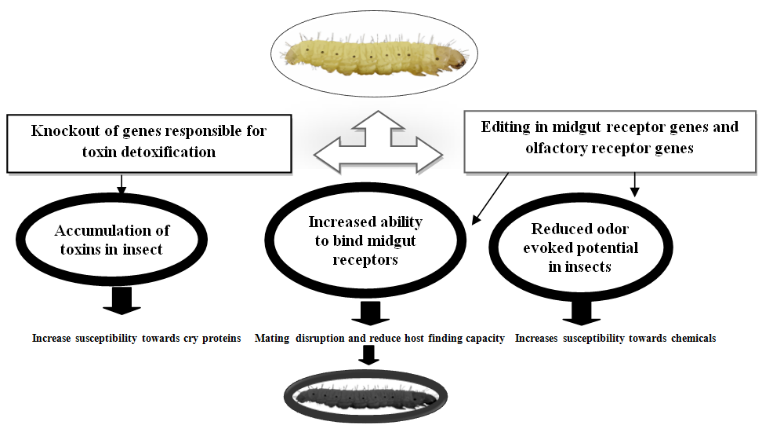

Fig. 3. Genome editing in insect.

Garczynski *et al*. (2017) conducted a study to knockout CpomOR1 gene using CRISPR-Cas9 gene editing system in codling moth for determination of physiological functions. Codling moth contains CpomOR1 gene which is an odorant receptor and is a prime candidate for codlemone receptor based on its high expression in male antennae. The study showed unexpected results that fecundity and fertility were effected with editing of females genome by CRISPR-Cas system producing non viable eggs as it was not possible for edited male to locate and mate edited female if this gene was disrupted so far this they further demonstrated the study to determine if mating was occurred between edited males and females and they found that both appeared mated due to presence of spermatophores in female abdomen. Thus they concluded that these are present in antennae of male and also in the abdominal segments of females (Li *et al.,* 2016). Lucusts are important agricultural pests world wide and is regarded as study model for entomology. The availability of genome sequence information about locust provide an opportunity to promote the study of locust biology and develop potential methods for pest control. A successful genome editing was achieved to induce targeted heritable mutagenesis in migaratory locust by CRISPR Cas9. In migratory locust Orco an olfactory co-receptor which is essential for localization of odorant receptors in insect. A target sequence of gRNA was designed to disrupt the gene encoding Orco so that examine the odorant receptors pathway in the locust. The mixture of Cas9-mRNA was microinjected into locust eggs to establish orco mutants and results showed that Orco mutants lost attraction response towards aggregation pheromones and also odor evoked potential was not detected in basiconic sensilla and trichod sensilla. The results concluded that chemosensory respone of insects is mostly dependent on Orco.

Table 1: Insect genes successfully modified by CRISPR/Cas9 system.

Insect	Targed genes	Delivery method	References
Ceratitis capitata	Eye pigmentation gene white eye (We)	Microinjection	Meccariello *et al.* (2017)
Trobolium castaneum	E GFP1	mRNA injection+DNA	Gilles *et al.* (2015)
Helicoverpa armegera	HaCad	mRNA+DNA	Wang *et al.* (2016)
Plutella xylostella	Pxabd-A	mRNA+DNA	Huang *et al.* (2016)
Agrotis ipsilon	yellow-Y Gene	Microinjection	Chen *et al.* (2016)
Locust migratoria	Orco	Microinjection	Li *et al.* (2016)

CONCLUSION

Crispr/cas technology has exploded in popularity due to its ease of use, low cost, and versatility as a genome editing tool. The CRISPR Cas-9 system is a flexible technology that has already sparked new uses in biology and other domains. This technology has the potential to transform the pace and direction of agricultural research since it allows scientists to precisely and swiftly implant desired features into plants. Crop protection through genetic modification offers a promising alternative to reducing pesticide use that has no discernible impact on human health or the environment. In the future, researchers will focus on using genome editing technologies to improve agricultural productivity, nutritional value, insect pest and disease resistance, and other features. Much research is still needed on its use in agriculture with the aim to eventually use this technology under field conditions for management of insect pest and diseases.

FUTURE SCOPE

Development of new pest management strategies having no negative impact on environment, humans and natural enemies and provide effective control of insect pests are much needed.

Conflict of Interest. None.

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