BIOTECHNOLOGICAL APPROACHES IN INSECT PEST MANAGEMENT

Abstract

The global population is projected to reach almost 10 billion by 2050, demanding a 50% increase in agricultural production. In light of the increasing occurrence of pest and disease outbreaks, which jeopardize food security, it is crucial to adopt innovative approaches. Conventional approaches such as chemical pesticides have been found to be inadequate, prompting a shift towards biotechnology alternatives. Biotechnological interventions, such as gene transformation and genetic engineering, provide innovative approaches for controlling insect pests. The advancements in gene editing technologies, such as CRISPR-Cas9, offer possibilities for managing insect pests. RNA interference (RNAi) methods, including double-stranded RNA (dsRNA), have demonstrated potential in specifically eliminating pest species while leaving non-target species. The gene-drive approach modifies the inheritance of specific genes, providing a potent tool for managing insect pests. The book chapter explores the diverse applications of biotechnology in insect pest management, covering gene editing, RNAi, and gene-drive technologies. It highlights successful cases of gene editing in various insect species, such as fruit flies and the migratory locust, and discusses the potential for CRISPR-Cas9 to modify plants for insect resistance. In summary, the incorporation of biotechnology in agriculture provides inventive remedies to tackle the difficulties presented by rising insect prevalence, thereby promoting sustainable and robust food supply for the growing global population.

Keywords: Agricultural Production, Biotechnological Interventions, Genetic Engineering, CRISPR-Cas9

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"If you know the enemy and know yourself, you need not fear the result of a hundred battles. If you know yourself but not the enemy, for every victory gained, you will also suffer defeat. If you know neither the enemy nor yourself, you will succumb in every battle."

- Sun Tzu,

World's population is projected to be almost 10 billion by 2050, leading to almost a 50% increase in the demand for agricultural production (Nishimoto, 2019). It is anticipated that food production must increase by at least 50 percent to fulfil the dietary requirements of the projected 2050 population (Dijk et al. 2021). According to the changing climatic scenario, the world's population would rise by 2 billion in the next 30 years, from the present 7.7 billion to 10 billion by 2050. (Kumari et al., 2022; Zsögön et al. 2022). The increase in pest and disease outbreaks in plants and animals is a growing threat to food security as these outbreaks have a significant impact on food production and can lead to economic, social, and environmental consequences (Sundström et al., 2014; Ristaino et al., 2021). Management of Insect pest is an ongoing challenge for farmers, agricultural industries and entomologists (Dent and Binks 2020).

Insect pests cause widespread damage to agriculture and are a major concern for many economically significant crops. (Isman, 2019). According to studies by Joshi et al. (2020), Mateos Fernandez et al., (2021) and Kumari et al., (2022) insect pests cause an estimated 25–30% loss in agricultural production. Conventional control method like chemical insecticides, have been unsuccessful to prevent the significant infestation caused by insect pests (Dhananjayan et al., 2020). Advances in biology have been used to develop innovative control options. Regarding the promotion of sustainable agricultural methods, such as integrated pest management, conservation agriculture, integrated crop-livestock-energy systems, and agroforestry, a thorough understanding of the necessity for technical approaches in research and development is must. (Seifu et al., 2020).

According to a global meta-analysis of 147 biotech crop studies, over the past 20 years, the use of genetically modified organisms (GMOs) has reduced the use of chemical pesticides by almost 37 percent and increased crop yields by 22 percent, which has resulted in a 68 percent increase in farmer profits (Klümper and Qaim, 2014; Coupe and Capel 2016; Yali, 2022). GMO crops were cultivated on almost 181.5 million hectares in 2014, up from 1.7 million ha in 1996 in 28 countries (Aldemita et al., 2015). GMO crops are grown by more than 18 million farmers, which mostly includes from farmers in China and India (Aldemita and Hautea 2018). The insertion of transgenes into crops for pest management has been a key element in the drastic changes in insect pest management over the last 15 years. (Duke, 2021; Alemu, 2020).

Science and research on the genetic management of insect pests are at an exciting stage right now as there has never been a greater need for innovative ideas to combat rising agricultural pests. Disease-carrying mosquitoes are being targeted using molecular approaches, which are now beginning to include pest species that are significant to agriculture (Friedman et al., 2020). To make it possible for these biological and genetics-based solutions to be implemented safely and effectively, regulatory issues are being given attention. Together, these advancements have increased the likelihood of achieving significant agricultural and social advantages.

Since transgenic *Bacillus thuringiensis* (Bt) was first used in the Integrated Pest Control (IPM) program more than 20 years ago, biotechnological methods of insect pest control have revolutionized the idea of genetic modification in insect pest management (Books, 2019). Although insect management for crop security has been much enhanced by transgenic crops, the technology quickly ran into a big problem with insect resistance, which led researchers to focus on more recent biotechnological techniques to insect pest control (Books, 2019). The breeding of insect tolerance to pesticides and the transgenic insertion of novel genes are only a few examples of the key biotechnological interventions for insect pest management. Gene modification, genome editing, RNA interference, and marker-assisted selection are examples of biotechnological methods. (Talakayala et al., 2020; Gandhi et al., 2022; Kumari et al., 2022).

I. GENE TRANSFORMATION AND GENETIC ENGINEERING

To provide crop plants with resistance to insect pests, certain DNA segments or genes can be incorporated into plants using gene transformation and genetic engineering. Typically, the released DNA fragment encodes a protein with insecticidal properties (Daniell et al., 2016; Latham et al., 2017; Rani et al., 2022). The production of an insecticidal protein found in the inserted DNA segment confers resistance to plant against particular insect pests (Gatehouse, 2013; Kumari et al., 2022). Lepidoptera, Coleoptera, and Dipteran insect pests along with few other insect pests have been successfully controlled using this method (Birkett & Pickett, 2014; Nitnavare et al., 2021; Kamatham et al., 2021). In addition, many methods for shielding plants from insect attack have been investigated, typically present in many plants, lectins bind to carbohydrates in the midguts of phytophagous insects, thereby upsetting their digestive processes (Konno and Mitsuhashi, 2019; Chettri et al., 2021). Protease inhibitors used to prevent insects from digesting food have also been introduced via transgenic methods (Singh et al. 2020; Divekar et al., 2022). Similarly, lepidopterans, coleopterans, dipterans, and hemipterans were not attracted to transgenic plants expressing α-amylase inhibitors (Singh et al. 2020).

Chitin breakdown in the exoskeleton and gut lining is also greatly aided by insect chitinases and chitinase-like proteins. These have demonstrated insecticidal properties and have been successfully cloned into plants. (Holen, 2022; Kumari et al., 2022; Shobade, 2022). Because insect chitinases stop insect growth and development, they have been developed as biopesticides and transgenes for crop protection. The development of agrochemicals that target this enzyme has been impeded by the absence of structural data on some insect chitinases and the paucity of study on this enzyme. (Jiang et al., 2020; Sharma et al., 2023). Their use in biotechnological processes will be accelerated by greater knowledge of their biochemistry and structural characteristics.

II. GENOME EDITING

Insect resistance poses a threat to the agricultural biotech sector, which has prompted biotech companies to work tirelessly to find new, economical, and environmentally responsible methods for managing insect pests or methods to overcome the problem of insect resistance. Genetic pest management methods now have more options owing to recent developments in gene editing technology. By using the internal workings of the cell, gene editing, also known as genome editing technology, successfully modifies the function of genes. By adding, removing, or swapping DNA bases, in the genome, this method modifies a particular target DNA sequence (Bortesi and Fischer, 2014; Legros et al., 2021; Molla et al., 2021). Genome editing has been referred to as a "new breeding technology" since it has the capacity to combat insect pests in the current setting of constrained agricultural areas and an increased load of insect pests on crop plants. (Razzaq et al., 2019; Vats et al., 2019).

These technologies depend on nucleases to cleave particular genomic target sequences. Double-stranded breaks (DSBs) promote DNA repair by a non-homologous end-joining (NHEJ) mechanism, which introduces small insertions and deletions (indels) at specific locations (Curtin et al., 2012; Malzahn et al., 2017). A DSB causes a frame shift after an indel in the coding sequence, which causes gene inactivation and mutation. Targeted genome editing and direct plant modification are both possible applications of this method. Only a few of the engineered nucleases that have been developed include homing endonucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), oligonucleotide-directed mutagenesis (ODM), and clustered regularly interspaced short palindromic repeats (CRISPR) (Shalem et al., 2015; Bennett et al. 2020; Kumari et al., 2022).

III. RNA INTERFERENCE (RNAi) FOR PEST CONTROL

Through a technique known as RNA interference, double-stranded RNA (dsRNA) is introduced into a cell to suppress unwanted genes and, in some cases, the synthesis of new genes (Silver et al., 2021; Abo-al-ela et al., 2021). Recent years have seen a significant amount of research into the integration of RNAi gene-editing strategies for gene silencing into pest control and crop protection programs. RNAi is used to selectively eradicate pest insects without affecting non-target species by precisely targeting the genes necessary for the pest insect's development, growth, and reproduction (Mamta & Rajam 2017; Munawar et al., 2023). RNAi, a naturally occurring cellular defense system mediated by double-stranded RNA (dsRNA), regulates a wide variety of insects. This is especially true of sap-sucking insects. However, transgenic crops cannot control this insect population (Kunte et al., 2020; Chung et al., 2021). Because gene silencing only affects afflicted cells, the delivery strategy is crucial for accomplishing this effect.

Injection, feeding, soaking, and transgenic plants that produce dsRNA are a few of the delivery techniques that have been researched in various groups of species. (Suzuki et al., 2017; Christiaens et al., 2018; Books, 2019). It has been established that direct dsRNA injection into target tissues is delicate and time-consuming. Using the feeding/ingestion method, insects that consume transgenic plants producing hairpin RNA absorb dsRNA, However, different animals respond differently to RNAi via dsRNA intake (Books, 2019). Additionally, it is challenging to estimate the amount of dsRNA ingested by insects after oral administration, and more material is needed for delivery (Lin et al., 2017; Kunte et al., 2020). The soaking approach involves submerging the insects in a solution that contains dsRNA, however it is only effective with particular insect species and developmental stages that can quickly absorb dsRNA from the solution. (Kunte et al., 2020; Nitnavare et al., 2021). RNAi technology has successfully suppressed target genes in a number of insect orders; however, due to the current research gap, this approach is less realistic as a long-term technique of controlling insect pests (Jain et al., 2021).

IV. GENE - DRIVE APPROACH

The term "Gene Drive" refers to gene editing technology that increases the likelihood that a certain gene will be passed down to an organism's progeny through biased inheritance (DiCarlo et al., 2015; Books, 2019). A specific DNA sequence may be passed down by sexual reproduction from one generation to the next, maybe throughout the whole local population, and eventually throughout all related populations of a species (Collins, 2018). This potential is increased by the gene drive approach. Gene drives were conceptualised in response to the actions of a group of naturally occurring genes known as selfish genetic elements, or "selfish genes" (Oberhofer et al., 2019). According to McFarlane et al. (2018), Selfish genes are genetic segments that, even if they do not help the organism, can enhance and promote their own inheritance to the following generation. Additionally, according to Hammond et al. (2016) and Books, (2019) the site-specific endonucleases used in Gene Drive technology modify selfish genes to spread traits throughout populations. As a result, gene drivers could spread a particular gene across members of the same species.

The Gene Drive technique is a promising new biotechnological method for controlling insect pests, particularly invading insect species, agricultural insects, and insects that spread disease. It is the most effective tool for controlling or eliminating insects that spread illnesses because it affects sexually reproducing organisms. Insect resistance to transgenic Bt or pesticides can be eliminated using this method, which is also used to erase resistance genes in insects (Min et al., 2018; Wedell et al., 2019).

The Gene Drive technology is used to influence the law of natural inheritance (Mendelian inheritance), which stipulates that all sexually reproducing organisms receive 50% of their genetic composition from their parents (Books, 2019; Bresson et al., 2020). This indicates that there is only a 50% chance of a certain gene being passed from parent to child. More than half of the kids will inherit the desired gene thanks to Gene Drive technology, which disturbs this process to locate the targeted gene's wild-type form and replace it with the desired/altered gene. By affixing Gene Drives to a desired/altered gene and delivering it into an organism's genome, gene-drive modification may be accomplished out (Akbari et al., 2013; Books, 2019). Therefore, the wild-type gene that would have been acquired from the wild-type parent would be recognized and molecularly deleted (cleaved) when an organism with the gene drives mates with the wild-type organism. The changed gene, Gene Drive, which functioned as a template for producing healthy cells, will then be multiplied to restore the injured cell. The organism may pass on these copies to the next generation now that it has two copies of the same gene i.e., one on each chromosome (Books, 2019; Doss, 2022). The same process is used in every generation, which allows the altered genes to spread throughout the population (Books, 2019; Legros et al., 2021; Palmer et al., 2022). Unfortunately, the CRISPR-Cas9 system and Gene Drive technology work better together than they do alone (Champer et al., 2017; Books, 2019). Researchers may propagate these alterations throughout wild-type populations using gene-driven technologies. Two significant limitations of Gene Drive technology have prevented it from becoming successful (Esvelt et al., 2014):

- It is challenging to make precise and desirable changes in the genome.
- Even after achieving the intended modification, it is challenging to fast propagate into the natural population without introducing a significant number of changed individuals.

V. CRISPR-Cas9 APPLICATIONS FOR INSECT PEST MANAGEMENT

In order to manage insect pests and safeguard crops, CRISPR-Cas9 is employed in both medicine and agriculture. Additionally, CRISPR-Cas9 technology is used to develop drives that stop harmful pest behaviours like the swarming of *Locusta migratoria* a migratory locust, as well as drives that sensitise pests to make them vulnerable to specific chemicals like pesticides (Sun et al., 2017). Drives may be constructed to modify or eliminate vector-borne insects, and it may even be possible to learn how to reprogram an insect's olfactory system to deter crop consumption or to make mosquitoes lose the scent of the crops (Esvelt et al., 2014; Singh et al., 2020).

In Drosophila i.e., fruit flies (Diptera: Drosophilidae), one of the first documented uses of the CRISPR-Cas9 system in insects, mutations were effectively inserted into the yellow gene (Lei et al. 2016; Singh et al., 2020). This technique was also successfully used to target the BmBLOS2 gene in the silkworm *Bombyx mori* (Lepidoptera: Bombycidae) (Liu et al. 2014). The genome of the Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae), was altered using the CRISPR-Cas9 gene editing method by Garczynski et al. (2017). The scientists aimed to create stable populations of altered codling moths by rearing emerging neonates to maturity by pairing males with females with mutations in CpomOR1. These results suggest that changed females' fecundity and fertility were impaired, resulting in the production of non-viable eggs. Uncertainty still exists about the precise mechanism by which CpomOR1 influences the fecundity and fertility of codling moths.

The locust is a serious agricultural pest on a global scale. Their swarming behaviour may damage crops across wide distances, often with significant financial repercussions. The migratory locust was modified using the CRISPR-Cas9 system in the research by Li et al. (2016) and Gui et al. (2020), and a target sequence of guide RNA was made to damage the gene encoding the odorant receptor co-receptor (Orco). Experiments were also conducted on the role of the locust odorant receptor pathway. Target-gene editing appeared to work as evidenced by the diminished electrophysiological responses to several scents in mutants of the Orco gene. Mutant locusts thus lack the capacity to react favourably to aggregated pheromones in crowded conditions.

Since sick and unhealthy plants attract insects, keeping healthy plants is an essential part of an Integrated Pest Management program. CRISPR-Cas9 systems, according to Lei et al. (2018), Jaganathan et al. (2018), and Moon et al. (2022), can be used to modify plants so that they either emit or do not emit particular enzymes that can fend off insect pests from coming into contact with the plant or draw in specific insect predators to feed on insect species that attack the plant. For effective control of insects that spread illness, CRISPR-Cas9, a gene editing technique, has a number of applications. These methods can be used to eradicate vector-borne illnesses such West Nile virus, Zika virus, dengue, Lyme disease, and malaria. (Hammond et al., 2016; Hillary et al., 2021; Muller et al., 2023). CRISPR-Cas9 can also be used to reduce population sizes of certain species, through the use of population suppression targets. Furthermore, CRISPR-Cas9 enabled Gene Drive systems can make a population more susceptible to harmless substances, thus creating species-specific insecticides (Gong, 2017; Books, 2019).

VI. CONCLUSION AND FUTURE OUTLOOKS

The primary cause of declining agricultural output is insects. Farmers are more likely to use chemical pesticides because they offer an immediate answer to the problem of insect infestation. The potential adoption of alternative pest control strategies has acquired major relevance as a result of the rapidly growing public awareness of the risks that indiscriminate pesticide usage brings to human and animal health as well as the environment. Numerous insect-resistant plant species have been created thanks to biotechnology, including varieties of maize, rice, cotton, canola, soybean, tobacco, apple, and potato (Paoletti et al., 2020). In a number of crops, new plant resistance traits that offer good defence against invasive and harmful pests are presently being developed using biotechnological methods (Kumari et al., 2022). The emergence of biotechnological methods like genome editing and genetic engineering will expedite the creation of insect-resistant crops in the present and the future.

Additionally, novel approaches for producing insect-resistant crops are provided by RNA interference and genome editing using CRISPR/Cas9. Therefore, the production of novel insect-resistant agricultural plants has been shown to be economically viable, pesticide-resistant, and ecologically safe (Kamatham et al., 2021). Modern methods, such as RNAi and CRISPR, can be used to quit or alter vulnerable genes to create plants that are resistant to insects. The persistence of plant-resistant components will be enhanced and augmented by new developments that offer more effective treatment for emerging pests. Biotechnology has clearly made insect pest management techniques much more distinctive, but many of these techniques require regulatory frameworks that may not currently exist for some products or in some areas. Additionally, it requires the cooperation of both producers and consumers, which calls for open discussions about the possible impact of new technology on social transformation. In conclusion, the development of crops using biotechnology that are more nutritious, resistant to diseases and pests, and have low production costs is an example of a novel scientific application that may be used for the good of society. From this viewpoint, biotechnology is a scientific discipline that, if applied morally and sensibly, has the potential to offer great benefits.

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