

2.3 Target insect populations

Bt toxins are highly effective against a range of insect pests, particularly those in the orders Lepidoptera and Coleoptera. Lepidopteran pests, such as the cotton bollworm (*Helicoverpa armigera*), the diamondback moth (*Plutella xylostella*), and the cabbage looper (*Trichoplusia ni*), are among the primary targets of Cry1Ac and Cry1F toxins (Kain et al., 2022; Xiao et al., 2023). Coleopteran pests, such as the Colorado potato beetle (*Leptinotarsa decemlineata*), are targeted by other Cry proteins, such as Cry3A (Ren et al., 2021). The effectiveness of Bt toxins against these pests has led to their widespread use in transgenic crops, such as Bt cotton and Bt maize, which express these toxins to provide continuous protection against insect damage (Jurat-Fuentes et al., 2021; Heckel et al., 2021; Kain et al., 2022).

Bt toxins are a diverse group of insecticidal proteins with specific modes of action and target insect populations. Understanding the types, mechanisms, and target pests of Bt toxins is crucial for developing effective pest management strategies and mitigating the risk of resistance development.

3 Genetic Mechanisms of Resistance

The genetic resistance mechanisms of insect populations to Bt toxins include point mutations, deletions and insertions, gene silencing, gene amplification, and possible epigenetic modifications. These mechanisms alter the target sites or expression levels of proteins that interact with Bt toxins, thereby reducing the efficacy of these biopesticides and posing a significant challenge to their sustainable use in agriculture. In studies on the mode of action of Bt toxins, mutations in target genes are considered one of the key factors in the development of insect resistance (Figure 2). Research has shown that certain gene mutations in insects can alter the binding capacity of the toxin to its receptor, rendering the toxin inactive (Wang et al., 2019).

Figure 2 provides a detailed illustration of the receptor screening process for Bt toxins in insect cells. By overexpressing receptor proteins, the binding abilities of the toxin to different receptors were evaluated. These mutations weaken the binding capacity of Bt toxins to the receptors, leading to the development of resistance in insects. The varying binding effects of different toxins and receptors depicted in the figure offer critical experimental evidence for studying resistance mechanisms.

3.1 Mutations in target genes

3.1.1 Point mutations

Point mutations in target genes are a common mechanism by which insects develop resistance to Bt toxins. For instance, in the fall armyworm (*Spodoptera frugiperda*), point mutations in the *ABCC2* gene have been linked to resistance to the Cry1F protein (Guan et al., 2020). Similarly, a single point mutation in the cadherin gene of the cotton bollworm (*Helicoverpa armigera*) results in mislocalization of the cadherin protein, which underpins resistance to Cry1Ac toxin (Xiao et al., 2017). These mutations alter the structure and function of the proteins that Bt toxins target, thereby reducing the efficacy of the toxins.

3.1.2 Deletions and insertions

Deletions and insertions in target genes may also confer resistance to Bt toxins in insects. For example, in *Plutella xylostella* (diamondback moth), CRISPR/Cas9-mediated deletion mutations in the *PxABCC2* and *PxABCC3* genes resulted in high resistance to the Cry1Ac toxin (Guo et al., 2019). Studies have found multiple point mutations in target genes such as acetylcholinesterase 1 (*ace-1*), including A201S, G227A, and F290V, which are associated with resistance to organophosphates and pyrethroids (Figure 3). Similarly, in some populations of *Spodoptera frugiperda* in Brazil, a 12-base pair insertion mutation was found in the *ABCC2* gene, which is associated with resistance to Bt proteins (Guan et al., 2020). These genetic changes may lead to the production of truncated, non-functional proteins that are unable to effectively bind Bt toxins.

The study by Guo et al. (2019) illustrated four mutant alleles (r1-r4) of the *ABCC2* gene associated with resistance to Cry1F toxin, with r4 being a newly discovered mutation in this study. The figure details the positions of these mutations in both the genomic and protein structures, highlighting how these mutations may result in loss of function. This provides an important reference for understanding how deletions and insertions in target genes can confer resistance to Bt toxins in insects.