



# Biotechnological Innovations in Insect Pest Management: From RNAi to CRISPR

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**Abstract**— The increasing resistance of insect pests to conventional chemical insecticides, coupled with environmental and human health concerns, has necessitated the development of innovative and sustainable pest management strategies. In this context, biotechnological approaches have emerged as powerful tools for precise and eco-friendly insect pest control. This review comprehensively examines recent advances in biotechnological innovations for insect pest management, with a particular focus on RNA interference (RNAi) and CRISPR-Cas-based genome editing technologies. RNAi has demonstrated significant potential through sequence-specific gene silencing, offering targeted pest suppression with minimal non-target effects, although challenges related to delivery efficiency, stability, and resistance development remain. CRISPR-Cas technology represents a transformative advancement, enabling precise genome modifications for functional genomics, population suppression, gene drive systems, and the development of pest-resistant crop varieties. In addition to RNAi and CRISPR, the review highlights complementary biotechnological tools such as transgenic crops expressing insecticidal proteins, marker-assisted selection, and symbiont-mediated pest control strategies. The regulatory, biosafety, and ethical considerations associated with the deployment of these technologies are also discussed. Finally, the review emphasizes future prospects and research priorities for integrating biotechnological innovations into integrated pest management (IPM) programs to achieve sustainable and resilient agricultural production. Overall, the advancements discussed underscore the critical role of biotechnology in shaping the next generation of insect pest management strategies.



**Keywords**— Insect pest management; Biotechnology; *Bacillus thuringiensis*; Transgenic crops; RNA interference (RNAi); CRISPR/Cas9; Host plant resistance; Integrated pest management

## Introduction

The rapid expansion of the global population necessitates a substantial increase in both the quantity and quality of food production. However, agricultural productivity is significantly constrained by insect pests, which are responsible for approximately 18% of global crop losses, excluding additional damage caused by plant diseases and weeds (Oerke, 2006; sharma *et al.*, 2017). In the absence of effective control measures, yield reductions can reach nearly 50% in wheat and more than 80% in cotton production [Oerke, 2006; sharma *et al.*, 2017]. Therefore, effective insect pest management remains a critical

requirement for ensuring food security and agricultural sustainability.

Since the 1950s, insect pest control has relied predominantly on so-called “conventional” synthetic insecticides. These chemicals revolutionized agricultural production during the mid-20th century and were widely adopted due to their remarkable effectiveness. However, in recent decades, Integrated Pest Management (IPM) has emerged as a more sustainable and ecologically sound approach, producing satisfactory outcomes despite practical challenges [Deguine *et al.*, 2021; Zhou *et al.*, 2024]. Ideally, IPM follows a three-layered pyramidal

structure. The foundation consists of cultural practices, field manipulation, and the use of resistant host plants. The second tier incorporates biological and microbiological control agents, semiochemicals, and emerging biotechnological tools. Chemical control serves as the final option and is applied only when necessary.

The continuous pressure exerted by insect pests necessitates the development of effective management strategies that minimize environmental damage and reduce reliance on chemical pesticides (Gurr *et al.*, 2004). Historically, insect pest management has evolved considerably. Early control methods involved manual removal and destruction of insects, followed by cultural and biological strategies. The discovery and widespread adoption of chemical insecticides in the mid-1940s revolutionized agricultural industries during the 1950s and early 1960s. Their unparalleled effectiveness led to extensive use, increasing food production and controlling insect-borne diseases. However, excessive reliance on chemical pesticides eventually resulted in what became known as the “Chemical crisis,” characterized by escalating application rates due to the development of insect resistance (“History of IPM” n.d.). Public awareness of the environmental consequences of pesticide overuse increased significantly after the publication of *Silent Spring* in 1962 (Unsworth 2010).

Growing recognition of the adverse effects associated with heavy pesticide use shifted attention toward more comprehensive pest management strategies, leading to the formal adoption of Integrated Pest Management (IPM) in agriculture during the 1970s (“History of IPM” n.d.). IPM is defined as a multifaceted pest management approach that integrates various control strategies to maintain pest populations at tolerable levels while minimizing environmental impact (Alston 2011). Since its establishment, IPM has been widely regarded as one of the most effective and sustainable frameworks for insect pest management. Nevertheless, IPM is not without limitations. Many of these limitations arise from the difficulty in identifying and implementing the most appropriate combination of tactics under diverse agroecological conditions. These challenges have driven continued research efforts and stimulated the development of advanced biotechnological approaches for more precise and sustainable insect pest management.

In this review, we will explore emerging biotechnological approaches for insect pest management, highlighting their mechanisms, applications, and potential contributions to modern sustainable agriculture.

## CRISPR-Cas9 Applications for Insect Pest Management

CRISPR-Cas9 has emerged as a highly promising tool in modern insect pest management (Mir *et al.*, 2018). This genome-editing technology enables precise and targeted modification of genetic material, thereby offering significant opportunities for the development of pest-resistant crops, genetically engineered biological control agents, and direct genetic manipulation of pest populations. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems represent a next-generation genetic control strategy with broad potential applications in sustainable agriculture.

In the context of insect pest management, CRISPR-Cas9 technology is being explored for several innovative applications, including gene drive systems, induction of sex-specific sterility, and the development of pest-resistant crop varieties. These approaches provide highly specific, efficient, and potentially long-term solutions for controlling economically important insect pests while reducing reliance on conventional chemical insecticides.

### Gene drive systems and related CRISPR applications

One of the most promising applications of CRISPR in pest management is the development of gene drive systems, which are gene-editing technologies designed to increase the probability that a specific gene is inherited by offspring through biased inheritance, thereby enabling the rapid spread of desirable traits within populations (Lei-Chen *et al.* 2016). Drawing on the idea of selfish genetic elements that promote their own transmission regardless of host benefit (McFarlane *et al.* 2018), CRISPR-based gene drives have been engineered to introduce traits such as sterility or disease resistance, facilitating their swift propagation across generations (Hammond *et al.* 2016). Through targeted gene editing in pest species, gene drives can disseminate characteristics like infertility or reduced disease transmission; a well-known example involves malaria-resistant mosquitoes, where gene drives impair the insects’ capacity to transmit the pathogen (Curtis, 1968). These CRISPR-mediated modifications are inherited by nearly all progeny, leading to rapid suppression of pest populations and improved control of vector-borne diseases. Advances in site-specific genome editing have enabled successful demonstrations of gene drives in yeast (DiCarlo *et al.*, 2015), flies (Gantz and Bier, 2015), and mosquitoes (Gantz *et al.*, 2015), underscoring the broad applicability of this strategy for population management. Overall, CRISPR-based gene drives represent a potent approach for pest suppression, disease mitigation, and conservation, expanding genetic control strategies across diverse species (Esvelt *et al.*, 2014; Webber *et al.*, 2015).

In addition to gene drives, CRISPR/Cas9 technology has significantly improved the sterile insect technique (SIT) by enabling precise modification of reproduction-associated genes to induce sterility with minimal off-target effects; editing sex-specific or fertility-related genes generates sterile individuals whose mating with wild counterparts produces non-viable offspring, progressively reducing pest populations. Diseases such as yellow fever, dengue, chikungunya, and Zika are transmitted by *Aedes aegypti* Linnaeus (Qsim et al., 2017), and traditional SIT methods relied on releasing radiation-sterilized males to compete with wild males (Benedict and Robinson, 2003; Shaw and Catteruccia, 2019; Dame et al., 2009), whereas CRISPR now enables targeted gene knockouts, such as  $\beta$ 2-tubulin (B2t) mutation in *A. aegypti* causing complete male infertility (Chen et al., 2021). Similarly, CRISPR disruption of sex-specific doublesex (*dsx*) variants in *Spodoptera frugiperda* J.E. Smith reduced fecundity and fertility (Gu et al., 2022), while editing Serine protease 2 (Ser2) in *Spodoptera litura* Fabricius induced male sterility without affecting females (Bi et al., 2022). In *Bombyx mori* Linnaeus, mutation of *BmSfp62* impaired sperm motility and altered seminal protein gene expression, leading to male sterility (Xu et al., 2022), and knockout of *Hcdsx* in *Hyphantria cunea* caused genital abnormalities, partial sex reversal, and reduced fecundity through altered splicing and downstream gene expression (Li et al., 2020). Furthermore, CRISPR-mediated genetic modification of natural enemies can enhance biological control by improving traits such as feeding efficiency or reproductive capacity, as demonstrated in parasitic wasps (Bai et al., 2024), with cinnabar and vermilion frequently targeted for phenotypic studies (Liu et al., 2017; Zhang et al., 2024). In *Harmonia axyridis* Pallas, which exhibits multiple elytral colour forms (Gautier et al., 2018; Xiao et al., 2020) and preys on aphids (Osawa, 2000; With et al., 2002) synchronizing oviposition with aphid peaks (Hironori and Katsuhiko, 1997; Osawa, 2000), editing the dopa decarboxylase (DDC) gene resulted in melanin-deficient mutants with extended development yet increased predation (Wu et al., 2022). Additionally, CRISPR/Cas9 offers a precise alternative to conventional breeding for pest-resistant crops, which is often laborious and time-

consuming (Scheben et al., 2017); initiatives such as the i5k project have generated extensive genomic resources for insects (Thomas et al., 2020; Majhi et al., 2023), supporting identification of novel targets for CRISPR-based crop protection. CRISPR enables modification of plants to enhance resistance against pests (Wang et al., 2018a) and supports development of pest- and disease-resistant genotypes (Molla et al., 2021), with edited crops demonstrating improved resistance to pests such as *Chilo suppressalis* and *Nilaparvata lugens*, thereby reducing reliance on chemical pesticides.

### RNA Interference

Extensive research has been conducted on the application of RNA interference (RNAi) as a highly specific strategy for insect pest control [Zhu et al., 2020]. RNAi is a conserved molecular mechanism present in nearly all eukaryotic organisms and is responsible for sequence-specific gene silencing. The process is initiated by double-stranded RNA (dsRNA), either exogenous or endogenous, that is homologous to a target gene. Once inside the cell, dsRNA molecules are processed into small interfering RNAs (siRNAs) of approximately 21–23 nucleotides in length. These siRNAs are subsequently incorporated into the RNA-induced silencing complex (RISC), which utilizes the siRNA as a guide to recognize and degrade complementary messenger RNA (mRNA), thereby suppressing expression of the target gene [Meister and Tuschl, 2024].

Despite its considerable potential, RNAi efficiency varies significantly among different insect orders. It has been shown to be highly effective in Coleoptera, whereas its efficacy in Lepidoptera is comparatively lower. Intermediate and variable responses have been reported in Hemiptera and Diptera (Christiaens et al., 2021; Cooper et al., 2019; He et al. 2022). This variability is largely attributed to intrinsic biological factors, particularly differences in cellular uptake mechanisms and intestinal degradation of dsRNA (Christiaens et al., 2021).

Numerous studies have demonstrated the potential of RNAi-based approaches in insect pest management, and selected examples are summarized in Table 1.

Table 1. RNAi-mediated gene silencing in insect pests and target crops

| Sr. No. | Target insects                                   | Silenced gene                                      | Target crop | References             |
|---------|--|--|-------------|------------------------|
| 1       | <i>Diabrotica virgifera virgifera</i><br>LeConte | Suppression of target mRNA                         | Maize       | Baum et al. (2007)     |
| 2       | <i>Diabrotica v. virgifera</i>                   | <i>hunchback</i> (hb) and <i>brahma</i> (brm) gene | Maize       | Khajuria et al. (2015) |
| 3       | <i>Leptinotarsa decemlineata</i>                 | $\beta$ -actin gene                                | Potato      | Zhang et al. (2015)    |

|    |  |   |                    |                               |
|----|--|---|--------------------|-------------------------------|
| 4  | Lepidopteran                                     | dsRNA spray   | Maize              | Li <i>et al.</i> (2015)       |
| 5  | <i>Helicoverpa armigera</i>                      | Chitinase gene ( <i>HaCHI</i> )                         | Tomato,<br>Tobacco | Mamta <i>et al.</i> (2016)    |
| 6  | <i>Chilo suppressalis</i>                        | Aminopeptidase N genes ( <i>APN1</i> +<br><i>APN2</i> ) | Rice               | Qiu <i>et al.</i> (2017)      |
| 7  | <i>Leguminivora glycinivorella</i>               | SppPO-dsRNA   | Soybean            | Meng <i>et al.</i> (2017)     |
| 8  | <i>Helicoverpa armigera</i>                      | Juvenile hormone methyl transferase<br>(JHMT)           | Cotton             | Ni <i>et al.</i> (2017)       |
| 9  | <i>Diabrotica virgifera virgifera</i><br>LeConte | <i>Dvvrgr, dvbol</i>                                    | Maize              | Niu <i>et al.</i> (2017)      |
| 10 | <i>Leptinotarsa decemlineata</i>                 | <i>ECR</i> gene   | Potato             | Hussain <i>et al.</i> (2019)  |
| 11 | <i>Scirpophaga incertulas</i>                    | <i>AChE</i> – Acetylcholine esterase                    | Rice               | Kola <i>et al.</i> (2019)     |
| 12 | <i>Manduca sexta</i>                             | <i>v-ATPaseA</i> gene                                   | Tobacco            | Burke <i>et al.</i> (2019)    |
| 13 | <i>Bemisia tabaci</i>                            | <i>BATCTB</i> gene                                      | Tobacco            | Dong <i>et al.</i> (2020)     |
| 14 | <i>Aphis glycines</i>                            | <i>TREH, ATPD, ATPE, CHSI</i>                           | Soybean            | Yan <i>et al.</i> (2020)      |
| 15 | <i>Bemisia tabaci</i>                            | Phenolic glucoside<br>malonyltransferase                | Tobacco            | Xia <i>et al.</i> (2021)      |
| 16 | <i>Spodoptera littoralis</i>                     | Sl 102 immune gene                                      | Tobacco            | Di Lelio <i>et al.</i> (2022) |

### Marker-Assisted Selection

The use of molecular markers to assist phenotypic selections in crop improvement is known as marker-assisted selection (MAS). It involves selecting individuals based on their marker pattern (genotype) rather than their observable traits (phenotype) as shown in Fig 1. There are various types of molecular markers, such as single nucleotide polymorphism (SNP), have been recognised and have shown great promise in enhancing the efficiency and accuracy of conventional plant breeding. Molecular marker techniques are the most advanced method for transferring desired genes into desired crop plants in the required combination. It is the most widely used molecular techniques, and their application is a novel opportunity for increasing the yield of crop (Das *et al.*, 2017). MAS studies showed introgression of *Bph14* and *Bph15* through molecular marker-assisted selection (MAS) to enhance the resistance in Minghui 63 and its derived hybrids against BPH (Hu *et al.*, 2012). Resistance to bacterial blight (BB) and brown planthopper (BPH) was achieved in Yuehui9113 and F1 hybrids by pyramiding one BB resistance gene (*Xa21*) and two BPH resistance genes (*Bph14* and *Bph15*) in Yuehui9113 using a marker-assisted backcrossing (MABC) strategy combined with phenotypic selection (He *et al.*, 2019). Rice line, ASD7 expressing a BPH resistance gene *bph2* when crossed to a susceptible cultivar C418, a japonica restorer line and evaluated through marker-assisted selection (MAS)

exhibited significantly higher resistance against brown plant hopper *Nilaparvata lugens*, one of the most destructive pests of rice crop (Li-Hong *et al.*, 2006). Liu *et al.* (2016) investigated that the pyramiding of two brown plant hopper resistance genes *Bph3* and *Bph27* (t), into elite rice cultivars through marker-assisted pyramiding showed significantly enhanced resistance against BPH and reduction in the yield loss caused by BPH. Shabanmofred *et al.* (2015) developed rice cultivars through marker-assisted selection (MAS) that provided resistance in rice against biotypes 2 and 3 of brown plant hopper (BPH). Sharma *et al.* (2004) used marker-assisted pyramiding to successfully construct the *Bph1* and *Bph2* resistance genes on rice chromosome 12 to provide resistance against rice BPH. *et al.* As a result, using MAS to improve pest resistance would be very beneficial. There are various advantages of using MAS to enhance selection efficiency of insect resistant plants 1) It can be performed on seedling material, 2) less affected by environmental conditions, 3) MAS may be cost effective and faster than conventional phenotypic assays, 4) multiple markers can be evaluated using the same DNA sample etc. But the potential drawbacks of MAS are 1) Recombination between the marker and the gene of interest may occur, leading to inaccurate results 2) Incorrect estimates of QTL locations and effects may result in slower progress than expected, 3) Markers developed for MAS in one population may not be transferrable to other populations.

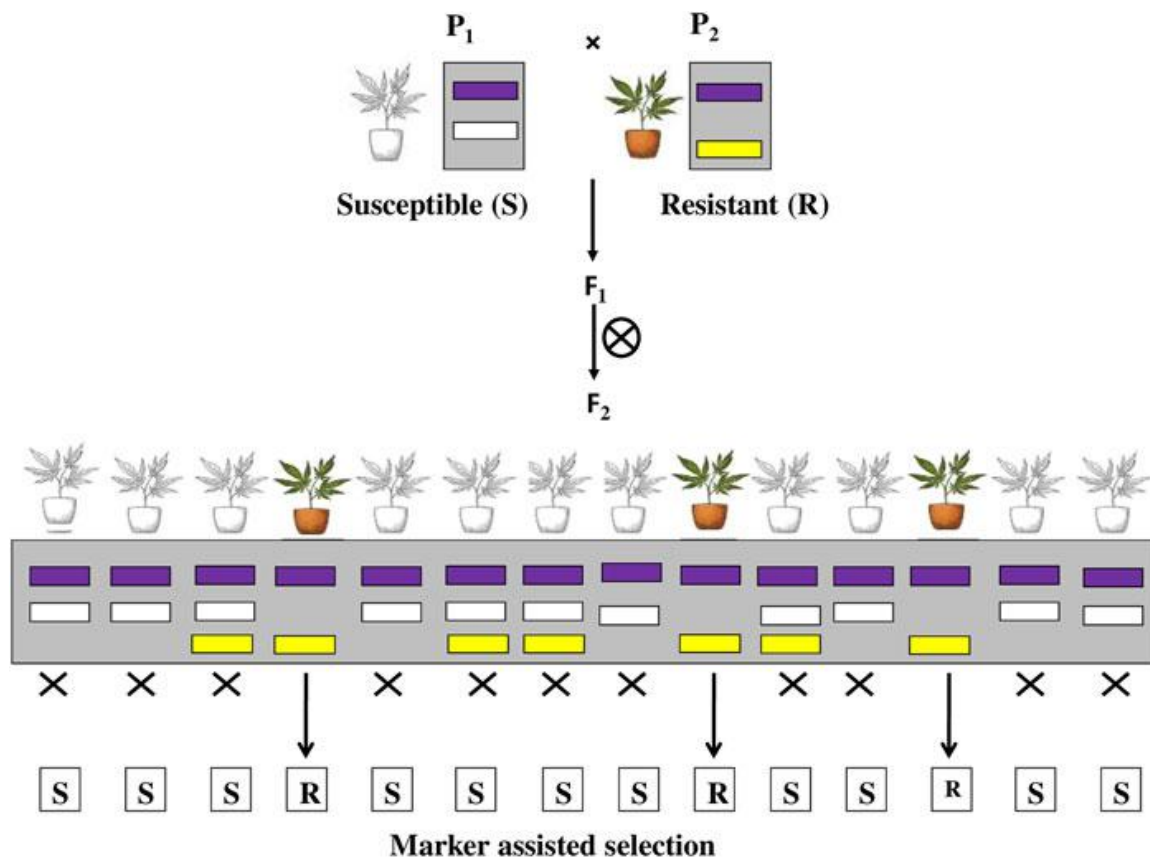


Fig. 1: Marker assisted selection

### Gene Transformation

Gene transformation, also referred to as genetic engineering of crops for insect resistance, involves the incorporation of specific DNA segments or genes into crop plants to confer protection against insect pests. The introduced DNA sequences typically encode proteins possessing insecticidal activity. Resistance in plants is achieved through the expression of these insecticidal proteins derived from the inserted genetic material, thereby providing targeted protection against specific insect pests (Gatehouse, 2013).

This technology has been evaluated against a broad spectrum of insect pests belonging to the orders Lepidoptera, Coleoptera, and Diptera (Birkett and Pickett, 2014). Among the most successful applications of genetic transformation is the development of genetically modified crops expressing insecticidal proteins from *Bacillus thuringiensis* (Bt), a soil-dwelling bacterium. Since their commercial introduction in 1996, Bt crops have been extensively cultivated worldwide and have played a significant role in modern agricultural pest management (Abbas, 2018).

The cry gene transformation technology involves the transfer of specific DNA sequences encoding Cry proteins

into crop plants through methods such as Agrobacterium-mediated transformation or particle bombardment (Juturu *et al.*, 2015). These transformation techniques enable stable integration and expression of insecticidal genes within the plant genome, resulting in enhanced resistance to target insect pests.

### Cry Genes

*Bacillus thuringiensis* (Bt) is a Gram-positive soil bacterium expressing insecticidal crystalline proteins (ICPs) that are exceptionally toxic to specific classes of pests (Panwar *et al.*, 2018). Insecticidal activity in insect-resistant Bt crops is expressed by the genes coding for parasporal crystal protoxins (Palma *et al.*, 2014). ICPs produced by transgenic plants have had a significant impact on the successful evolution of insect resistance. The crystal involves a protoxin protein which get solubilized in the larval midgut due to alkaline pH and subsequently cleaved enzymatically to an active toxin. The toxin diffuses through the peritrophic membrane covering the gut and binds to receptors present in the midgut epithelium (Paul and Das, 2020) making pores in the midgut epithelium. The gut gets paralyzed and then the pest stops feeding and dies within 2–3 days (Figure 2).

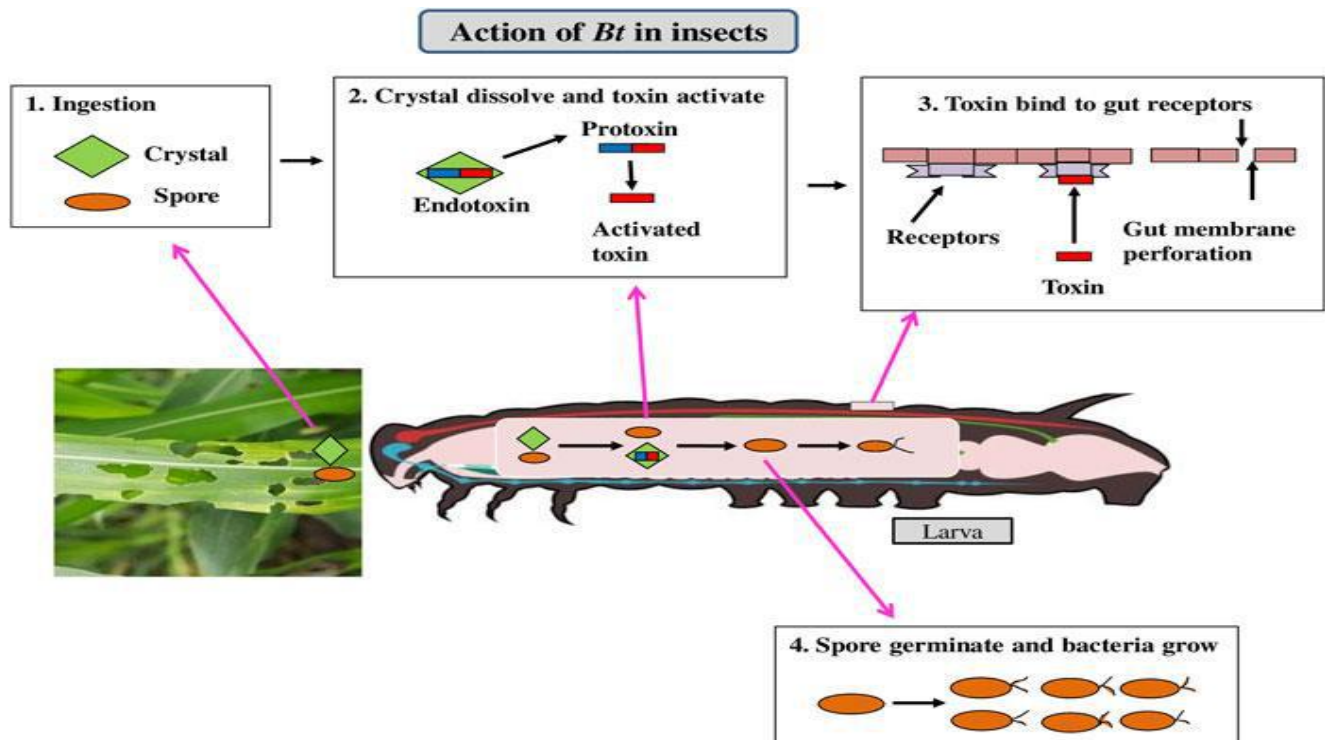


Fig. 2: Action of *Bt*. In insects

The first-generation *Bt* cotton, Bollgard I (BG I), expressing *cry1Ac*, was commercialized and released in 2002 for the management of major bollworm species, including *Pectinophora gossypiella*, *Earias vittella*, and *Helicoverpa armigera*, in cotton-growing regions of India. Subsequently, Bollgard II (BG II) was introduced in 2006 as a second-generation *Bt* cotton containing pyramided traits expressing *cry1Ac* and *cry2Ab* (MON15985 event). At present, BG II occupies nearly 95% of the total cotton cultivation area in India. Compared with BG I, which expresses only *cry1Ac*, BG II harboring both *cry1Ac* and *cry2Ab* demonstrates enhanced efficacy in pest management (Carrière *et al.*, 2015).

Another transgenic cotton variety, WideStrike cotton expressing *cry1Ac* + *cry1F*, was approved in the USA in 2004 by Dow AgroSciences, contributing to improvements in both crop yield and farmers' income. Both BG II and WideStrike cotton, with pyramided gene constructs, exhibit broader and more effective suppression of Lepidopteran, Coleopteran, and Dipteran pests than BG I.

Additionally, transgenic cotton containing *cry10Aa* displayed strong resistance against the cotton boll weevil (*Anthonomus grandis*), with 100% larval mortality observed in T1 generation bioassays following leaf consumption (Ribeiro *et al.*, 2017). Agrobacterium-mediated transformation has also been used to develop cotton lines expressing pyramided *cry1Ac* and *cry2Ab* genes within the T-DNA region, resulting in 93% larval

mortality of *S. litura* (Siddiqui *et al.*, 2019). Furthermore, transgenic rice lines engineered to express the *cry2AX1* gene showed effective resistance against rice leaf folder (*C. medinalis*) and rice yellow stem borer (*S. incertulas*) (Rajadurai *et al.*, 2018).

Transgenic cotton and brinjal varieties resistant to borers were approved for commercial cultivation in Bangladesh, while insect-resistant *Bt* soybean expressing *cry1Ac* + *cry1Ab* was authorized for production in Latin America in 2014 (Koch *et al.*, 2015). In another study, the introduction of a synthetic *cry1Ab* gene into tomato conferred effective resistance against the tomato leaf miner, *T. absoluta*, resulting in 100% mortality at the T0 generation within 4–5 days (Soliman *et al.*, 2021). Rice variety Bg94–1 developed through the transfer of the *cry2A* gene exhibited approximately 80% mortality in rice leaf folder populations (Gunasekara *et al.*, 2017).

Similarly, transgenic pigeon pea lines engineered with a combination of *cry1Ac* and *cry2Aa* demonstrated resistance to *H. armigera*, achieving 80%–100% larval mortality (Ghosh *et al.*, 2017). Expression of the *cry1Aa* gene in sweet potato provided resistance against the lepidopteran pest *Spodoptera litura* (Zhong *et al.*, 2019). In cotton event CH12, expression of the *cry2AX* gene resulted in 88% mortality of *H. armigera* at the T0 generation (Sakthi *et al.*, 2015), while expression of *cry2AX1* in the T3 generation of the same event produced 90% mortality (Jadhav *et al.*, 2020).

An industrially important non-edible castor developed through *Agrobacterium*-mediated transfer of the *cry1Aa* gene exhibited strong resistance against two lepidopteran pests, *Achaea janata* and *S. litura* (Muddanuru et al., 2019). Transgenic soybean expressing a *cry8*-like gene derived from *B. thuringiensis* conferred resistance to the coleopteran pest *Holotrichia parallela* (Qin et al., 2019). The transgenic cotton event MNH93 carrying *cry1Ab* showed 40–60% larval mortality against *H. armigera*, with a transformation frequency of 0.26% (Khan et al., 2011). Additional Bt-derived proteins, such as *cyt2Aa*, have been reported to provide resistance against aphids (Chougule et al., 2013), while *cry51Aa2* increased mortality of *Lygus* species in cotton (Gowda et al., 2016). The expression of cry genes across different crop species is summarized in Table 2.

Despite the successful application of cry gene technologies in developing insect-resistant crops, the evolution of resistance in target pest populations remains a major concern and can significantly threaten crop productivity. Other challenges limiting the long-term effectiveness of transgenic crops include secondary pest outbreaks, emergence of new pest biotypes, potential impacts on non-target organisms, environmental effects on transgene expression, biosafety concerns related to genetically modified foods, and various socio-economic and ethical considerations. Therefore, further research is needed to better understand plant–insect interactions and the mechanisms underlying resistance development in insects against cry genes.

Table 2. Transgenic crops carrying Bt genes for insect resistance.

| S. No. | Target insects  | Transgene              | Target crop  | References                 |
|--------|---|------------------------|--------------|----------------------------|
| 1      | <i>Chilo suppressalis</i> , <i>Cnaphalocrocis medinalis</i>     | <i>cry1Ab</i>          | Rice         | Fujimoto et al. (1993)     |
| 2      | <i>Scirpophaga incertulas</i> & <i>Chilo suppressalis</i>       | <i>cry1Ab</i>          | Rice         | Wünn et al. (1996)         |
| 3      | <i>Scirpophaga incertulas</i> , <i>Cnaphalocrocis medinalis</i> | <i>cry1Ab</i>          | Rice         | Ghareyazie et al. (1997)   |
| 4      | <i>Scirpophaga incertulas</i>                                   | <i>cry1Ac</i>          | Rice         | Nayak et al. (1997)        |
| 5      | <i>Scirpophaga incertulas</i>                                   | <i>cry1Ab/cry1Ac</i>   | Rice         | Tu et al. (2000)           |
| 6      | <i>Cnaphalocrocis medinalis</i> , <i>Scirpophaga incertulas</i> | <i>cry2A/cry1Ac</i>    | Rice         | Maqbool et al. (2001)      |
| 7      | <i>Helicoverpa armigera</i>                                     | <i>cry1Ab + NptII</i>  | Cotton       | Khan et al. (2011)         |
| 8      | <i>Heliothis</i> sp.  | <i>cry1Ab</i>          | Cotton       | Khan et al. (2013)         |
| 9      | <i>Helicoverpa armigera</i>                                     | <i>cry2AX</i>          | Cotton       | Sakthi et al. (2015)       |
| 10     | <i>Helicoverpa armigera</i>                                     | <i>cry1Ac + cry2Aa</i> | Pigeon pea   | Ghosh et al. (2017)        |
| 11     | <i>Helicoverpa armigera</i>                                     | <i>cryIIAa</i>         | Chickpea     | Sawardekar et al. (2017)   |
| 12     | <i>Cnaphalocrocis medinalis</i>                                 | <i>cry2A</i>           | Rice         | Gunasekara et al. (2017)   |
| 13     | <i>Tuta absoluta</i>  | <i>cry1Ac</i>          | Tomato       | Selale et al. (2017)       |
| 14     | <i>Anthonomus grandis</i>                                       | <i>cry1Aa</i>          | Cotton       | Ribeiro et al. (2017)      |
| 15     | <i>Helicoverpa armigera</i>                                     | <i>cry2Aa</i>          | Pigeon pea   | Baburao & Sumangala (2018) |
| 16     | <i>Helicoverpa armigera</i>                                     | <i>cry2Aa</i>          | Pigeon pea   | Singh et al. (2018)        |
| 17     | <i>Scirpophaga incertulas</i> , <i>Cnaphalocrocis medinalis</i> | <i>cry2AXI</i>         | Rice         | Rajadurai et al. (2018)    |
| 18     | <i>Spodoptera litura</i>  | <i>cry1Aa</i>          | Sweet potato | Zhong et al. (2019)        |
| 19     | <i>Spodoptera litura</i>  | <i>cry1Ac + cry2Ab</i> | Cotton       | Siddiqui et al. (2019)     |

|    |   |                  |         |                                |
|----|---|------------------|---------|--------------------------------|
| 20 | <i>Holotrichia parallela</i>            | <i>cry8-like</i> | Soybean | Qin <i>et al.</i> (2019)       |
| 21 | <i>Achaea janata, Spodoptera litura</i> | <i>cry1Ac</i>    | Castor  | Muddanuru <i>et al.</i> (2019) |
| 22 | <i>Helicoverpa armigera</i>             | <i>cry2AX1</i>   | Cotton  | Jadhav <i>et al.</i> (2020)    |
| 23 | <i>Tuta absoluta</i>                    | <i>cry1Ab</i>    | Tomato  | Soliman <i>et al.</i> (2021)   |

## Conclusion and Future Outlook

Insect pests continue to represent one of the most significant constraints to global agricultural productivity. Historically, farmers have relied heavily on chemical insecticides due to their rapid and visible effectiveness. However, increasing awareness of the adverse impacts of indiscriminate pesticide use on human and animal health, biodiversity, and environmental quality has intensified the search for safer and more sustainable pest management strategies. Within this framework, host plant resistance has emerged as an environmentally sound and economically viable cornerstone of Integrated Pest Management (IPM) programmes. The development and deployment of insect-resistant crop varieties provide long-term and cumulative suppression of pest populations while minimizing ecological disruption.

Substantial progress has been achieved in identifying resistant germplasm sources across diverse crop species. Nevertheless, conventional breeding for insect resistance remains laborious and time-intensive, particularly because many resistance traits are quantitatively inherited and controlled by multiple loci. Recent advances in biotechnology have revolutionized this field by offering precise, efficient, and multi-mechanistic approaches for pest control. Modern biotechnological tools facilitate the introduction of insecticidal genes, novel bioactive compounds, and engineered gene expression systems to enhance plant defence responses beyond the limitations of traditional breeding.

Several insect-resistant crops developed through biotechnological interventions—including maize, rice, cotton, canola, soybean, tobacco, apple, and potato—have demonstrated effective protection against economically destructive insect pests. The integration of advanced techniques such as genetic transformation, genome editing, marker-assisted selection, somaclonal variation, anther culture, embryo culture, and protoplast fusion is expected to further accelerate the development of improved resistant cultivars. The expression of bacterial delta-endotoxins, vegetative insecticidal proteins, and endogenous plant defence molecules such as lectins and protease inhibitors has significantly enhanced resistance levels in a shorter timeframe compared to conventional approaches.

More recently, RNA interference (RNAi) and CRISPR/Cas9-mediated genome editing have emerged as highly promising technologies for targeted and precise insect pest management. These approaches enable the silencing or modification of genes critical for insect survival, development, reproduction, or plant–insect interactions, thereby offering new opportunities for durable and species-specific resistance. Despite these technological advancements, the rapid evolution of resistance in insect populations against insecticidal toxins remains a persistent challenge that threatens the sustainability of current strategies.

Future research must prioritize a deeper mechanistic understanding of plant–insect interactions and resistance evolution to enhance the durability of engineered traits. The development of gene pyramiding strategies, resistance management frameworks, and integrated deployment within IPM systems will be essential to delay resistance development. Furthermore, comprehensive environmental risk assessments—particularly regarding impacts on non-target organisms and ecosystem dynamics—are critical prior to commercial release.

In addition to scientific considerations, socioeconomic and regulatory dimensions must not be overlooked. The implications of adopting biotechnological solutions, especially for smallholder and resource-limited farmers in developing countries, require careful evaluation. Robust regulatory frameworks, transparent risk–benefit analyses, and active engagement among researchers, policymakers, producers, and consumers are essential for responsible implementation. Public awareness and open dialogue will play a decisive role in fostering trust and acceptance of these emerging technologies.

In conclusion, biotechnology represents a powerful and transformative tool for advancing sustainable insect pest management. By enhancing crop resistance, reducing dependence on chemical pesticides, lowering production costs, and strengthening global food security, biotechnological innovations hold immense promise. When deployed responsibly, ethically, and within comprehensive regulatory and ecological safeguards, these approaches can substantially contribute to addressing current and future pest management challenges in agriculture.

## REFERENCES

- [1] Abbas, M. S. T. (2018). Genetically Engineered (Modified) Crops (*Bacillus Thuringiensis* Crops) and the World Controversy on Their Safety. *Egypt. J. Biol. Pest Control* 28 (1), 1–12. doi:10.1186/s41938-018-0051-2
- [2] Bai X, Yu K, Xiong S, Chen J, Yang Y, Ye X, Yao H, Wang F, Fang Q, Song Q, Ye G. 2024. CRISPR/Cas9-mediated mutagenesis of the white gene in an ectoparasitic wasp, *Habrobracon hebetor*. *Pest Management Science* 80(3): 1219-1227.
- [3] Benedict M Q, Robinson A S. 2003. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends in Parasitology* 19(8): 349-355.
- [4] Bi H, Xu X, Li X, Wang Y, Zhou S, Huang Y. 2022. CRISPR/Cas9 mediated Serine protease 2 disruption induces male sterility in *Spodoptera litura*. *Frontiers in Physiology* 13: 931824.
- [5] Birkett, M. A., and Pickett, J. A. (2014). Prospects of Genetic Engineering for Robust Insect Resistance. *Curr. Opin. Plant Biol.* 19, 59–67. doi:10.1016/j.pbi.2014.03.009
- [6] Carrière, Y., Crickmore, N., and Tabashnik, B. E. (2015). Optimizing Pyramided Transgenic Bt Crops for Sustainable Pest Management. *Nat. Biotechnol.* 33 (2), 161–168. doi:10.1038/nbt.3099
- [7] Chen J, Luo J, Wang Y, Gurav AS, Li M, Akbari OS, Montell C. 2021. Suppression of female fertility in *Aedes aegypti* with a CRISPR targeted male-sterile mutation. *Proceedings of the National Academy of Sciences* 2021: 2105075118.
- [8] Chougule, N. P., Li, H., Liu, S., Linz, L. B., Narva, K. E., Meade, T., et al. (2013). Retargeting of the *Bacillus Thuringiensis* Toxin Cyt2Aa against Hemipteran Insect Pests. *Proc. Natl. Acad. Sci. U.S.A.* 110 (21), 8465–8470. doi:10.1073/pnas.1222144110
- [9] Christiaens O., Sweet J., Dzhambazova T., Urru I., Smaghe G., Kostov K.V., Arpaia S. Implementation of RNAi-based arthropod pest control: Environmental risks, potential for resistance and regulatory considerations. *J. Pest Sci.* 2021;95:1–15. doi: 10.1007/s10340-021-01439-3.
- [10] Cooper A.M.W., Silver K., Zhang J., Parka Y., Zhua K.Y. Molecular mechanisms influencing efficiency of RNA interference in insects. *Pest Manag. Sci.* 2019;75:18–28. doi: 10.1002/ps.5126.
- [11] Curtis C F. 1968. Possible use of translocations to fix desirable genes in insect pest populations. *Nature* 218(5139): 368-369.
- [12] Dame D A, Curtis C F, Benedict M Q, Robinson A S, Knols B G. 2009. Historical applications of induced sterilisation in field populations of mosquitoes. *Malaria Journal* 8: 1-10.
- [13] Das, G., Patra, J. K., and Baek, K.-H. (2017). Insight into MAS: a Molecular Tool for Development of Stress Resistant and Quality of Rice through Gene Stacking. *Front. Plant Sci.* 8, 985. doi:10.3389/fpls.2017.00985
- [14] Deguine, J.P.; Aubertot, J.N.; Flor, R.J.; Lescourret, F.; Wyckhuys, K.A.G.; Ratnadass, A. Integrated pest management: Good intentions, hard realities. A review. *Agron. Sustain. Dev.* 2021, 41, 38.
- [15] DiCarlo J E, Chavez A, Dietz S L, Esvelt K M, Church G M. 2015. Safeguarding CRISPR-Cas9 gene drives in yeast. *Nature Biotechnology* 33(12): 1250-1255.
- [16] Esvelt K M, Smidler A L, Catteruccia F, Church G M. 2014. Concerning RNA-guided gene drives for the alteration of wild populations. *eLife* 3: 03401.
- [17] Gantz V M, Bier E. 2015. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. *Science* 348(6233): 442-444.
- [18] Gantz V M, Jasinskiene N, Tatarenkova O, Fazekas A, Macias V M, Bier E, James A A. 2015. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. *Proceedings of the National Academy of Sciences* 112(49): 6736-6743.
- [19] Gatehouse, J. A. (2013). “Genetic Engineering of Crops for Insect Resistance,” in *Sustainable Food Production*. Editors P. Christou, R. Savin, B. A. Costa-Pierce, I. Misztal, and C. B. A. Whitelaw (New York: Springer).
- [20] Gautier M, Yamaguchi J, Foucaud J, Loiseau A, Ausset A, Facon B, Gschloessl B, Lagnel J, Loire E, Parrinello H, Severac D. 2018. The genomic basis of color pattern polymorphism in the harlequin ladybird. *Current Biology* 28(20): 3296-3302.
- [21] Ghosh, G., Ganguly, S., Purohit, A., Chaudhuri, R. K., Das, S., and Chakraborti, D. (2017). Transgenic Pigeonpea Events Expressing Cry1Ac and Cry2Aa Exhibit Resistance to *Helicoverpa Armigera*. *Plant Cell Rep.* 36 (7), 1037–1051. doi:10.1007/s00299-017-2133-0
- [22] Gowda, A., Rydel, T. J., Wollacott, A. M., Brown, R. S., Akbar, W., Clark, T. L., et al. (2016). A Transgenic Approach for Controlling *Lygus* in Cotton. *Nat. Commun.* 7 (1), 12213. doi:10.1038/ncomms12213
- [23] Gu J, Wang J, Bi H, Li X, Merchant A, Zhang P, Zhang Q, Zhou X. 2022. CRISPR/Cas9-mediated mutagenesis of sex-specific doublesex splicing variants leads to sterility in *Spodoptera frugiperda*, a global invasive pest. *Cells* 11(22): 3557.
- [24] Gunasekara, J., Jayasekera, G., Perera, K., and Wickramasuriya, A. (2017). Development of a Sri Lankan Rice Variety Bg 94-1 Harbouring cry2A Gene of *Bacillus Thuringiensis* Resistant to Rice Leafhopper [*Cnaphalocrocis Medinalis* (Guenée)]. *J. Natn. Sci. Found. Sri Lanka* 45 (2), 143. doi:10.4038/jnsfsr.v45i2.8180
- [25] Gurr GM, Wratten SD, Altieri MA (Eds.). *Ecological engineering for pest management: Advances in habitat manipulation for arthropods*. CSIRO Publishing; 2004
- [26] Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russell S, Burt A. 2016. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology* 34(1): 78-83.
- [27] He L., Huang Y., Tang X. RNAi-based pest control: Production, application and the fate of dsRNA. *Front.*

- Bioeng. Biotechnol. 2022;10:1080576. doi: 10.3389/fbioe.2022.1080576.
- [28] He, C., Xiao, Y., Yu, J., Li, J., Meng, Q., Qing, X., et al. (2019). Pyramiding *Xa21*, *Bph14*, and *Bph15* Genes into the Elite Restorer Line Yuehui9113 Increases Resistance to Bacterial Blight and the Brown Planthopper in Rice. *Crop Prot.* 115, 31–39. doi:10.1016/j.cropro.2018.09.001
- [29] Hironori Y, Katsuhiko S. 1997. Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. *Biocontrol* 42(1-2): 153-163.
- [30] Hu, J., Li, X., Wu, C., Yang, C., Hua, H., Gao, G., et al. (2012). Pyramiding and Evaluation of the Brown Planthopper Resistance Genes *Bph14* and *Bph15* in Hybrid Rice. *Mol. Breed.* 29 (1), 61–69. doi:10.1007/s11032-010-9526-x
- [31] Jadhav, M. S., Rathnasamy, S. A., Natarajan, B., Duraiagaraja, S., and Varatharajalu, U. (2020). Study of Expression of Indigenous *Bt* Cry2ax1 Gene in T<sub>3</sub> Progeny of Cotton and its Efficacy against *Helicoverpa Armigera* (Hubner). *Braz. Archives Biol. Technol.* 63 (2), 2020. doi:10.1590/1678-4324-2020180428
- [32] Juturu, V. N., Mekala, G. K., and Kirti, P. B. (2015). Current Status of Tissue Culture and Genetic Transformation Research in Cotton (*Gossypium* spp.). *Plant Cell Tiss. Organ Cult.* 120 (3), 813–839. doi:10.1007/s11240-014-0640-z
- [33] Khan, G. A., Bakhsh, A., Riazuddin, S., and Husnain, T. (2011). Introduction of *cry1Ab* Gene into Cotton (*Gossypium Hirsutum*) Enhances Resistance against Lepidopteran Pest (*Helicoverpa Armigera*). *Span. J. Agric. Res.* 9, 296–302. doi:10.5424/sjar/20110901-136-10
- [34] Koch, M. S., Ward, J. M., Levine, S. L., Baum, J. A., Vicini, J. L., and Hammond, B. G. (2015). The Food and Environmental Safety of *Bt* Crops. *Front. Plant Sci.* 06, 283. doi:10.3389/fpls.2015.00283
- [35] Lei CHEN, Gui WANG, Ya-Nan ZHU, Xiang H, Wen WANG. 2016. Advances and perspectives in the application of CRISPR/Cas9 in insects. *Zoological Research* 37(4): 136.
- [36] Li X, Liu Q, Liu H, Bi H, Wang Y, Chen X, Wu N, Xu J, Zhang Z, Huang Y, Chen H. 2020. Mutation of doublesex in *Hyphantria cunea* results in sex specific sterility. *Pest Management Science* 76(5): 1673-1682.
- [37] Li, Y., Zhang, J., Chen, D., Yang, P., Jiang, F., Wang, X., et al. (2016). CRISPR/Cas9 in Locusts: Successful Establishment of an Olfactory Deficiency Line by Targeting the Mutagenesis of an Odorant Receptor Co-receptor (Orco). *Insect Biochem. Mol. Biol.* 79, 27–35. doi:10.1016/j.ibmb.2016.10.003
- [38] Li-Hong, S. U. N., Chun-Ming, W. A. N. G., Chang-Chao, S. U., Yu-Qiang, L. I. U., Hu-Qu, Z. H. A. I., and Jian-Min, W. A. N. (2006). Mapping and Marker-Assisted Selection of a Brown Planthopper Resistance Gene *bph2* in rice (*Oryza sativa* L.). *Acta Genetica Sinica* 33 (8), 717–723.
- [39] Liu H, Liu Q, Zhou X, Huang Y, Zhang Z. 2017. Genome editing of Wnt-1, a gene associated with segmentation, via CRISPR/Cas9 in the pine caterpillar moth, *Dendrolimus punctatus*. *Frontiers in Physiology* 7: 666.
- [40] Majhi P K, Bhoi T K, Sahoo K C, Mishra N, Tudu S, Das S, Ray M, Singh S, Samal I, Behera P P, Saini V. 2023. Understanding the genetics and genomics of vegetable grafting to ensure yield stability. In:
- [41] McFarlane G R, Whitelaw C B A, Lillico S G. 2018. CRISPR-based gene drives for pest control. *Trends in Biotechnology* 36(2): 130-133.
- [42] Meister G., Tuschl T. Mechanisms of gene silencing by double-stranded RNA. *Nature.* 2024;431:343–349. doi: 10.1038/nature02873.
- [43] Mir A, Alterman J F, Hassler M R, Debacker A J, Hudgens E, Echeverria D, Brodsky M H, Khvorova A, Watts J K, Sontheimer E J. 2018. Heavily and fully modified RNAs guide efficient SpyCas9 mediated genome editing. *Nature Communications* 9(1): 2641.
- [44] Molla K A, Sretenovic S, Bansal K C, Qi Y. 2021. Precise plant genome editing using base editors and prime editors. *Nature Plants* 7(9): 1166-1187.
- [45] Muddanuru, T., Polumetla, A. K., Maddukuri, L., and Mulpuri, S. (2019). Development and Evaluation of Transgenic castor (*Ricinus communis* L.) Expressing the Insecticidal Protein Cry1Aa of *Bacillus Thuringiensis* against Lepidopteran Insect Pests. *Crop Prot.* 119, 113–125. doi:10.1016/j.cropro.2019.01.016
- [46] Oerke, E.C. Crop losses to pests. *J. Agric. Sci.* 2006, 144, 31–43. [Google Scholar] [CrossRef]
- [47] Osawa N. 2000. Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): Resource tracking and population characteristics. *Population Ecology* 42: 115-127.
- [48] Palma, L., Muñoz, D., Berry, C., Murillo, J., and Caballero, P. (2014). *Bacillus Thuringiensis* Toxins: an Overview of Their Biocidal Activity. *Toxins* 6 (12), 3296–3325. doi:10.3390/toxins6123296
- [49] Panwar, B. S., Ram, C., Narula, R. K., and Kaur, S. (2018). Pool Deconvolution Approach for High-Throughput Gene Mining from *Bacillus Thuringiensis*. *Appl. Microbiol. Biotechnol.* 102 (3), 1467–1482. doi:10.1007/s00253-017-8633-6
- [50] Paul, S., and Das, S. (2020). Natural Insecticidal Proteins, the Promising Bio-Control Compounds for Future Crop Protection. *Nucleus* 64 (1), 7–20. doi:10.1007/s13237-020-00316-1
- [51] Qiu, L., Sun, Y., Jiang, Z., Yang, P., Liu, H., Zhou, H., et al. (2019). The Midgut V-ATPase Subunit A Gene Is Associated with Toxicity to Crystal 2Aa and Crystal 1Ca -expressing Transgenic Rice in Chilo Suppressalis. *Insect Mol. Biol.* 28 (4), 520–527. doi:10.1111/imb.12570
- [52] Qsim M, Ashfaq U A, Yousaf M Z, Masoud M S, Rasul I, Noor N, Hussain A. 2017. Genetically modified *Aedes aegypti* to control dengue: a review. *Critical Reviews in Eukaryotic Gene Expression* 27(4).
- [53] Rajadurai, G., Kalaivani, A., Varanavasiyappan, S., Balakrishnan, N., Udayasuriyan, V., Sudhakar, D., et al. (2018). Generation of Insect Resistant Marker-free

- Transgenic Rice with a Novel *cry2AX1* Gene. *Electron. Journ. Plan. Breed.* 9 (2), 723–732. doi:10.5958/0975-928x.2018.00086.8
- [54] Ribeiro, T. P., Arraes, F. B. M., Lourenço-Tessutti, I. T., Silva, M. S., Lisei-de-Sá, M. E., Lucena, W. A., et al. (2017). Transgenic Cotton Expressing Cry10Aa Toxin Confers High Resistance to the Cotton Boll Weevil. *Plant Biotechnol. J.* 15 (8), 997–1009. doi:10.1111/pbi.12694
- [55] Sakthi, A. R., Naveenkumar, A., Deepikha, P. S., Balakrishnan, N., Kumar, K. K., Devi, E. K., et al. (2015). Expression and Inheritance of Chimeric *cry2AX1* Gene in Transgenic Cotton Plants Generated through Somatic Embryogenesis. *Vitro Cell.Dev.Biol.-Plant* 51 (4), 379–389. doi:10.1007/s11627-015-9695-8
- [56] Scheben A, Wolter F, Batley J, Puchta H, Edwards D. 2017. Towards CRISPR/Cas crops—bringing together genomics and genome editing. *New Phytologist* 216(3): 682-698.
- [57] Shabanimofrad, M., Yusop, M. R., Ashkani, S., Musa, M. H., Adam, N. A., Haifa, I., et al. (2015). Marker-assisted Selection for Rice Brown Planthopper (*Nilaparvata Lugens*) Resistance Using Linked SSR Markers. *Turk J. Biol.* 39 (5), 666–673. doi:10.3906/biy-1406-78
- [58] Sharma, P. N., Torii, A., Takumi, S., Mori, N., and Nakamura, C. (2004). Marker-assisted Pyramiding of Brown Planthopper (*Nilaparvata Lugens* Stål) Resistance Genes *Bph1* and *Bph2* on Rice Chromosome 12. *Hereditas* 140 (1), 61–69. doi:10.1111/j.1601-5223.2004.01726.x
- [59] Sharma, S.; Kooner, R.; Arora, R. Insect Pests and Crop Losses. In *Breeding Insect Resistant Crops for Sustainable Agriculture*; Arora, R., Sandhu, S., Eds.; Springer: Singapore, 2017.
- [60] Shaw W R, Catteruccia F. 2019. Vector biology meets disease control: using basic research to fight vector-borne diseases. *Nature Microbiology* 4(1): 20-34.
- [61] Siddiqui, H. A., Asif, M., Asad, S., Naqvi, R. Z., Ajaz, S., Umer, N., et al. (2019). Development and Evaluation of Double Gene Transgenic Cotton Lines Expressing Cry Toxins for Protection against Chewing Insect Pests. *Sci. Rep.* 9 (1), 1–7. doi:10.1038/s41598-019-48188-z
- [62] Soliman, H. I. A., Abo-El-Hasan, F. M., El-Seedy, A. S., and Mabrouk, Y. M. (2021). *Agrobacterium*-mediated Transformation of Tomato (*Lycopersicon esculentum* mill.) Using a Synthetic Crylab Gene for Enhanced Resistance against *Tuta Absoluta* (Meyrick). *J. Microbiol. Biotechnol. Food Sci.* 7, 67–74. doi:10.15414/jmbfs.2017.7.1.67-74
- [63] Thomas G W, Dohmen E, Hughes D S, Murali S C, Poelchau M, Glastad K, Anstead C A, Ayoub N A, Batterham P, Bellair M, Binford G J. 2020. Gene content evolution in the arthropods. *Genome Biology* 21: 1-14.
- [64] Wang G, Dong Y, Liu X, Yao G, Yu X, Yang M. 2018a. The current status and development of insect-resistant genetically engineered poplar in China. *Frontiers in Plant Science* 9: 1408.
- [65] Webber B L, Raghu S, Edwards O R. 2015. Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *Proceedings of the National Academy of Sciences* 2015: 10565-10567.
- [66] Webber B L, Raghu S, Edwards O R. 2015. Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *Proceedings of the National Academy of Sciences* 2015: 10565-10567.
- [67] With K A, Pavuk D M, Worchuck J L, Oates R K, Fisher J L. 2002. Threshold effects of landscape structure on biological control in agroecosystems. *Ecological Applications* 12(1): 52-65.
- [68] With K A, Pavuk D M, Worchuck J L, Oates R K, Fisher J L. 2002. Threshold effects of landscape structure on biological control in agroecosystems. *Ecological Applications* 12(1): 52-65.
- [69] Wu MM, Chen X, Xu Q X, Zang L S, Wang S, Li M, Xiao D. 2022. Melanin synthesis pathway interruption: CRISPR/Cas9-mediated knockout of dopa decarboxylase (DDC) in *Harmonia axyridis* (Coleoptera: Coccinellidae). *Journal of Insect Science* 22(5): 1.
- [70] Xiao D, Chen X, Tian R, Wu M, Zhang F, Zang L, Harwood J D, Wang S. 2020. Molecular and potential regulatory mechanisms of melanin synthesis in *Harmonia axyridis*. *International Journal of Molecular Sciences* 21(6): 2088.
- [71] Xu X, Chen J, Du X, Yao L, Wang Y. 2022. CRISPR/Cas9-mediated disruption of seminal fluid protein Sfp62 induces male sterility in *Bombyx mori*. *Biology* 11(4): 561.
- [72] Zhang X, Singh A, Soriano Martinez K, Ferree P M. 2024. Direct parental (DIPA) CRISPR in the jewel wasp, *Nasonia vitripennis*. *G3: Genes, Genomes, Genetics* 14(7): 095.
- [73] Zhong, Y., Ahmed, S., Deng, G., Fan, W., Zhang, P., and Wang, H. (2019). Improved Insect Resistance against *Spodoptera Litura* in Transgenic Sweetpotato by Overexpressing Cry1Aa Toxin. *Plant Cell Rep.* 38 (11), 1439–1448. doi:10.1007/s00299-019-02460-8
- [74] Zhou, W.; Arcot, Y.; Medina, R.F.; Bernal, J.; Cisneros-Zevallos, L.; Akbulut, M.E.S. Integrated Pest Management: An Update on the Sustainability Approach to Crop Protection. *ACS Omega* 2024, 9, 41130–41147.
- [75] Zhu K.Y., Palli S.R. Mechanisms, Applications, and Challenges of Insect RNA Interference. *Annu. Rev. Entomol.* 2020;65:293–311. doi: 10.1146/annurev-ento-011019-025224.