The Story of the Pink Bollworm: *Pectinophora gossypiella* (Saunders)

Abstract: Cotton, a vital commercial fibre crop, is prone to heavy infestations by insect pests, with bollworms being the most damaging globally. Helicoverpa armigera and other Heliothines have developed significant resistance to conventional insecticides, rendering cotton cultivation uneconomical in many regions by the mid-1990s. The introduction of Bt cotton, expressing Cry1Ac toxins, marked a breakthrough in bollworm management, drastically reducing pesticide usage and increasing yields. However, resistance to Cry toxins, particularly Cry1Ac, emerged over time, as first reported in pink bollworm (*Pectinophora gossypiella*) in Gujarat, India, by 2010. Factors contributing to resistance include mutations in genes such as *PgCad1*, ABC transporters, and others. Additionally, declining toxin expression in Bt cotton hybrids and improper pest management practices exacerbate resistance. Integrated pest management strategies, such as timely sowing, field sanitation, pheromone traps, and mating disruption tools, have been recommended to mitigate resistance. Recent data highlights increasing PBW infestations in North India, driven by factors like shorter cotton seasons and limited prior exposure to Bt toxins. Studies focusing on genetic and phenotypic polymorphism in Indian PBW populations have emphasized the need for sustainable resistance management. Adopting dual-toxin Bt cotton (Cry1Ac + Cry2Ab) and stringent IRM strategies remains critical to preserving the efficacy of transgenic technology.

Keywords: Cotton, *Bacillus thuringiensis*, Cry toxin, Pink bollworm, Resistance, Management

Introduction

Cotton is an important commercial fibre crop which is attacked by large number of insect pests throughout the season. Among them, the complex of *American*, pink, spotted, and spiny bollworms are said to be the most dreaded leading to heavy loss and requiring huge protection costs worldwide (Rajendran *et al*., 2018). However, in the USA cotton is attacked by *Heliothis virescens* (Fabricius) *and Helicoverpa zea* (Boddie) (Blanco, 2012), in India by *Helicoverpa armigera* (Hübner), and in Australia by *H. armigera* along with *H. punctigera* Wallengren (Downes and Mahon, 2012) are most significant bollworms. Out of the cost of cotton production in India, a lion shares of >43 per cent was speared targeting insect pest management, of which 80 per cent for bollworm control itself, particularly *H. armigera* (Kranthi, 2012). Heliothines caused a serious threat to upland cotton production worldwide including India due to their high degree of resistance to classical insecticides (organophosphates, carbamates and pyrethroids) in the late 1980s (Razaq, 2019). In India, *H. armigera* showed 15-fold resistance to quinalphos, 5 to 6500-fold to cypermethrin, >30 fold to methomyl, 16 to 3200-fold to fenvelerate, and 2 to 59-fold to endosulfan. Similarly, *Erias vittella* Fabricius also exhibited 70 to 362 folds resistance to these insecticides (Armes *et al.*, 1996). Hence by the mid-1990s cotton cultivation mostly turned uneconomical in major cotton growing countries (Kranthi, 2012, and Narayanamoorthy and Kalamkar, 2006) and more so in the Indian subcontinent and China. The use of insecticides having novel chemistry was too costly to rely upon. Meanwhile, efforts to popularize IPM strategies to manage bollworms remained enforcement activity rather than self-adaption by farmers (Kranthi, 2012). Hence as best-bet technology to manage bollworms, MON 531 (Cry1Ac gene) event-based *Gossypium hirsutum* varieties DP20B, DP50B, DP90B, NuCotn 33, and NuCotn 35 were planted successfully in the USA on a commercial scale during 1996 (Perlak *et al.*, 2001). By 2000 *Bt* cotton genotypes were approved in subsequently, other countries like Australia (1996), Argentina (1997), China (1997), Mexico (1998), South Africa (1998), Indonesia (2001) and Colombia (2002) (Steven *et al.*, 2008). In all these countries *H. armigera*, *H. zea*, *H. virescense*, *Earias* spp, and pink bollworm, *Pectinophora gossypiella* (Saunders) were controlled successfully. This enforced India also to accept transgenic technology by 2002 (Mohan and Manjunath, 2002) and three *G. hirsutum Bt* cotton hybrids (MECH 12, MECH 162, and MECH 184) were authorized for commercial cultivation (Khadi, 2007). Due to great bioefficacy against bollworms, the *Bt* cotton area increased drastically from 0.29 lakh ha in 2002-03 to 33.53 lakh ha in 2006-07 (Anonymous, 2020). In this period success of *Bt* transgenic was characterized by cent per cent bollworm check, a 40-60 percent reduction in pesticide usage in cotton (Khadi, 2007), and net profit enhanced to 78 per cent (Rao and Dev, 2009).

The resistance development to cry1Ac is an expected phenomenon from many of the laboratory studies refers no change in the susceptibility during the first 4-5 generations of *H. armigera* under selected pressure against Cry1Ac but, initial indications of resistance were clear after the 6th selection regimen and by the end of 10th generation, resistance increased 76-folds as reflected by the LC_{50} values (Kranthi *et al.*, 2000). Hence, to avoid/slowdown resistance development to Cry1Ac toxin in filed population growing of refugia imposed by EPA, US and all other Government agencies including GEAC in India, as resistance management strategies. Another IRM strategy for sustainability of transgenic technology under the threat potential of resistance development was keeping refugia also in use (Andow, 2008). This was the cultivation of genotypes expressing two toxins *i.e.*, Cry1Ac + Cry2Ab simultaneously. The Cry1Ac + Cry2Ab (MON 15985) cotton was progressively replacing Cry1Ac varieties (DP20B, DP50B, DP90B, NuCotn 33 and NuCotn 35) in the United States during 2003 and NuCotn 37, Deltapine varieties in Australia during 2004. The replacement was spurred by the idea that evolution of resistance would be delayed substantially by two-toxins relative to one toxin (Ali and Luttrell, 2007). In India also during 2006, Bollgard II was launched which has two genes, Cry1Ac + Cry2Ab with unrelated mode of action that aids in delaying resistance. By 2013, Bollgard II occupied 91 per cent of *Bt* cotton (Choudhary and Gaur, 2010) and by 2015, more than 95 per cent of *Bt* cotton with staked genes (Kranthi, 2016 and Komarlingam, 2018). However, the major gene in action is cry1Ac itself in all these genotypes (Anonymous, 2003). So far, over more than two decades field resistance to cry toxin has not been observed in any Heliothines including *H. armigera* in part of the world. But in 2010, pink bollworm, *P. gossypiella* showed resistance to Bollgard I event (Cry1Ac) in Gujarat, with a resistance ratio of 44 (Dhurua and Gujar, 2011) and subsequently to Bollgard II (Cry1Ac + Cry2Ab) with 40-80 per cent of the bolls harboured surviving larvae as recorded from Amreli and Bhavnagar districts of Gujarat during 2014 (Naik *et al*., 2018). From 2014 onwards, reports have highlighted the outbreak of pink bollworm on Bollgard I and Bollgard II in states namely, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, and Andhra Pradesh with a resistance ratio of 1387 to Cry1Ac and 4196 to Cry2Ab in 2017 from central and southern India (Naik *et al*., 2018). The PBW is essentially a monophagous pest, relying exclusively on cotton as its primary host, with no significant alternate hosts in Indian cotton ecosystems (Ingram, 1994). This fact was continued through a systematic survey in Karnataka (Rakhesh *et al*., 2023). PBW primarily feeds on developing seeds. The rapid and widespread adoption of *Bt* cotton, produced substantial quantities of *Cry* toxins in raw seeds (Kranthi *et al*., 2005) and bolls (Knight *et al*., 2013), leading to high selection pressure causing resistance. The exchange of seeds, transportation of seed cotton paves a way for the spreading of resistant population from one place to another place (Naik *et al*., 2020).

The resistance development might be due to various reasons

- Cry toxin expression is highest in leaves followed by squares, bolls, and flowers (Kranthi *et al*., 2005, Willrich *et al*., 2009, and Mahalakshmi and Prasad, 2013).
- Toxin expression decline after 90 days after sowing (Iqbal *et al*., 2013, and Bhullar and Gill, 2015).
- Feeding of segregating seeds in F_1 *Bt*-cotton hybrids (Shahid *et al*., 2021).
- Non-application of refugia (Shahid *et al*., 2021).
- Extending crop beyond time (Fand *et al*., 2019 and Krishna *et al*., 2020).
- Lack of timely and appropriate management initiatives (Krishna *et al*., 2020).
- Hybrids with varying flowering and fruiting periods (Fand *et al*., 2019 and Krishna *et al*., 2020).
- Cultivation of long duration hybrids (Fand *et al*., 2019).
- Long term storage of raw cotton in ginneries (Kumar *et al*., 2020).
- Nitrogen deficiencies and rainfed condition lowered the levels of toxin expression (Kranthi, 2012).

This resulted in more damage (40-95 %) with 20-30 per cent yield loss, despite the use of novel chemical insecticides and integrated pest management strategies by the farmers (Fand *et al*., 2019).

Following field scale survival many efforts have been made to know the resistance level in Indian PBW populations. The flower infested PBW from Junagadh, Amreli, Jalna, Wardha, Yavtmal, and Rajkot showed 3.6, 26.4, 3.2, 5.6, 1.4, 3.4- and 3.6-fold resistance to Cry1Ac, respectively; Adilabad, Prakasham, Wardha, Yavtmal and Jalna population showed 28.67, 18.67, 67.67, 20.67 and 630.67-fold resistance to Cry2Ab, respectively. Whereas, the boll infested PBW from Rajkot (Gujarat) recorded 371.8-fold to Cry1Ac and 4214.3-fold resistance to Cry2Ab (Naik *et al*., 2021) Similarly, Cry toxin resistance levels in pink bollworm across cotton-growing zones in India is represented in Table 1. Previous studies reported no incidence of pink bollworm (PBW) on Bt cotton in North India (Naik *et al*., 2018; Shantala, 2020). However, in recent years, the severity of green boll infestation by PBW has been increasing in the northern cotton-growing zones. The infestation levels have ranged from 10 to 100 percent in Punjab, with an average larval incidence of 25.20 %, and in Haryana, with 27.90 % larval incidence. In Rajasthan, infestation levels ranged from 10 to 30 percent, with an average larval incidence of 11.20 % (Prasad and Kumar, 2022; Sham, 2023 and Rakhesh 2024). The lower adaptability of PBW to Cry toxins in Northern India could be attributed to several factors. Notably, PBW populations in the region had not been exposed to BG-I cotton prior to the simultaneous introduction of both BG-I and BG-II for commercial cultivation in 2006. Additionally, the cotton-growing season in North India is relatively short, lasting only five to six months to accommodate subsequent wheat cultivation. This creates a closed season, significantly reducing selection pressure by limiting the number of PBW generations exposed to Bt cotton each year (Naik *et al*., 2018).

Mutations in specific genes are primarily responsible for Cry toxin resistance in the pink bollworm (*Pectinophora gossypiella*). The *PgCad1* gene, which encodes cadherin proteins, often exhibits mutations or deletions that reduce or eliminate the binding of Cry1Ac toxin to midgut receptors, a critical step for the toxin's action. Similarly, mutations in ATP-binding cassette (ABC) transporter genes impair the transport or activation of Cry toxins, thereby contributing to resistance. Alterations in aminopeptidase N (APN) genes further disrupt the binding of Cry toxins, compounding the resistance mechanism. Additionally, mutations in the alkaline phosphatase (ALP) gene, which is associated with receptor function, can hinder the effectiveness of Cry toxins in the insect gut. These genetic changes collectively enable the pink bollworm to withstand the toxic effects of Cry proteins (Tabashnik *et al*., 2004; Morin *et al.*, 2004; Malthankar and Gujar, 2015; Agrawal *et al*., 2020; Shanthala, 2020, and Fabrick *et al*., 2021). In addition to conventional diet incorporation bioassays used to assess resistance levels, efforts have been made to investigate Cry toxin resistance polymorphism in Indian pink bollworm (PBW) strains. However, these studies have predominantly focused on sequencing the mitochondrial *COI* gene (Naik *et al*., 2020). This has served as evidence for resistant population expansion. The Cry toxin resistance in PBW has been linked to certain mutations in cadherin genes and this is being validated presently in respect of Indian strains (Shanthala, 2020; Sham, 2023 and Rakhesh, 2024).

Integrated pest management practices:

- Timely sowing and narrowing sowing window (Henneberry *et al*., 1982).
- Implement field sanitation, clearing old stalks, and eliminating unopened and partly opened boll (Gutierrez *et al*., 2015).
- Select verified *Bt* cotton seeds for sowing.
- Monitoring of PBW through trap catches (8-10 moths for 3 consecutive days, Qureshi *et al.*, 1993) and 10 per cent flower/ green boll damage (ETL).

- Deploy pheromone traps for mass trapping of pink bollworm (>20 traps/ha) at 45 DAS.
- Spraying of Ovicides at 60 DAS (Udikeri *et al*.,2022).
- Two release of *T. bactre* @ 60000 per acre between 75-85 DAS (Udikeri *et al*.,2022).
- Use of mating disruption tools (SPLAT/ PB rope L).
- Synthetic pyrethroid spray after 100 DAS (Gopalaswamy *et al*., 2000 and Prasad *et al*., 2007).
- Timely crop termination (Javaid, 1995 and Khakwani *et al*., 2022)

Conclusion

Cotton is a crucial commercial crop, but it faces constant threats from a wide range of insect pests, with the bollworms *viz*., American, pink, spotted and spiny bollworms being among the most destructive. These pests cause significant yield losses and incur high control costs globally. In India, the primary bollworm species affecting cotton crops are *Helicoverpa armigera* and *Pectinophora gossypiella*, with resistance to chemical insecticides becoming a major concern since the late 1980s. This resistance, especially to organophosphates, carbamates, and pyrethroids, led to economic losses in cotton production, particularly in major growing countries like India and China. The introduction of Bt cotton, with its Cry1Ac toxin, revolutionized bollworm management by offering a more sustainable control method. Bt cotton significantly reduced pesticide use and increased profits, leading to its widespread adoption. However, the emergence of resistance in pink bollworm populations, particularly in Gujarat, raised concerns. Resistance to Cry1Ac and Cry2Ab toxins was observed in various cotton-growing regions, highlighting the need for robust resistance management strategies. Efforts to mitigate resistance include the development of Bt cotton varieties expressing multiple toxins, such as Cry1Ac and Cry2Ab, to delay the evolution of resistance. Integrated pest management (IPM) practices, such as timely sowing, field sanitation, the use of pheromone traps, and crop termination, are also essential for maintaining the efficacy of Bt cotton. Despite these challenges, ongoing research and management strategies remain critical for the sustainability of cotton production in India.

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Table 1. Cry toxin resistance levels in pink bollworm across cotton-growing zones in India

Cry toxins	Cotton growing zones			Year	Authors
	Southern	Central	Northern		
Resistance ratio against Cry1Ac (-folds)	18-47	18-121	Nil	2013	Naik *et al.*, 2018
	39	36-767	Nil	2014	
	36-551	13-1581	Nil	2015	
	19-343	36-2868	Nil	2016	
	798	704-2060	Nil	2017	
	-	372	-	2020	Naik *et al.*, 2021
	711-817	419-740	719-1042	2021	Sham, 2023
	386-858	535-960	306-856	2022	Rakhesh, 2024
Resistance ratio against Cry2Ab (-folds)	1	2-31	Nil	2013	Naik *et al.*, 2018
	1	3-423	Nil	2014	
	15-1604	36-2465	Nil	2015	
	192-5003	287-3779	Nil	2016	
	1570	1306-9366	Nil	2017	
	-	4214	-	2020	Naik *et al.*, 2021
	613-784	335-907	871-1038	2021	Sham, 2023
	809-1584	970-2096	630-1225	2022	Rakhesh, 2024