Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

Molecular profiling of resistance alleles in *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) collected from different locations

P. Likhitha[1*], V. Chinna Babu Naik[2], M. P. Moharil[3], D. B. Undirwade[1], U. S. Kulkarni[1] and A. V. Kolhe[1]

Abstract

Background After the commercialization of insect-resistant transgenic *Bt* cotton Bollgard I & II, India ranks first in the world in cotton production. Cotton insecticide consumption was drastically reduced as nearly 95% of the cotton area was replaced with Bollgard II. However, the benefits of transgenic cotton appear to have been diminished as the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) developed field resistance to Cry1Ac expressing *Bt* cotton in India in 2008. In 2015, there was an unusual survival of *P. gossypiella* on Bollgard II cotton in some parts of Gujarat and Maharashtra, which is a cause for concern.

Results In the present study, PCR analysis and gel visualization of BGII resistant field population of *P. gossypiella* showed that the presence of r1, r2, r3, r1s, r2s, r3s, and ss mutated cadherin alleles, which produced amplicon sizes of 750 bp, 700 bp, 730 bp, 12,700 bp, 750 bp, 980 bp, 600 bp and 1600 bp, respectively, when seven different types of cadherin allele(s) specific primers were used. The r1 mutant allele was detected in Amaravati, Wardha, Yavatmal, and Nagpur, but not in Akola and Buldhana, using primers r1disfor and Int-540. The r2 mutant allele was detected in Akola, Wardha, and Nagpur, but not in Buldhana, Amaravati, and Yavatmal, using primers r2disback and Cad2366. The r3 mutant allele was detected in all locations using primers r3disback and Cad3221. Amplicons of sizes 750 bp, 700 bp and 730 bp were obtained for r1, r2, and r3 mutant alleles, respectively. The sizes of the amplicons were 1270 bp for r1s, 750 bp for r2s, and 980 bp for r3s.The absence of bands for r1, r2, and r3 cadherin alleles in individuals indicated the possibility of having the ss allele, which was confirmed using primers Cad3324 and Int-651. The presence of ss mutant allele was observed in field-collected *P. gossypiella* populations from BG II cotton in various locations, with a 600 bp and 1600 bp size amplicons produced using the same primers.

Conclusions PCR analysis revealed the presence of r1, r2, r3, r1s, r2s, r3s, and ss mutated cadherin alleles in field-collected populations of Vidarbha which provide evidence to field-evolved resistance of *P. gossypiella* to BGII cotton.

Keywords *Bt* cotton, Cadherin receptor, Bollgard I & II, PCR analysis, Gel visualization, *Pectinophora gossypiella*

*Correspondence:
P. Likhitha
likhithaneelu@gmail.com
[1] Department of Agriculture Entomology, Post Graduate Institute, Dr. PDKV-Akola, Akola, Maharashtra 444001, India
[2] Department of Crop Protection, Central Institute for Cotton Research, Nagpur, Maharashtra 440010, India
[3] Biotechnology Centre, Post Graduate Institute, DR PDKV, Akola, Maharashtra 444001, India

Background

India is the largest cotton cultivator globally, and prior to the use of genetically modified *Bt* cotton, cotton yields were impacted by insect pests such as the bollworm complex. The adoption of *Bt* cotton hybrids, which produce Cry1Ac (BG I), has significantly impacted the management of bollworms in India, making it the world's largest

producer of cotton. *Bacillus thuringiensis(Bt)* is a bacterium that produces Cry proteins or endotoxins, and scientists have introduced genes from *Bt* into cotton plants to create transgenic *Bt*-cotton. The Cry genes from *Bt* var. *kurstaki* were transferred to cotton using Agrobacterium and CaMV 35S promoter. These genes produce crystalline endotoxins that disrupt the osmotic balance of susceptible insects' midgut, causing epithelial cell lysis, starvation, and ultimately death. Currently, there are over 700 Cry gene sequences that have been identified, coding for 78 different Cry proteins and 323 Cry protein holotypes (Mathew et al. 2018). *Pectinophora gossypiella* (Saunders) is a common pest of *Bt* cotton in India and worldwide, causing damage ranging from 10 to 50%. Cry1Ac and Cry2Ab are the *Bt* toxins widely used in insect-resistant transgenic crops. *Bt* cotton expressing the Cry1Ac gene (BG I) has controlled all bollworms, including *P. gossypiella*, since 2002. To delay bollworm resistance to BG I, a second-generation *Bt* cotton (BGII) expressing Cry1Ac + Cry2Ab was introduced, replacing over 95% of traditional cotton cultivation in India and reducing pesticide use. *P. gossypiella* has become more aggressive due to the development of insecticide and Cry toxin resistance. Survivability of *P. gossypiella* on Bollgard cotton was observed in Gujarat, (Monsanto Report, 2010) and larval survival on Bollgard II was significantly higher in some districts of Saurashtra. An outbreak of *P. gossypiella* in Maharashtra in 2016 (Naik et al. 2017) caused significant damage and yield losses in *Bt* cotton, as well as increased management costs in several states. Cry1Ac-resistant *P. gossypiella* has a higher survivability rate on BG I compared to dual-stacked cotton (Kranthi 2016). *P. gossypiella* populations in India have developed resistance to Cry1Ac, with an LC_{50} of 0.04 g (Murali-mohan et al. 2009). Cry2Ab-resistant *P. gossypiella* also showed cross-resistance to Cry1Ac, but Cry1Ac-resistant strains had low cross-resistance to Cry2Ab. After 5 years of exposure to Cry1Ac, the frequency of resistant individuals increased to 56% in Gujarat state. Cry toxins bind to specific receptors in the midgut of lepidopteran insects, causing cell lysis and death. Cadherin, aminopeptidase N, and alkaline phosphatase are crucial receptors for Cry toxin toxicity (Zhao et al. 2017). Insects can develop resistance to Cry toxins by altering their binding sites (Ferre and van Rie 2002). Despite a decade of exposure, *P. gossypiella* populations in the United States did not develop resistance to Bt cotton (Tabashink et al. 2006). Mutations in the cadherin gene confer Cry1Ac resistance in *P. gossypiella*, and 13 cadherin-resistant alleles have been identified in resistant *P. gossypiella* populations from the United States, India, and China (Morin et al. 2003). Alternative splicing of mRNA in India also results in cadherin mutations that confer Cry1Ac resistance in *P. gossypiella* (Fabrick et al. 2014). A mutation in the trans-membrane region of cadherin was found to induce Cry1Ac resistance in *P. gossypiella* (Wang et al. 2018). Current study aims to profile molecular resistance alleles in *P. gossypiella* populations from different locations.

Methods

Sample collection

Pink bollworm-infested mature green cotton bolls were collected at random from commercial/farmer cotton fields of BG-II in the Vidarbha districts of Akola, Buldhana, Amaravati, Wardha, Yavatmal, and Nagpur during October–November 2020, representing all cotton growing zones of Vidharba.

Genomic DNA extraction protocol

To extract DNA from the *P. gossypiella* larvae, 200 µl of ice-cold homogenization buffer was added to crush insects. Twenty-five µl of lysis buffer was then added and the mixture was incubated at room temperature for 20 min. Following this, 25 µl of 20% Sodium dodecyl sulfate (SDS) was added and the mixture was incubated at 65° C for 50 min. RNAase was then added at a concentration of 5 µl per tube and the mixture was incubated at 37° C for 15 min. Subsequently, 30 µl of potassium acetate buffer was added and the mixture was incubated at 4°C for 30 min (3 M for Potassium and 5 M for acetate). An equal volume of Phenol: Chloroform 1:1 [Phenol (5 ml); Chloroform Isoamylalcohol (5 ml)] and 500 µl Phenol: Chloroform Isoamylalcohol was added, and the mixture was centrifuged at 12,000 rpm for about 1 min. The aqueous layer was then collected and mixed with ice-cold 600 µl Isoproponal and incubated at -20° C overnight. The mixture was centrifuged at 12,000 rpm for 10 min and the supernatant was removed. The resulting pellet was washed with 500 µl of 70% ethanol, air-dried, and dissolved in 50 µl of TE buffer. Before being utilized in a PCR reaction, the DNA was diluted to a concentration of 100 ng/ml. The DNA concentration was measured using a NanoDrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and the quality was verified through 0.8% agarose gel electrophoresis. This ensured that the DNA was suitable for use as a template in the PCR reaction.

Quantification of genomic DNA

The isolated genomic DNA was quantified using the ethidium bromide fluorescent quantification method, as described by Maniatis et al. (1982). The band intensity of the isolated DNA in agarose, dissolved in 40 ml of 1XTAE buffer containing ethidium bromide at a concentration of 0.5 µg per ml, was compared to the band intensity of a reference λ DNA Hind III digest. In addition to

this method, the quality and concentration of the DNA were also determined, using a spectrophotometer and by gel electrophoresis with 1.2% agarose and known concentrations of uncut λ DNA.

$$\text{DNA } (\mu g/\mu l) = \frac{\text{OD260} \times 50(\text{Dilution factor}) \times 50\ \mu l/\text{ml}}{1000}$$

The purity of the DNA was determined by measuring the ratio of OD260 to OD280. A ratio of 1.6 or less indicates that the sample may contain proteins and/or other UV absorbers, while a ratio greater than 2.0 suggests the presence of contamination from chloroform or phenol.

PCR amplification

Mutations in the cadherin gene were amplified from 6 populations of *P. gossypiella,* using the cadherin gene-specific primers (Table 1) provided below as described by Morin et al. (2003).

PCR reactions were performed with a total volume of 20 µl, comprising 2.5 µl of 10×PCR buffer, 0.4 µl of dNTPs, 0.5 µl of each primer, 0.25 µl of Taq DNA polymerase (M/S Bangalore Genei Pvt. Ltd., Bangalore), 14.85 µl of water, and 1 µl of genomic DNA. A touchdown thermal cycling protocol, covering a range of annealing temperatures from 55 to 50°C, was also used for amplification in a Thermal Cycler (Bio-Rad, Hercules, CA). The cycling began with an initial denaturation at 94°C for 5 min. The cycling parameters were then set at 39 cycles of 94°C for 20 s, 55°C for 1 min, and 72°C for 1 min (Table 2), followed by a final cycle of 1 min at 55°C and a 7-min extension step at 72°C.

Table 1 PCR Primers used for determining cadherin genotypes

Allele	Sequence	Primers
r1	5′AATTACAATCCTCACTGACGAC 3′	r1disfor
	5′CTAACTATTTGCTTCTGACTG 3′	Int-540
r2	5′CTACAGACATTGATGGCCCAC 3′	Cad2366
	5′AGGTGCCAACGAGGTGAGAGT 3′	r2disback
r3	5′TGTCGCCTAACAACGTAACCG 3′	Cad3221
	5′CGTCCACACACGACAACGGTC 3′	r3disback
r1s	5′AGTTCGTTTTTCCTGAATCCGG 3′	Cad3386
	5′TGCCGATGTATTTGGTCACTT 3′	Int-651
r2s	5′CTACAGACATTGATGGCCCAC 3′	Cad2366
	5′AGTTGAGGTGCCAACGAGGTG 3′	r2allback
r3s	5′TGTCGCCTAACAACGTAACCG 3′	Cad3221
	5′TGCCGATGTATTTGGTCACTT 3′	Int-651
ss (control)	5′TCAGCAGATATCTCATGAGGTGT 3′	Cad3324
	5′TGCCGATGTATTTGGTCACTT 3′	Int-651

Results

Detection of r alleles using allele-specific cadherin gene primers

Three allele-specific PCR reactions were performed, each with an allele-specific primer, to selectively amplify the respective r allele. Different allele-specific primers namely; r1disfor and Int-540 for r1, r2disback, Cad2366 for r2 and r3disback, and Cad3221 for r3 were used during the present investigation. After the PCR reaction, electrophoresis was carried out to see the presence of r1, r2, and r3 mutant allele(s) in field-collected *P. gossypiella* population collected from BG II cotton. Visualization of gel images states the presence of r1, r2, and r3 mutant alleles in BG II resistant field populations of collected *P. gossypiella*. A 750 bp size amplicons were obtained when primers r1disfor and Int-540 were used to detect the presence of the r1 mutant allele. The r1 mutant allele is detected in locations of Amaravati, Wardha, Yavatmal, and Nagpur except for Akola and Buldhana (Fig. 1). A 700 bp size amplicons were produced when primers r2disback and Cad2366 were used to detect the presence of r2 mutant allele. r2 mutant allele is detected in locations of Akola, Wardha, and Nagpur, except for Buldhana, Amaravati, and Yavatmal (Fig. 2). A 730 bp size amplicons were produced when primers r3disback and Cad3221 were used to detect the presence of r3 mutant allele. r3 mutant allele was detected in locations of Akola, Buldhana, Amaravati, Wardha, Yavatmal, and Nagpur (Fig. 3).

Discrimination between resistant homozygotes and heterozygotes using allele-specific cadherin gene primers

PCR reactions were done to discriminate between homozygous resistant (r1r1, r2r2, or r3r3) alleles and heterozygous resistant (r1s, r2s, or r3s) alleles. Primers used were Cad3386 and Int-651, Cad2366 and r2allback and Int-651and Cad3221 and Int-651. After the PCR

Table 2 Different cycles of DNA amplification of pink bollworm population through PCR Primers

Cycle	Denaturation		Annealing		Extension	
First cycle	94° C	5 min	–	–	–	–
2–40 cycles	94° C	20 s	55° C	1 min	72° C	1 min
Last cycle	–	–	55° C	1 min	72° C	7 min

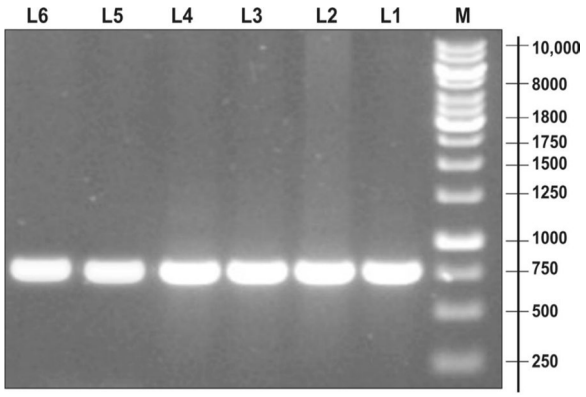

Fig. 1 PCR amplified r1 cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using r1disfor &Int-540. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati, Lane 4 = Warda, Lane 5 = Yavatmal,Lane 6 = Nagpur

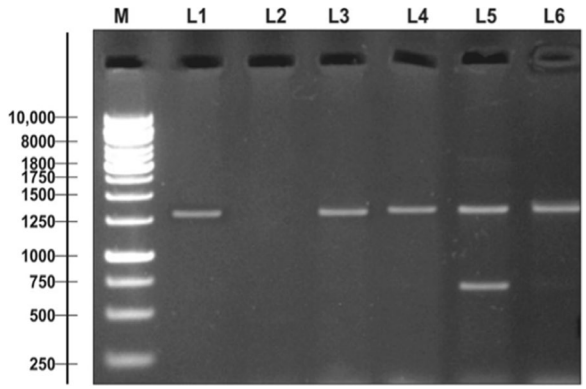

Fig. 4 PCR amplified r1s cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using Cad3386 &Int-651. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati, Lane 4 = Warda, Lane 5 = Yavatmal, Lane 6 = Nagpur

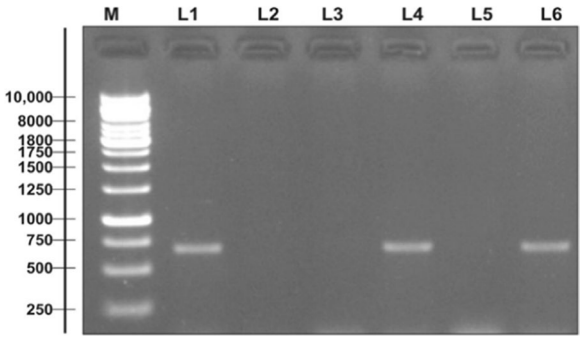

Fig. 2 PCR amplified r2 cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using Cad2366 & r2disback. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati, Lane 4 = Warda, Lane 5 = Yavatmal, Lane 6 = Nagpur

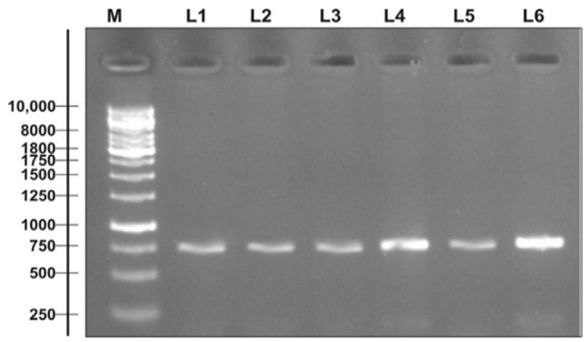

Fig. 5 PCR amplified r2s cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using Cad2366 & r2allback. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati, Lane 4 = Warda, Lane 5 = Yavatmal, Lane 6 = Nagpur

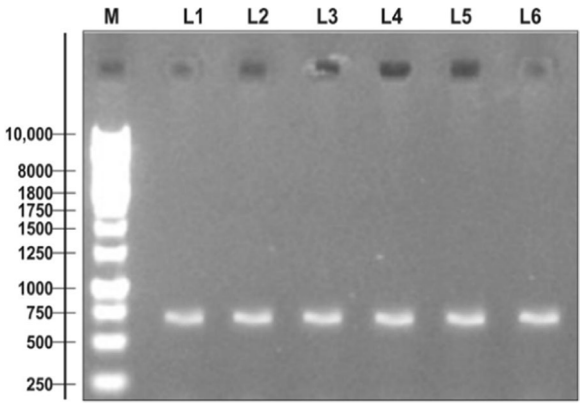

Fig. 3 PCR amplified r3 cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using Cad3221 &r3disback. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati, Lane 4 = Warda, Lane 5 = Yavatmal, Lane 6 = Nagpur

reaction, electrophoresis was carried out to see the presence of r1s, r2s and r3s mutant allele(s) in the field-collected *P. gossypiella* population collected from BG II cotton. Visualization of gel images states the presence of r1s, r2s, and r3s mutant allele in BG II resistant field populations of *P. gossypiella*. A 1270 bp size amplicon was produced when primers Cad3324 and Int-651 were used to detect the presence of the r1s mutant allele. r1s mutant allele was detected in locations of Akola, Amaravati, Yavatmal, and Nagpur, except for Buldhana and Wardha (Fig. 4). A 750 bp size amplicons were produced when primers Cad2366 and r2allback were used to detect the presence of r2s mutant allele. r2s mutant allele was detected in locations of Akola, Amaravati, Wardha, Yavatmal, and Nagpur except for Buldhana

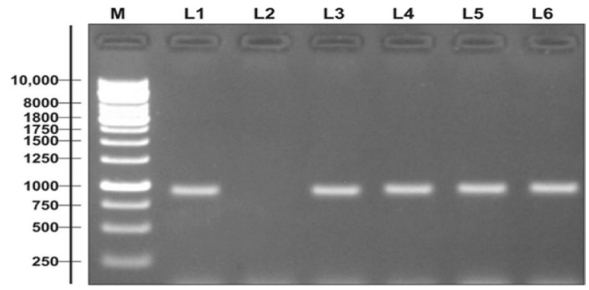

Fig. 6 PCR amplified r3s cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using Cad3221 &Int-651. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati,Lane 4 = Warda, Lane 5 = Yavatmal, Lane 6 = Nagpur

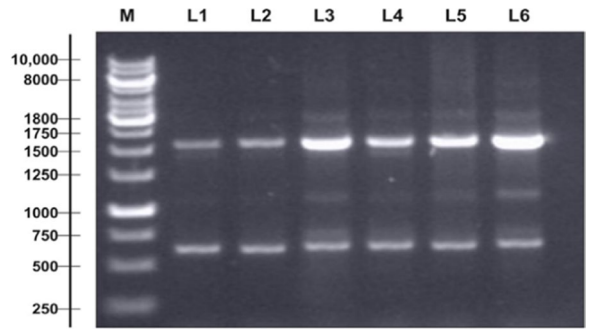

Fig. 7 PCR amplified ss cadherin gene in BG II resistant populations of *Pectinophora gossypiella* using Cad3324 & Int-651. M = 1 Kb plus DNA ladder, Lane 1 = Akola, Lane 2 = Buldana, Lane 3 = Amravati, Lane 4 = Warda, Lane 5 = Yavatmal, Lane 6 = Nagpur

(Fig. 5). A 980 bp size amplicons were produced when primers Cad3221 and Int-651 were used to detect the presence of r3s mutant allele. r3s mutant allele was detected in locations of Akola, Amaravati, Wardha, Yavatmal, and Nagpur except for Buldhana (Fig. 6).

Individuals that produce no bands when tested for their presence of r1, r2, and r3 cadherin alleles could be ss. However, it was again reconfirmed using primers Cad3324 and Int-651. After the PCR reaction, electrophoresis was carried out to see the presence of ss mutant allele(s) in the field-collected *P. gossypiella* population from BG II cotton. Visualization of gel images states the presence of ss mutant allele in BG II resistant field-collected populations of *P. gossypiella* in Akola, Buldhana, Amaravati, Wardha, Yavatmal, and Nagpur locations. A 600 bp and 1600 bp size amplicons were produced when primers Cad3324 and Int-651 were used to detect the presence of the ss mutant allele (Fig. 7).

Discussion

Findings of the present study suggested that mutations in the cadherin receptor had a major role in BG II resistance in populations of *P. gossypiella*. The findings are consistent with the presence of the same resistant alleles in Cry1Ac-resistant Amreli populations of *P. gossypiella* in India reported by Dhurua and Gujar (2011). Similarly, Morin et al. (2004) found that in many insects, reduced binding of Cry toxins to different receptors such as cadherin, alkaline phosphatase, and aminopeptidase N is the most accepted Mode 1 type of resistance to Cry1Ac toxin. *P. gossypiella* resistance to Cry1Ac toxin in the United States has been linked to mutations in cadherin receptors r1, r2, and r3. Fabrick et al. (2014) discovered eight different mutated cadherin alleles in Cry1Ac resistance, namely r5 to r12, they further found that these alleles are associated with mutations disrupting the extracellular domain of cadherin receptors that bind to Cry1Ac toxin. Mechanisms underlying *P. gossypiella* resistance against Cry2Ab and thereby to BG II were unknown. Similarly, Wang et al. (2018) found *P. gossypiella* to be 220 times more resistant to Cry1Ac and 2.1 times more resistant to Cry2Ab in China. In Cry1Ac-resistant *P. gossypiella*, the new mutant allele r13 disrupts cadherin cellular trafficking in the trans-membrane region. As a result, single-point mutations in *H. armigera* mislocalized cadherin receptors in Cry1Ac-resistant individuals, affecting membrane receptor trafficking. Although different alleles are responsible for Cry1Ac resistance in *P. gossypiella* studies on trans-membrane mutations in cadherin receptors need to be prioritized to elucidate the mechanism of Cry2Ab and BG II resistance in *P. gossypiella* in India. Similarly, Wang et al. (2019) discovered the most common resistance allele (r13) in the United States, accounting for more than 71% of all resistance alleles detected. Correspondingly Wang et al. (2019) discovered that the r16 allele, which disrupts cadherin localization mediates Cry1Ac resistance in *P. gossypiella*. Likewise, Wang et al. (2020) discovered that the r14 allele of the *P. gossypiella* cadherin gene (PgCad1) was recessive and was tightly linked to r14, which reduced translation, increased degradation and/or mislocalized cadherin. Host specificity and toxicity of different groups of *Bt* Cry toxin vary. Although the molecular interactions of Cry toxins are not fully understood, Cry toxins' binding to specific midgut proteins varies depending on the toxins and hosts. To be effective against insects, *Bt* Cry toxins must bind to GPI-anchored receptors found in brush border membrane vesicles in the insect's midgut. The most widely accepted theory in the laboratory and field-selected Cry1Ac-resistant strains of many insects were that toxin binding to the midgut epithelium was reduced (Wu 2014). Caccia et al. (2010) discovered that binding site alteration is a mechanism of resistance in both Cry1A and Cry2A class proteins in many insects. Previous researchers reported mutations in cadherin receptors for Cry1Ac resistance, but little work has been reported on enzymatic activity

in various insects. Earlier reduced membrane-bound alkaline phosphate levels in Cry1Ac-resistant *Helicoverpa virescens* were linked to resistance development, according to Jurat-Fuents and Adang (2004). Cry1Ac resistance was conferred in Indian populations of *P. gossypiella* by reduced binding of Cry1Ac to larval brush border membrane vesicles (BBMV) (Ojha et al. 2014). Previous and present data on the mechanism of resistance in *P. gossypiella* to Cry2Ab and thereby to BG II suggest that more research is needed to identify the new sights and more emphasis may be placed on the role of different resistant alleles, alkaline phosphatases, ABC transporters and gut inhabiting symbionts to accelerate monitoring and management of field-evolved resistance to Cry2Ab.

Conclusion

PCR analysis of the field population of *P. gossypiella* revealed the presence of various mutated cadherin alleles (r1, r2, r3, r1s, r2s, r3s, and ss) that produced different amplicon sizes when seven different types of cadherin allele-specific primers were used. The gel visualization of the results suggested that the field population has evolved resistance to BGII cotton.

Abbreviations

DNA	Deoxyribose nucleic acid
dNTP	Deoxyribo nucleoside triphosphate
PCR	Polymerase chain reaction
Taq	*Thermus aquaticus*
µl	Microlitre

Acknowledgements

The first author expresses gratitude to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and the Director, CICR- Nagpur for providing financial assistance, support, and reliability throughout the study. The first author also expresses appreciation for the scholarship award from Jawaharlal Nehru Memorial Fund, New Delhi during the course of the study.

Author contributions

The current work is a part of PL's Ph.D. research aspect. The experimental paradigm was developed and conceptualized by VCN, MPM, DBU, USK, and AVK. PL was responsible for collecting field samples, performing laboratory procedures, drafting and editing the manuscript. All of the contributors reviewed and agreed upon the final manuscript.

Funding

The present research work was supported and funded by Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India-444001.

Availability of data and materials

All results obtained during this research are reported in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 25 January 2023 Accepted: 13 May 2023
Published online: 17 May 2023

References

Caccia S, Hernandez-Rodriguez CS, Mahon RJ, Downes S, James W (2010) Binding Site alteration is responsible for field-isolated resistance to *Bacillus thuringiensis* Cry2A insecticidal proteins in two *Helicoverpa Species*. PLoS ONE 5(4):9975

Dhurua S, Gujar GT (2011) Field-evolved resistance to *Bt* toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag Sci 67(8):898–903

Fabrick JA, Ponnuraj J, Singh A, Tanwa RK, Unnithan GC, Yelich AJ, Li X, Carrirere Y, Tabashink BE (2014) Alternate splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to *Bt* cotton in India. PLoS ONE 9:97900

Ferre J, Van Rie J (2002) Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. Annu Rev Entomol 47:501–533

Jurat-Fuents JL, Adang MJ (2004) Characterization of a Cry1Ac- receptor alkaline phosphatase in susceptible and resistant *Heliothis virescens* larva. Eur J Biotechnol Biosci 271:3127–3135

Kranthi KR (2016) Pink bollworm strikes *Bt*- Cotton Statistics and News.

Liu YC, Wanf QS, Zhang SS, Lou CX, Ding SY (1997) Insecticide resistance in field strains of *Pectinophora gossypiella* in China and effects of synergists on deltamethrin and parathion-methyl activity. J Pest Sci 50:183–186

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual, Cold spring harbour laboratory, Cold spring harbor. USA, pp 23–25

Mathew LG, Ponnuraj J, Mallappa B, Chowdary LR, Zhang J, Tay WT, Walsh TK,Gordon KHJ, Heckel DG, Downes S (2018) ABC transporter mis-splicing associated with resistance to *Bt* toxin Cry2Ab in laboratory and field-selected pink bollworm Sci Rep 8: 13531

Mohan KS, Ravi KC, Suresh PJ, Sumerford D, Head GP (2016) Field resistance to the *Bacillus thuringiensis protein* Cry1Ac expressed in Bollgard® hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders) populations in India. Pest Manag Sci 72:738–746

Monsanto (2010) Cotton in India. http://www.monsanto.com/newsviews/Pages/india-pink-bollworm.aspx Subsequently revised to Pink bollworm resistance to GM cotton in India. Accessed 29 April 2014.

Morin S, Bigg RW, Sisterson MS, Shriver L, Ellers-Kirk C, Higginson D, Holley D, Gahan LJ, Heckel DG, Carriere Y, Dennehy TJ, Brown JK, Tabashink BE (2003) Three cadherin alleles associated with resistance to *Bacillus thuringiensis* in pink bollworm. Proc Natl Acad Sci USA 100:5004–5009

Morin S, Henderson S, Fabrick JA, Carriers Y, Dennehy TJ, Brown JK, Tabashink BE (2004) DNA-based detection of *Bt* resistant alleles in pink bollworm. Insect Biochem Mol Biol 34:1225–1233

Muralimohan K, Kamath S, Mohan KS, Ravi KC, Deeba G, Sivasupramanian S, Head GP (2009) Mass rearing diet for the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and its susceptibility to insecticidal *Bt* proteins. Int J Trop Insect Sci 29:102–107

Naik VCB, Kranthi S, Kumbhare S, Nagrare VS (2017) A manual on pink bollworm resistance monitoring and management, ICAR-CICR—Central Institute for Cotton Research, Shankar Nagar, Nagpur 36.

Ojha A, Sree KS, Sachdev B, Rashmi MA, Ravi KC, Suresh PJ, Mohan KS, Bhatnagar RK (2014) Analysis of resistance to Cry1Ac in field- collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) population. GM Crops and Foods 5:280–286

Prasada Rao GMV, Kranthi S, Kranthi K (2021) Pink Bollworm Resistance to *Bt* Cotton and Management Considerations.The ICAC Recorder.

Tabashink BE, Fabrick JA, Henderson S, Biggs RW, Yafuso CM, Nyboer ME, Mandardt NM, Coughlin LA, Sollome J, Carrire Y, Dennehy TJ, Morrin S (2006) DNA screening reveals pink bollworm resistance to *Bt* cotton remain rare after a decade of exposure. J Econ Entomol 99:1525–1530

Wang L, Ma Y, Wan P, Liu K, Xiao Y, Wang J, Cong S, Xu D, Wu K, Fabrick JA, Li X (2018) Resistance to *Bacillus thuringiensis* linked with a cadherin

trans-membrane mutation affecting cellular trafficking in pink bollworm from china. Insect Biochem Mol Biol 94:28–35
Wang L, Ma Y, Guo X, Wan P, Liu K, Cong S, Wan J, Xu D, Xiao Y, Li X, Tabashnik BE, Wu K (2019) Pink Bollworm resistance to *Bt* Toxin Cry1Ac associated with an insertion in cadherin exon 20. Toxins 11:186
Wang L, Ma Y, Wei W, Wan P, Liu K, Xu M, Cong S, Wang J, Xu D, Xiao Y, Li X, Tabashnik BE, Wu K (2020) Cadherin repeat 5 mutation associated with *Bt* resistance in a field-derived strain of pink bollworm. Sci Rep 10:16840
Wu Y (2014) Detection and mechanisms of resistance evolved in insects to Cry toxins from *Bacillus thuringiensis*. Adv Insect Physiol 47:297–342
Zhao M, Yuan X, Wei J, Zhang W, Wang B, Myint Khaing M, Liang G (2017) Functional roles of cadherin, aminopeptidase-N and alkaline phosphatase from *Helicoverpa armigera* (Hübner) in the action mechanism of *Bacillus thuringiensis* Cry2Aa. Sci Rep 10(7):46555

Publisher's Note