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Reason of Resistance Development in Pink Bollworms against Cry Proteins

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Introduction

Transgenic crops delivering Bt toxins are grown in millions of hectares. Although, the Bt crops increased the yield and reduced the use of conventional insecticide, however, their effectiveness would be decreased by evolution of resistance by insect pests. The first case of insecticide resistance has been reported in synthetic insecticide (DDT) within 6 years of its introduction (1948). The first case of insect-resistance to Bt crops was reported in Mississippi and Arkansas between 2003 and 2006 in bollworm *Helicoverpa zea.* This case was reported after 7 years after being introduced by Bt cotton. The *bollworm* resistance was discovered when an entomologist's team form University of Arizona investigated published data from monitoring studies of six main caterpillar pests of Bt crops in U.S, Australia, China and Spain. The different cases of resistance to Bt toxins has been stated in different Bt crops. The number of resistant species has been increased worldwide, 13 cases of field-developed resistance to 5 Bt toxins in transgenic corn and cotton. Most of the resistance reported cases belong to *Cry1A* family.

Mode of action of Cry toxins

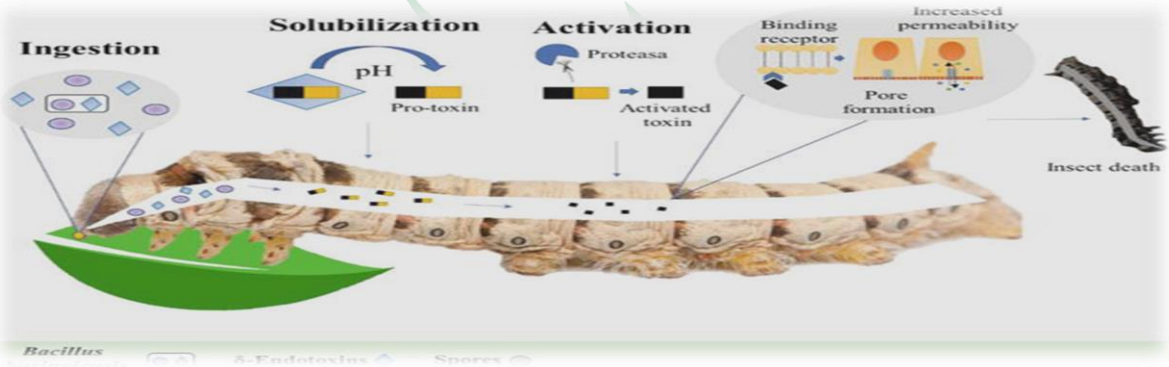

Figure 1: Mode of action of Cry toxins

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1. **Ingestion**
2. **Solublization**
3. **Proteolytic activation**
4. **Binding to target site**

It is widely accepted that the primary action of Cry toxins is to lyse midgut epithelial cells in the target insect by forming pores in the apical microvilli membrane of the cell.

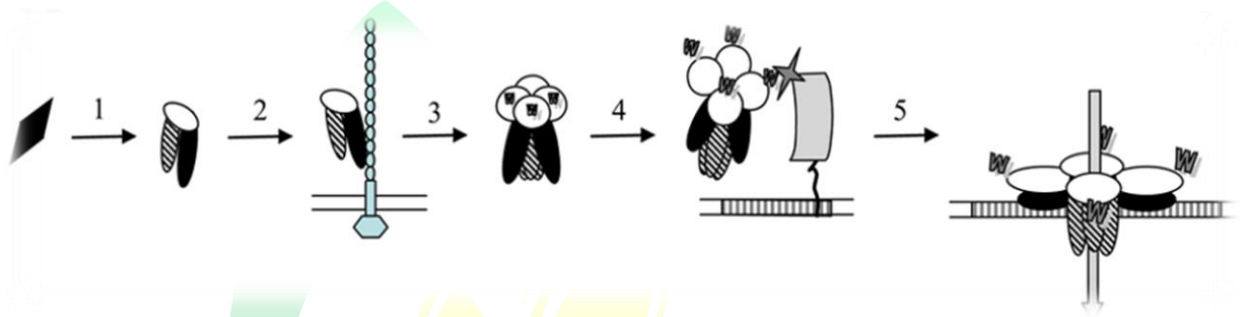

Figure 2: Mode of action of cry toxins at the molecular level

(1) Solubilization and activation of the toxin; (2). Binding of monomeric Cry toxin to the first receptor (CADR or GCR), conformational change is induced in the toxin and α-helix 1 is cleaved; (3) Oligomer formation; (4) Binding of oligomeric toxin to second receptor (GPI-APN or GPI-ALP), a conformational change occurs and a molten globule state of the toxin is induced; (5) insertion of the oligomeri toxin into lipid rafts and pore formation.

Findings that indicate the Reason of Resistance Development in Pink Bollworms Against Cry Proteins:

Fabrick and Tabashnik (2012) compared the genetic basis of resistance to Bt toxin Cry1Ac in two independently derived, laboratory-selected strains (Two resistance strain AZP-R and Bt4-R, one susceptible strain; APHIS-S) of a major cotton pest, the pink bollworm (*Pectinophora gossypiella* [Saunders]). Previous work showed that AZP-R had three recessive mutations (r^1, r^2, and r^3) in the pink bollworm cadherin gene (PgCad1) linked with resistance to Cry1Ac and Bt cotton producing Cry1Ac. Survival at the diagnostic concentration of Cry1Ac (10 mg Cry1Ac per mL diet) was 90% for resistant strain AZP-R and 0% for the susceptible strain APHIS-S, confirming previous results. Survival of resistant strain Bt4R was 75%, which suggests that it might have included a mixture of resistant and susceptible individuals. Survival of F_1 progeny from mass crosses between Bt4R and a

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susceptible strain did not differ between reciprocal crosses (i.e., R Bt4R6= APHIS-S versus R APHIS-S6= Bt4R), indicating autosomal inheritance. Survival was 0% for the F_1 progeny from crosses between Bt4R and a susceptible strain, indicating completely recessive resistance at the diagnostic concentration. Survival was also 0% for the F_1 progeny of a cross between AZP-R and the susceptible strain, which confirmed that resistance of AZP-R was completely recessive at the diagnostic concentration. The results reported here from complementation tests for allelism show that the same locus confers resistance in Bt4R and AZP-R. Molecular analysis revealed a new mutant cadherin allele (r4) in Bt4R.

Malthankar and Gujar (2015) Explored the ability of pink bollworm, to evolve resistance to Cry 2 Ab and characterized it in terms of inheritance. Sixteen day bioassay of Bt Cry 2Ab toxin against 5 day old pink bollworm larvae show medium lethal concentration (LC 50) ranging 0.16 – 1.44µ g/g diet for five different population collected from Srivilliputtur (TN), Jalgaon (MH), Bharuch (GJ), New Delhi and Sri ganganagar (RJ). The 5-day old larvae of pink bollworm population, Jalgaon-R showing the highest resistance to Cry2 Ab (LC 50 1.44µ g/g). Sixteen day bioassay of *Cry2Ab* against pink bollworm population show differences susceptibility. Jalgaon-R population was the least susceptible with LC 50 1.44µ g/g and Bharuch non Bt was the most susceptible. Besides Jalgaon are Jalgaon-S and Sri ganganagar population showed les susceptibility to Cry 2 Ab based on LC 50 data, the population which was found least susceptible to Cry 2 Ab was selected for evolution of resistance.

Akhtar *et al*. (2018) in this review, illustrated by examples, there were rare instances where dominant resistant alleles were found. Reason of resistance in fields may include different concentration of Bt protein used in different regions, this low concentration causes less binding of toxins to insect midgut. Mostly resistance development was observed under laboratory conditions against Cry proteins which are inserted in transgenic cotton. Under laboratory conditions, cadherin was found to be responsible for development of resistance in bollworm. Resistance developed in the boll worms was related to recessive alleles. Seven transgenic cotton varieties and genotypes were tested for field performance against insect pests infestation under laboratory and field conditions. Response of target insects varied from different cotton cultivars and genotypes

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Wang *et al.*(2018) reported the first case of a cadherin transmembrane mutation associated with insect resistance to a Bt crop. They used three strains of pink boll worm 1.AQ47 (Resistant) 2.AZP (Resistant) 3.APHIS (Susceptible)

Inheritance of resistance:

They used virgin adults in reciprocal mass crosses. We placed 20 APHIS-S females and 20 AQ47 males in one plastic box and 20 APHIS-S males × 20 AQ47 females in another, allowed the adults allowed to mate, and collected the F_1 eggs. We conducted bioassays as described above for AQ47, APHIS-S, and their F_1 progeny.

Genetic linkage between resistance to Cry1Ac and r^{13}: The test for genetic linkage between resistance to Cry1Ac and r^{13}, they generated F_1 progeny from a single-pair cross between a male from APHIS-S strain and a female from AQ47. They tested progeny from five backcross families, which were each produced by a single-pair cross between a male F_1 with a female from the resistant strain. Because crossing over in Lepidoptera occurs only in males, we used F_1 males to generate backcross families and to test the hypothesis that resistance is tightly linked with *PgCad1 (*Heckel *et al., 1999).* Sequencing of the full-length cDNA from AQ47 revealed that r^{13} has a 207 bp deletion and encodes a cadherin protein lacking 69 amino acids, including all 23 amino acids of the transmembrane domain, 40 amino acids of the membrane proximal region, and six amino acids of the cytoplasmic domain.

Future prospect

Bt is the most successfully used bio-pesticide in the agriculture sector, and its insecticidal protein genes are used to control insect pests in transgenic crops. Although, transgenic plants provide distinct opportunities for management of pest populations, but the development of insect-resistance threatens the continuous success of the transgenic crops delivering Bt toxins. The resistance to Bt crops could affect the long-term future of Bt applications. Furthermore, different strategies have been used to increase pest control efficiency and delay the evolution of resistance to Bt crops.

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