ISSN: 2643-671X

Research Article

Journal of Agriculture and Horticulture Research

The Expression of Cry1ac in *Gossypium Hirsutum* Against Chewing Insects Via Agrobacterium Mediated Genetic Transformation

Babur Ali Akbar[1], Muhammad Awais Arshad[2*], Muhammad Huzaifa Mahmood[3], Nadia Iqbal[4], Muhammad Faisal[5], Muhammad Umar[6], Saba Khan[2], Farwah Murtaza[2], Shah Rukh[7], Muhammad Usman Hayat[6] and Hafiz Muhammad Aftab Ayoub[6]

[1]*Centre of Agricultural Biochemistry and Biotechnology, Plant Transgenic Laboratory, University of Agriculture Faisalabad Pakistan*

[2]*CAB-International, Rawalpindi, Pakistan*

[3]*Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 44-50, 40127 Bologna, Italy*

[4]*National Institute for Biotechnology and Genetics Engineering, NIBGE-PIEAS*

[5]*Department of Entomology, Faculty of Agriculture, University of Agriculture Faisalabad, Pakistan*

[6]*Department of Agronomy, Faculty of Agriculture, University of Agriculture Faisalabad, Pakistan*

[7]*Department of Computer Science, Faculty of Sciences, University of Agriculture Faisalabad, Pakistan*

[*]Corresponding Author
Muhammad Awais Arshad, CAB-International, Rawalpindi, Pakistan.

Submitted: 2025, Jan 09 **Accepted:** 2025, Feb 17 ; **Published:** 2025, Mar 05

Citation: Akbar, B. A., Arshad, M. A., Mahmood, M. H., Iqbal, N., Faisal, M., et al. (2025). The Expression of Cry1ac in *Gossypium Hirsutum* Against Chewing Insects via Agrobacterium Mediated Genetic Transformation. *J Agri Horti Res, 8*(1), 01-10.

Abstract

Cotton (Gossypium hirsutum), a crop of immense global economic importance, faces substantial yield and quality losses due to insect pests such as Spodoptera exigua and Pectinophora gossypiella. Conventional pesticide strategies are increasingly unsustainable owing to environmental concerns, economic inefficiencies, and pest resistance. This study investigates the potential of cry1Ac, a gene derived from Bacillus thuringiensis (Bt), in developing insect-resistant transgenic cotton. Utilizing Agrobacterium-mediated transformation, cry1Ac was introduced into cotton under the regulation of a wound-inducible promoter (AoPR1), enabling localized expression and mitigating ecological risks associated with constitutive expression. Molecular analyses confirmed successful gene integration and expression, while bioassays demonstrated enhanced resistance, with transgenic lines achieving 80–90% pest mortality compared to negligible effects in controls. Insights into resistance mechanisms, including mutations in pest cadherin genes such as PgCad1, were explored alongside emerging RNA interference (RNAi)-based approaches for resistance management. Field evaluations corroborated the effectiveness of transgenic cotton in controlling target pests, while also identifying challenges posed by non-target pest adaptations and climatic variability. This research underscores the significance of wound-inducible promoters and integrative pest management strategies, offering a sustainable framework for developing resilient cotton varieties capable of addressing evolving pest pressures.

Keywords: Cotton (*Gossypium Hirsutum*), Agrobacterium-Mediated Transformation, Cry1Ac Gene, *Bacillus Thuringiensis* (Bt), Pink Bollworm (*Pectinophora Gossypiella*), Pest Resistance, RNA Interference (RNAi), Sustainable Agriculture, Transgenic Plants.

1. Introduction

Archaeological evidence indicates that cotton has been used by humans for more than 4000 yr. The history of cotton cultivation is at least 3000 years old. There are four cultivated cotton species, two diploid species and two tetraploid species [1]. Among four cotton crops, G. *hirsutum* and G. *arboreum* are cultivated worldwide in more than 90% of the total land under cotton cultivation [2]. Cotton is a product having great economic importance for humanity with its widespread and compulsory usage and having added value and creating employment opportunities for the producer countries [3]. As the raw material, cotton is utilized by several industries including the ginnery industry with processing practices, the textile industry with the usage of its fiber, the oil and feed industry with the usage of its seed, and the paper industry with the usage of its linter [4]. Cottonseed is among the leading oilseed groups along with soybean, rapeseed, peanut and sunflower. Because of specific diseases, problems arising out of pests and weeds cause significant product losses, and the measures to be taken against them lead to the cost of the product to increase and this affects the cotton sector especially in economic terms [5]. At the present time, the volume of pesticide usage is about 3,000,000 tonnes globally, and nearly 40 billion USD is spent each year. Herein, cotton producers around the world spend 2.6 billion USD each year for pesticide usage. This corresponds to more than 10% of worldwide pesticide usage and nearly 25% of the world's insecticide usage each year [6].

Reducing insecticide usage, maintaining of crop yield and earliness of maturity, keeping of susceptibility of pests to new selective insecticides, being practical and workable in the context of the whole farming system, and being effective both for conventional cotton and transgenic (Bt) varieties are the pest management challenges standing in front of the cotton industry and integrated management system could provide effective solutions for these challenges [7]. Conventional methods of pest control, including chemical pesticides, have been widely utilized but come with associated drawbacks such as environmental pollution, harmful effects on non-target organisms, and the development of pest resistance [8]. To address these issues, researchers have been exploring alternative strategies, including genetic engineering, to enhance plant resistance against insect pests while minimizing ecological harm [9-12]. One such approach involves the introduction of specific insecticidal genes into the plant genome, a process known as genetic transformation. Among the genes commonly employed for this purpose is cry1Ac, which encodes a protein toxic to certain insect pests. Cry1Ac is derived from the soil bacterium *Bacillus thuringiensis* (Bt) and has been widely used in genetically modified (GM) crops to confer resistance against lepidopteran and coleopteran pests [13].

To tackle the problems of insect's threat, many different foreign gene(s) have been introduced into cotton genome successfully to encode resistance against insect pests. Among these BT [14-16]. In the context of cotton cultivation, the expression of cry1Ac via Agrobacterium-mediated genetic transformation presents a promising strategy for enhancing resistance against chewing insects. By introducing the cry1Ac gene into *Gossypium hirsutum*, researchers aim to equip the plant with the ability to produce the Cry1Ac protein, which acts as a potent insecticide against susceptible pests upon ingestion. This study aims to investigate the efficacy of cry1Ac expression in Gossypium hirsutum against chewing insects, with a focus on assessing the impact of genetic transformation on pest resistance, plant performance, and agronomic traits. By elucidating the mechanisms underlying cry1Ac-mediated insect resistance in cotton, this research contributes to the development of sustainable pest management strategies in agriculture [17]. The majority of transgenic crops available commercially worldwide typically incorporate foreign genes controlled by the CaMV 35S promoter. This promoter is renowned for its ability to trigger robust gene expression across various tissue types and at different growth stages of the crop, ensuring consistently high levels of gene activity [18]. The constant expression of introduced gene may also increase the potential risk of evolvement of resistance in target insects. We have already started witnessing reports of resistance evolvement in pests against Bt genes [19]. See the table in which pest resistant against Bt genes pink bollworm table. The analysis of GUS reporter gene activity in transgenic tobacco plants revealed that the AoPR1 promoter becomes active in response to various stimuli, including wounding, pathogen invasion, and hydrogen peroxide treatment. Thus, focused gene expression is crucial for future value-added crop development, as it may enhance public acceptance of transgenic traits [17,20]. This study aimed to create insect-resistant cotton lines with localized expression of the insecticidal gene cry1Ac, regulated by the wound-responsive promoter AoPR1, offering a promising approach for insect management.

2. Materials and Methods

2.1. Plant Material

Gossypium hirsutum CEMB Klean Cotton (CKC-01), FH NIAB-878 was selected for this study on account of its germination and resistant against chewing insects.

2.2. Delinting and Screening of Seeds

Delinting is a process used to completely eliminate fuzz from seed coat from cotton seed .100ml / kg is a concentration of sulphuric acid (H2SO4). When seeds were poured in a beaker 20 ml of acid was added and (80 ml distilled water) whole the solution was mixed by continuously stirring of spatula for 7-10 times until the lint was removed. Wash the seeds for 5-7 times with tap water to completely remove the residue of acid. Some of the seed was floated and some seeds sink. Only sinker seed was selected for further processing. After that damaged seed was removed. Dirt and other trash must be removed. Select healthy seeds having no injuries and defects or disease free cotton seed was taken for soaking.

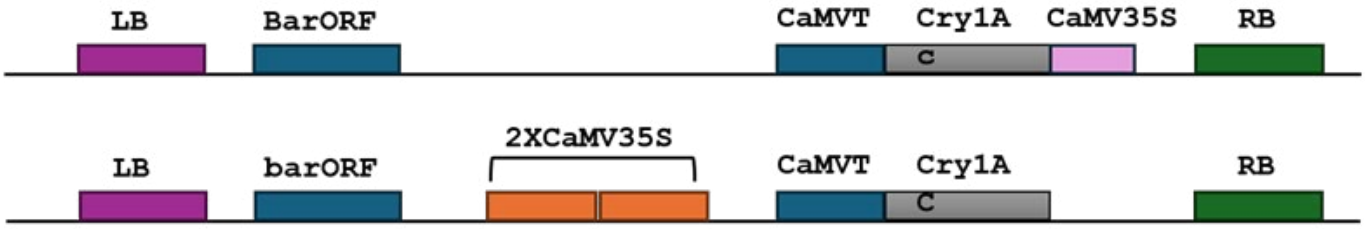
2.3. Soaking and Washing

Put the selected seed in a flask. Add some autoclaved distilled water along with One drop of SDS and 5 to 10 ml mercuric chloride. Rinse the seeds gently to disinfect them and discard the water. Wash seeds three to four times with autoclave distilled water. Discard water and dry the class on the burner to remove moisture

from the flask. Cover the mouth of the flask with aluminum foil and incubate it overnight at 37°C. Take a soaked flask to the laminar air flow cabinet and remove contaminated seeds and the seeds having fungal growth from the flask. After that next day for washing purpose add distilled water along with 5 to 10 ml of mercuric chloride. Rinse the seeds with distilled water and mercury chloride gently and discard the solution. Wash seeds three to four times with distilled water. Discard water and remove moisture from the flask by heating it on the burner. Cover the mouth of flask with aluminum foil and incubate it overnight at 37°C.

2.4. Construction of Recombinant Vector

The Full- length *cry1Ac* gene cassette under CaMV35S promotor and *NOS* terminator with *XhoI* and *SacI* restriction sites were chemically synthesized by BioBasic Inc and provided in pUC57 cloning vector. The recombinant vector containing gene cassette was transformed into the competent cells of *E. coli* (strain Top 10) through the heat shock method. The DNA (500 ng) of recombinant plasmid was restricted with FastDigest *XhoI* and FastDigest *SacI* restriction enzymes The excised product was resolved on 0.8% agarose gel and purified through Thermo Scientific Gene JET Gel Extraction Kit. Schematic representation of plant constructs is shown below



2.5. Preparation of Cultures for Plant Transformation

Prepare YEP media. Take 25 ml of it in falcon tubes and add 25 µlRifamycine+ Kanamycine. as a selection. Add a small microliter volume of Agrobacterium stock. (Always use freshly prepared solutions) Put it on a shaker overnight. Next day centrifuge the overnight grown culture. Discard supernatant and dissolve pallets in simple MS media.

2.6. Agrobacterium Mediated Genetic Transformation of Cotton

Healthy and well germinated seeds to the cutting bench. Excised the embryo and injure it in case of indirect method of agrobacterium-mediated transformation. In case of direct method of agrobacterium mediated transformation injures the embryo along with seed. Put injured cotton embryos and seeds in a prepared transformation culture and place it on rotatory Shaker for at 28 °C for 2 h. For indirect methods of Agrobacterium mediated transformation cotton embryos were co-cultivated with MS-zero broth in a rotary shaker at 28 °C for 2 h and transferred on MS medium plates containing 250 µg/mL cefotaxime to avoid bacterial contamination. After 3 to 4 days transfer embryos grown in petri plates to test tubes having growth media until 2 to 3 leaf stage. When plants reach 2 to 3 leaf stage then transfer them to pots with well-prepared soil and cover them with polythene bags. For direct transformation sow the seeds with autoclave loamy soil containing an equal volume of peat moss, fungicide, sand and clay and wrapped with transparent bags to maintain the humidity. Acclimatize plants by gradually increasing their time to interact with the atmosphere in the lab.

2.7. Molecular Analysis of Putative Transgenic Cotton Plants

Polymerase chain reaction (PCR) was conducted using gene-specific primers to amplify fragments of *cry1Ac, AoPR1*, and *BAR* genes from putative transgenic cotton plants. Genomic DNA was extracted. PCR reactions were carried out in a total volume of 50µL, comprising 10X reaction buffer, 5ng of DNA template, 25 mM MgCl2, 0.7 nmol of each dNTP, 25pmol of each primer, and 5 unit of Taq DNA polymerase along Tris buffer with 10mM conc. Primer sequences, annealing temperatures, and product sizes are detailed in Table 1. Plasmid DNA served as the positive control, while DNA from untransformed plants served as the negative control. Amplified DNA fragments were separated by electrophoresis on a 2.0% agarose gel and visualized through ethidium bromide staining under ultraviolet (UV) light.

2.8. Protein Expression Analysis

A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was employed to measure the accumulated levels of cry1Ac protein in the leaves of putative transgenic plants. Transformants leaves contain construct collected for protein quantification. Optical density (OD) measurements at 430 nm were utilized to determine the concentration of cry1Ac protein by comparison with a standard cry1Ac protein reference.

2.9. Detection of Transgenes

Foreign gene copy number and localization in transgenic cotton plants were assessed via Fluorescence in situ Hybridization (FISH), with fluorescent signal detection conducted using a fluorescent microscope equipped with a blue filter for 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) dye.

2.10. Insect Mortality Bioassays

The transgenic cotton plants of T0 generation were exposed to leaf-detach insect bioassays to assess the individual efficacy of insecticidal toxins against *Spodoptera exigua.* One fresh leaf of non-transgenic and transgenic cotton plants was placed in petri dishes containing wet filter papers. Three 2nd instar larvae of *Spodoptera exigua* were released on cotton leaves. The sealed petri plates were put in a culture room at 25 ± 2 °C and 16 h light: 8 h of the dark cycle along with ≈60% humidity. The percent mortality was calculated on the third day of infestation.

Treatment Group	Initial Insect Count	Final Insect Count	Mortality (%)
Control	23	20	13%
Transgenic	25	4	84%

Table 1: Insects Mortality Bioassays on 3rd Day of Infestation

2.11. Treatment Group:

This column refers to the different experimental groups or conditions being tested in the study. Each treatment group represents a unique set of conditions or interventions applied to the subjects (in this case, insects infesting cotton leaves).

2.12. Replicate

Replicate refers to the repetition of an experimental condition within the same treatment group. Each replicate helps to account for variability and to strengthen the statistical analysis of the results. It ensures that the findings are not due to random chance or specific conditions of a single experiment.

2.13. Initial Insect Count:

This represents the number of insects initially introduced or counted at the beginning of the experiment in each replicate of the treatment group. It serves as the baseline for assessing the effectiveness of the treatment.

2.14. Final Insect Count:

Final insect count indicates the number of surviving insects remaining after a specified period, in this case, on the 3rd day of infestation. It is crucial for evaluating the impact of the treatment on insect populations.

2.15. Mortality (%):

Mortality percentage is calculated by comparing the initial and final insect counts. It represents the proportion of insects that died during the course of the experiment, typically due to the treatment applied. It is calculated using the formula:

Mortality (%)=(1−Final Insect Count/Initial Insect Count)×100.

3. Results and Discussion

3.1. Evaluation of Primary Transformants

The primary putative transgenic plants that grew well in growth room and for the confirmation of presence of introduced cry1Ac gene in cotton genome along with promoter and selectable marker gene. The results were showed that required band of 412bp of cry1Ac in primary transformants marked and subjected to further analysis.

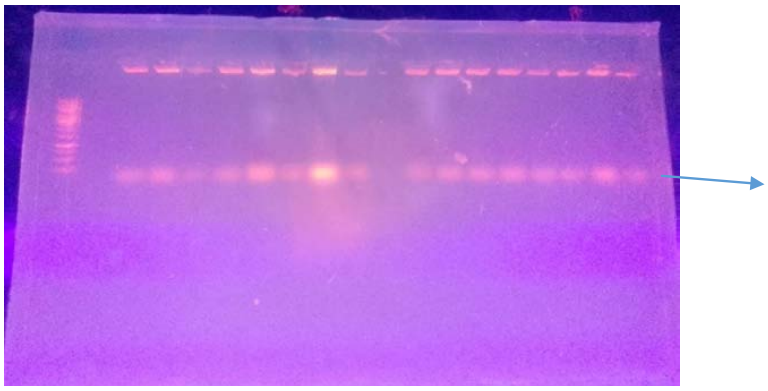


Figure 1: Molecular analysis of Transgenic Cotton Plants, Amplification of Cry1ac in Transformed Plants

Figure 2: Invitro conducted experiment to attack of chewing insects on non-transgenic and transgenic cotton leaves of selected cultivars. The transgenic plants showed low level of resistance against Spodoptera exigua.

Figure 3: *Pectinophora Gossypiella* Attack on BT Cotton

The resistant in pink bollworm against BT due to mutations in cadherin genes of threat agriculture pest. Different genes in mutations like PgCad1, ATP-binding cassette transporter protein PgABCA2, *r15A* and *r15B, PgCad1 alleles (r1-r20), PgABCC2*. Mutation in the Cadherin gene Is a key Factor for Pink Bollworm resistance to Bt Cotton in China [21,22]. Another study reported that Transposon insertion causes cadherin mis-splicing and confers resistance to Bt cotton in pink bollworm from China [23]. Previous work with laboratory- and field-selected pink bollworm indicated that resistance to Cry1Ac is caused by changes in the amino acid sequence of a midgut cadherin protein (*PgCad1*) that binds Cry1Ac in susceptible larvae. The ability of Pink bollworm and other major insects pest presents challenges for monitoring and managing resistance to Bt crops [24,25]. There could be the 5 cadherin repeated mutations associated with bt resistance in a field-derived strain of pink bollworm [26,27]. We report on a novel allele (r16) of the cadherin gene (*PgCad1*) in pink bollworm *(Pectinophora gossypiella*) associated with resistance to Bt toxin Cry1Ac, which is produced by transgenic cotton [28-30].

Figure 4: Attack of Army worm and American bollworm on BT cotton, the 45 days of cotton leaves shows that with the passage of time BT shows no resistance against chewing insects like Spodoptera frugiperda and American boll worm.

3.3. Graphical Stages of Agrobacterium Mediated Genetic Transformation of Cotton

Figure 5: (a) Delinting of seeds with pure sulphuric acid, (B) Embryo injury with surgical blades (C) shifting of embryo on MS media, (D) and E shows the embryo growing on media, (F) shifting the shoot on test tubes, (G) shows that prepare the sterilized antifungal soil with Thiophenate methyl and H shows shots formed from direct seed sowing in that soil, (I) shows that shifting the plants to the Invivo environment.

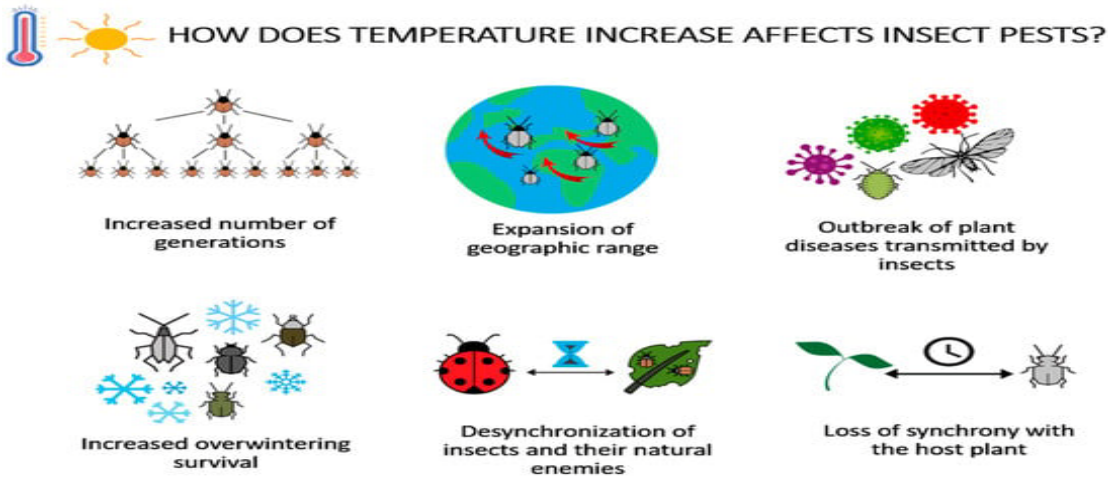
Treatment Group	Insect Species	Initial Insect Count	Final Insect Count	Mortality (%)
Non- transgenic	Species A	50	42	20%
	Species B	50	45	14
Transgenic	Species A	50	10	80%
	Species B	50	5	90

Table 2: Insects Mortality Rate On 7th Day of Infestation

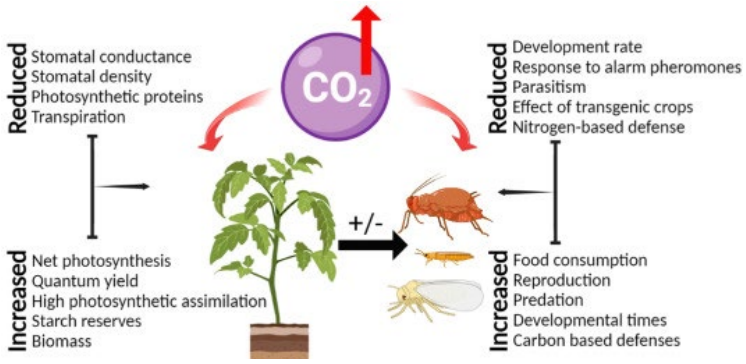
3.4. Reasons- the Downfall of Bt Cotton There are Many Factors for the Survival of Threat Pest Bemisia Tabaci.

Temperature: Temperature is a primary factor that determines the eco-geographical distribution and population development of invasive insects. Our previous studies have shown that CAT promotes whitefly adaptation to high temperature by eliminating ROS [31]. In this study, we investigated the role of CAT at different temperature 25 °C, 20 °C, and 4 °C. Silencing of BtCATs significantly increased the sensitivity of B. tabaci MED to low temperatures [32]. The results showed that invasive whiteflies had a significantly lower heat resistance after silencing BtCYP 4C1 and BtCar3. In addition, whiteflies had a higher cold tolerance after silencing BtCYP 4C1 [33]. These results indicate that BtCYP 4C1 and BtCar3 are key regulators in the temperature adaptation of B.

tabaci. Moreover, they may be key factors in influencing the geographical distribution and dispersal of B. tabaci as an invasive species in China [34]. In another study, the effects of elevated temperatures on BTQ's tolerance to the insecticide thiamethoxam were investigated. The high temperature influenced the tolerance of BTQ by affecting the activity of P450. Feeding on double-stranded RNA (dsRNA) of CYP6CM1 significantly reduced the mRNA levels of the target gene in the adults, and dramatically decreased tolerance to thiamethoxam induced by a temperature of 31 °C for 6 h [35-39].



Atmospheric Carbon Dioxide: Rise in atmospheric carbon dioxide concentration ([CO2]) and a warming climate are two of the most conspicuous characteristics of global climate change in this century. The independent effects of [CO2] enrichment on the biology and physiology of herbivorous insects are well studied [40]. In the Figure effect of Carbon dioxide that how it increased the genes of White fly that could be lethal for crop species.



PINK Bollworm: The pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), is a highly destructive insect pest of cotton across the global cotton-producing areas. The probable origin of pink bollworm is in the Indo-Pakistan region [41,42]. The commercial planting of Bt cotton effectively suppressed the population of pink bollworms. Furthermore, some field populations of pink bollworms have developed practical resistance to Bt cotton expressing Cry1Ac or even Bt cotton producing Cry1Ac and Cry2Ab, posing a serious threat to the sustainable use of transgenic Bt cotton [43]. Pink bollworm could be more serious threat for the survival of Cotton. Foliar pesticides like chlorpyrifos, esfenvalerate, lambda- cyhalothrin and bifenthrin used for pesticides on Pink bollworm. But due to their excessive used this pest have resistant against chemical insecticides. This chewing type pest contain insecticides detoxifying enzymes, so insects molecular biologist have taken a decision to knock off these genes by new emerging technology like RNA interference in the form of transgenic plants, to make the formulations as a foliar spray or inside the host insects.

RNAi demonstrated a successful gene silencing in insects that led to the development of novel approaches for insect pest management to knock off the genes encoding vacuolar ATPase (V-ATPase) subunits a and c from the midgut of pink bollworm. 200 ng of dsRNAs silenced both genes causing mortality of 18.9 to 26.7% [44,45].

Mutated Gene	Resistant against genes	References
PgCad1, ATP-binding cassette transporter protein PgABCA2	Cry1Ac, Cry2Ab	(Tabashnik and Carrière, 2019;Wang et al., 2020a;Fabrick et al., 2023) [46,47,30]
Cadherin alleles (r19 and r20)	Cry1Ac	(Wang et al., 2022) [21]
r15A and r15B	Cry1Ac	(Wang et al., 2019b) [23]
r14 allele of the pink bollworm cadherin gene (PgCad1)	Cry1Ac	(Wang et al., 2020b) [26]
r13PgCad1	Cry1Ac	(Wang et al., 2018) [48]
PgABCA2	Cry2Ab	(Mathew et al., 2018; Fabrick et al., 2021) [49,50]
PgCad1 alleles (r1-r20), PgABCC2	Cry1Ac, PgABCC2 confers low-level resistance to Cry1Ac	(Wang et al., 2024b) [41]

Table 3: Gene Mutations in Pink Bollworm

4. Conclusion

This research highlights the potential of genetic transformation, specifically the integration of the cry1Ac gene under wound-inducible promoters, as a sustainable and effective strategy for combating major insect pests in cotton cultivation. Transgenic cotton expressing cry1Ac demonstrated substantial resistance to chewing insects like Spodoptera exigua and Pectinophora gossypiella, achieving up to 90% mortality in bioassays. The use of a wound-responsive promoter (AoPR1) ensured localized expression, minimizing ecological concerns associated with constitutive expression of insecticidal genes. Despite these successes, the study underscores critical challenges, including the evolution of pest resistance through genetic mutations in targets such as the cadherin gene (PgCad1). Moreover, environmental factors like rising CO2 levels and fluctuating temperatures influence pest behavior and gene efficacy, complicating long-term pest control strategies. RNA interference (RNAi) technology offers a complementary approach to address these issues, enabling precise gene silencing to mitigate pest resistance mechanisms. In conclusion, the integration of cry1Ac and novel genetic strategies in cotton offers a promising avenue for sustainable pest management. However, to ensure long-term efficacy, future efforts must focus on integrating transgenic approaches with RNAi, developing species-specific strategies, and establishing comprehensive monitoring systems for resistance management. These advancements will be crucial for maintaining cotton yield and quality while reducing environmental impact and pesticide dependence [51].

References

1. Lee, J. A., & Fang, D. D. (2015). Cotton as a world crop: origin, history, and current status. *Cotton, 57*, 1-23.
2. Shim, J., Mangat, P. K., & Angeles-Shim, R. B. (2018). Natural variation in wild Gossypium species as a tool to broaden the genetic base of cultivated cotton. *J. Plant Sci. Curr. Res, 2*(005).
3. Majumdar, G., Singh, S. B., & Shukla, S. K. (2019). Seed production, harvesting, and ginning of cotton. *Cotton production,* 145-174.
4. Munir, H., Rasul, F., Ahmad, A., Sajid, M., Ayub, S., Arif, M., ... & Khan, M. A. (2020). Diverse uses of cotton: From products to byproducts. *Cotton production and uses: Agronomy, crop protection, and postharvest technologies,* 629-641.
5. Kaur, P., Bhagria, T., Mutti, N. K., Rinwa, A., Mahajan, G., & Chauhan, B. (2019). Cotton production in Turkey and Europe. K. Jabran, and BS Chauhan, 341-57.
6. Greenberg, S. M., & Parajulee, M. N. (2017). Integrated pest management in cotton. In *Integrated pest management in tropical regions* (pp. 134-174). Wallingford UK: CABI.
7. Tokel, D., Genc, B. N., & Ozyigit, I. I. (2022). Economic impacts of Bt (Bacillus thuringiensis) cotton. *Journal of Natural Fibers, 19*(12), 4622-4639.
8. Tahir, M., Arshad, M. A., Akbar, B. A., Bibi, A., Ain, Q. U., Bilal, A., ... & Pervaiz, R. (2024). Integrated nitrogen and irrigation management strategies for sustainable wheat production: Enhancing yield and environmental efficiency. *Journal of Pharmacognosy and Phytochemistry, 13*(4), 209-222.
9. Abbas, R. N., Arshad, M. A., Iqbal, A., Iqbal, M. A., Imran, M., Raza, A., ... & Hefft, D. I. (2021). Weeds spectrum, productivity and land-use efficiency in maize-gram intercropping systems under semi-arid environment. *Agronomy, 11*(8), 1615.
10. Rajak, P., Roy, S., Ganguly, A., Mandi, M., Dutta, A., Das, K., ... & Biswas, G. (2023). Agricultural pesticides–friends or foes to biosphere?. *Journal of Hazardous Materials Advances, 10,* 100264.
11. Khan, B. A., Nadeem, M. A., Nawaz, H., Amin, M. M., Abbasi, G. H., Nadeem, M., ... & Ayub, M. A. (2023). Pesticides: impacts on agriculture productivity, environment, and management strategies. In *Emerging contaminants and plants: Interactions, adaptations and remediation technologies* (pp. 109-134). Cham: Springer International Publishing.
12. Sood, P. (2024). Pesticides usage and its toxic effects-A review.
13. Yao, X., Duan, Y., Deng, Z., Zhao, W., Wei, J., Li, X., & An, S. (2023). ATP synthase subunit α from Helicoverpa armigera acts as a receptor of Bacillus thuringiensis Cry1Ac and synergizes Cry1Ac toxicity. *Journal of Agricultural and Food Chemistry, 71*(15), 6155-6163.
14. Perlak, F. J., Deaton, R. W., Armstrong, T. A., Fuchs, R. L., Sims, S. R., Greenplate, J. T., & Fischhoff, D. A. (1990). Insect resistant cotton plants. *Bio/technology, 8*(10), 939-943.
15. Sims, S. R. (1995). Bacillus thuringiensis var. kurstaki (CryIA (C)) protein expressed in transgenic cotton: effects on beneficial and other non-target insects.

16. Rasheed, H. U. (2024). Adaptation and Agricultural Significance of Syzygium cumini L. Saline Environments: A Global Perspective on Jamun Cultivation and Salt Stress Resilience. *Haya Saudi J Life Sci, 9*(5), 172-187.
17. Arshad, M. A., Rouf, S., Abbas, R. N., Aleem, K., Sarwar, A., Shahbaz, Z., ... & Masood, M. T. (2024). Environmental benefits and risks of herbicides use in forestry. *Review. Haya the saudi journal of life sciences, 9*(2), 23-35.
18. Amack, S. C., & Antunes, M. S. (2020). CaMV35S promoter–A plant biology and biotechnology workhorse in the era of synthetic biology. *Current Plant Biology, 24,* 100179.
19. Tabashnik, B. E., Brévault, T., & Carrière, Y. (2013). Insect resistance to Bt crops: lessons from the first billion acres. *Nature biotechnology, 31*(6), 510-521.
20. Arshad, M. A., Abbas, R. N., Khaliq, A., & Ahmed, Z. (2024). Assessing herbicide efficacy and susceptibility for weed management and enhancing production of non-GMO soybean cultivation. *Haya: Saudi Journal of Life Sciences. 2024a, 9*(5), 172-178.
21. Wang, L., Xu, D., Huang, Y., Zhou, H., Liu, W., Cong, S., ... & Wan, P. (2022). Mutation in the cadherin gene is a key factor for pink bollworm resistance to Bt cotton in China. *Toxins, 14*(1), 23.
22. Arshad, M. A., Rouf, S., Abbas, R. N., Shahbaz, Z., Aleem, K., Shahbaz, H., ... & Rehman, H. U. (2024). Navigating synergies: a comprehensive review of agroforestry system and agronomy crops. *Haya Saudi J. Life Sci, 9*, 97-113.
23. Wang, L., Wang, J., Ma, Y., Wan, P., Liu, K., Cong, S., ... & Tabashnik, B. E. (2019). Transposon insertion causes cadherin mis-splicing and confers resistance to Bt cotton in pink bollworm from China. *Scientific Reports, 9*(1), 7479.
24. Fabrick, J. A., Mathew, L. G., LeRoy, D. M., Hull, J. J., Unnithan, G. C., Yelich, A. J., ... & Tabashnik, B. E. (2020). Reduced cadherin expression associated with resistance to Bt toxin Cry1Ac in pink bollworm. *Pest Management Science, 76*(1), 67-74.
25. Pervaiz, R., Baloch, R., Arshad, M. A., Abbas, R. N., Shahzad, N., Hamid, M., ... & Akbar, M. M. (2024). Herbicide strategies for weed control in rice cultivation: Current practices and future directions. *Haya Saudi J Life Sci, 9*(4), 114-129.
26. Wang, L., Ma, Y., Wei, W., Wan, P., Liu, K., Xu, M., ... & Wu, K. (2020). Cadherin repeat 5 mutation associated with Bt resistance in a field-derived strain of pink bollworm. *Scientific Reports, 10*(1), 16840.
27. Gao, M., Wang, X., Yang, Y., Tabashnik, B. E., & Wu, Y. (2018). Epistasis confers resistance to Bt toxin Cry1Ac in the cotton bollworm. *Evolutionary Applications, 11*(5), 809-819.
28. Akhter, M. J., Abbas, R. N., Waqas, M. A., Noor, M. A., Arshad, M. A., Wajid Mahboob, W. M., ... & Umair Gull, U. G. (2017). Adjuvant improves the efficacy of herbicide for weed management in maize sown under altered sowing methods.
29. Wang, L., Ma, Y., Guo, X., Wan, P., Liu, K., Cong, S., ... & Wu, K. (2019). Pink bollworm resistance to Bt toxin Cry1Ac associated with an insertion in cadherin exon 20. *Toxins, 11*(4), 186.
30. Fabrick, J. A., Li, X., Carrière, Y., & Tabashnik, B. E. (2023). Molecular genetic basis of lab-and field-selected Bt resistance in pink bollworm. *Insects, 14*(2), 201.
31. Liang, P., Ning, J., Wang, W., Zhu, P., Gui, L., Xie, W., & Zhang, Y. (2023). Catalase promotes whitefly adaptation to high temperature by eliminating reactive oxygen species. *Insect Science, 30*(5), 1293-1308.
32. Ning, J., Liang, P., Wu, X., Wang, W., Wu, Q., Wang, S., ... & Xie, W. (2024). Silencing of catalase reduces unfavorable low-temperature tolerance capacity in whiteflies. *Pest Management Science, 80*(7), 3116-3125.
33. Rafeeq, H., Arshad, M. A., Amjad, S. F., Ullah, M. H., Imran, H. M., Khalid, R., ... & Ajmal, H. (2020). Effect of nickel on different physiological parameters of Raphanus sativus. *Int J Sci Res Publ, 10*, 9702.
34. Shen, X., Liu, W., Wan, F., Lv, Z., & Guo, J. (2021). The role of cytochrome P450 4C1 and carbonic anhydrase 3 in response to temperature stress in Bemisia tabaci. *Insects, 12*(12), 1071.
35. Forrest, J. R. (2016). Complex responses of insect phenology to climate change. *Current opinion in insect science, 17*, 49-54.
36. Guo, L., Su, M., Liang, P., Li, S., & Chu, D. (2018). Effects of high temperature on insecticide tolerance in whitefly Bemisia tabaci (Gennadius) Q biotype. *Pesticide Biochemistry and Physiology, 150,* 97-104.
37. Shrestha, S. (2019). Effects of climate change in agricultural insect pest. *Acta Scientific Agriculture, 3*(12), 74-80.
38. Nyamukondiwa, C., Machekano, H., Chidawanyika, F., Mutamiswa, R., Ma, G., & Ma, C. S. (2022). Geographic dispersion of invasive crop pests: the role of basal, plastic climate stress tolerance and other complementary traits in the tropics. *Current Opinion in Insect Science, 50*, 100878.
39. Barman, M., Samanta, S., Ahmed, B., Dey, S., Chakraborty, S., Deeksha, M. G., ... & Roy, D. (2023). Transcription dynamics of heat-shock proteins (Hsps) and endosymbiont titres in response to thermal stress in whitefly, Bemisia tabaci (Asia-I). *Frontiers in Physiology, 13*, 1097459.
40. Li, N., Li, Y., Zhang, S., Fan, Y., & Liu, T. (2017). Effect of elevated CO2 concentration and temperature on antioxidant capabilities of multiple generations of Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae). *Journal of Insect Physiology, 103*, 91-97.
41. Wang, L., Xu, M., He, L., Wei, W., Xu, D., Cong, S., ... & Wan, P. (2024). Mutation in PgABCC2 confers low-level resistance to Cry1Ac in pink bollworm. *Pest Management Science, 80*(7), 3326-3333.
42. Fand, B. B., Nagrare, V. S., Bal, S. K., Naik, V. C. B., Naikwadi, B. V., Mahule, D. J., ... & Waghmare, V. N. (2021). Degree day-based model predicts pink bollworm phenology across geographical locations of subtropics and semi-arid tropics of India. *Scientific Reports, 11*(1), 436.
43. Wang, L., Xu, D., Yang, Y., Liu, W., Cong, S., Li, W., ... & Wan, P. (2024). Impact of rutin on growth, development and oviposition of Bt-susceptible and Bt-resistant pink bollworm. *Entomological Research, 54*(1), e12702.
44. Mohammed, A. M. (2016). RNAi-based silencing of genes

encoding the vacuolar-ATPase subunits a and c in pink bollworm (Pectinophora gossypiella). *African Journal of Biotechnology, 15*(45), 2547-2557.

45. Abbas, A., Wang, Y., Muhammad, U., & Fatima, A. (2021). Efficacy of different insecticides against gram pod borer (Helicoverpa armigera) and their safety to the beneficial fauna. *Int J Biosci, 18*, 82-88.
46. Tabashnik, B. E., & Carrière, Y. (2019). Global patterns of resistance to Bt crops highlighting pink bollworm in the United States, China, and India. *Journal of economic entomology, 112*(6), 2513-2523.
47. Wang, J., Xu, D., Wang, L., Cong, S., Wan, P., Lei, C., ... & Wu, K. (2020). Bt resistance alleles in field populations of pink bollworm from China: Similarities with the United States and decreased frequency from 2012 to 2015. *Pest Management Science, 76*(2), 527-533.
48. Wang, L., Ma, Y., Wan, P., Liu, K., Xiao, Y., Wang, J., ... & Tabashnik, B. E. (2018). Resistance to Bacillus thuringiensis linked with a cadherin transmembrane mutation affecting cellular trafficking in pink bollworm from China. *Insect Biochemistry and Molecular Biology, 94*, 28-35.
49. Mathew, L. G., Ponnuraj, J., Mallappa, B., Chowdary, L. R., Zhang, J., Tay, W. T., ... & Fabrick, J. A. (2018). ABC transporter mis-splicing associated with resistance to Bt toxin Cry2Ab in laboratory-and field-selected pink bollworm. *Scientific Reports, 8*(1), 13531.
50. Fabrick, J. A., LeRoy, D. M., Mathew, L. G., Wu, Y., Unnithan, G. C., Yelich, A. J., ... & Tabashnik, B. E. (2021). CRISPR-mediated mutations in the ABC transporter gene ABCA2 confer pink bollworm resistance to Bt toxin Cry2Ab. *Scientific reports, 11*(1), 10377.
51. Arshad, M. A. (2021). A review on wheat management, strategies, current problems and future perspectives. *Haya Saudi J. Life Sci, 6*, 14-18.