



The Effectiveness and Performance of BT Cotton's Insecticidal Properties under Various Abiotic Stresses: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Over the past two decades, biotechnology has yielded Bt cotton, a huge success in the field of agriculture. In a number of countries, commercial Bt crop production has proven successful. The global food scarcity crisis has been alleviated by transgenic Bt crops, which have improved agricultural productivity. The Bt cotton crop displays variable levels of insect resistance to several environmental abiotic circumstances, including high temperatures, salt in the soil, water logging

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and scarcity, inadequate nitrogen supply, and humidity. It has been demonstrated that these components lessen the transgenic crop's endotoxin protein level, which in turn reduces the insecticidal efficacy of the crop. In unfavourable climatic conditions, plants have altered nitrogen metabolism and pain in their physiological systems. This article has discussed some of the primary stresses that Bt cotton encounters as well as the underlying mechanisms that explain its variable insecticidal efficacy under both single and combination pressures. While several important elements are covered in this study, such as gene silencing, post transcriptional changes, and protein breakdown, the most important aspect of this field that still need investigation is nitrogen metabolism. A thorough meta-analysis table provides an overview of the several significant research that have been carried out to examine the impacts of stress. A comprehensive study technique is needed to clarify the metabolic and physiological changes that transpire in transgenic cotton crops under high stress. In order to increase the production and performance of the cotton crop worldwide, more research is needed to address the unpredictable insect resistance of Bt crops.

Keywords: Abiotic stresses and Bt cotton; effectiveness against insects; environmental conditions for Bt cotton; field performance stressors transgenic cotton's; genetically modified.

1. INTRODUCTION

For the last 20 years, biotechnology has produced Bt cotton, a major hit in agriculture. Commercial Bt crop cultivation has been effective in several nations. The worldwide food scarcity situation has been resolved by transgenic Bt crops, which have positively enhanced agricultural output [1]. Under the effect of various environmental abiotic conditions such as high temperatures, salt in the soil, water logging and shortage, nitrogen insufficiency, and humidity, the Bt cotton crop exhibits varying degrees of insect resistance. These elements have been shown to weaken the transgenic crop's endotoxin protein level, which in turn lowers the crop's insecticidal effectiveness. Plants experience altered nitrogen metabolism and physiological system discomfort under adverse environmental circumstances. The cotton business is now experiencing several obstacles, such as the need to develop disease and pest resistance in cotton cultivars, competition from synthetic fibres in the market, a limited genetic basis of improved cultivars, and higher fibre quality for the textile industries. Since 145 bug species may be found in cotton, insect pests are a major cause of the crop's low yield [2]. In this case, using insecticides is thought to be crucial for controlling cotton pests. However, the use of pesticides led to the poisoning of food and the eradication of nontarget organisms. Additional hazards include the emergence of sucking pests environmental pollution and degradation consumer and farmer health concerns, and the adoption of resistance to synthetic pesticides [3]. Concerns over the widespread use of synthetic pesticides grew, prompting searches for more effective insect

control measures. In order to improve production while also changing plant defence mechanisms, biotechnological techniques, such as genetic engineering, were used. Many strategies were developed as a consequence of biotechnological attempts to produce insect-resistant cotton, some of which are already commercially accessible for cultivation. The use of certain fertilisers and agricultural practises that correlate to protect the crop from pests, diseases, and other abiotic factors has increased cotton output and quality in recent decades. The creation of Bt Cotton, a transgenic crop that generates a toxin useful in defending the crop against particular pests like bollworm, is one such accomplishment in the area of agricultural biotechnology [4]. *Bacillus thuringiensis* is the species of bacteria from which Bt protein is derived, and it has insecticidal qualities. The largest cotton-producing nations, notably Pakistan, the United States, and Mexico, have shown that the large-scale production of transgenic cotton has been a huge success [5].

2. BACKGROUND OF BT COTTON'S MODE OF ACTION

Several Bt genes, including Cry1Ac, Cry1Ab, Cry9C, and Cry2Ab, have been used in genetic engineering to protect certain crops from various insect species, including cotton, potato, brinjal, maize, soybean, and soybean [6]. Several studies have been suggested to investigate and ultimately determine the histology and mechanisms of the precise interaction between the Bt toxin and the gut wall of insects that are feeding on the crop. Put more simply, when an insect consumes Bt cotton, the toxins it ingests dissolve in its body and cause an alkaline (9 pH)

gut environment to activate [7]. In the end, the cry toxins destroy the gut epithelial cells, causing the eating insect to become infected and die in a matter of hours. Wild diploid species with the B, E, and F genomes are found in Africa and Arabia, whereas species with the D genome are found in South and Central America. Australia is home to diploid species that are represented in the genomes C, G, and K. An understanding of the genetic linkages across cotton species and cultivars is necessary to take advantage of the improved quality criteria that have been provided by varied germplasm resources [8]. Two tetraploid species, *G. hirsutum* L. [$n = 2x = 26$, (AD)1] and *G. barbadense* L. [$n = 2x = 26$, (AD)2], and two diploid species, *G. herbaceum* L. ($n = x = 13$, A1) and *G. Arboretum* L. ($n = x = 13$, A2), comprise the four cultivated species of cotton [9].

According to molecular research, allopolyploid *Gossypium* originated about 1.5 million years ago, during the mid Pleistocene [2, 11–13]. Allopolyploid cotton is believed to have originated via the transoceanic dispersion of A genome species to the New World by hybridization with indigenous diploid D genome species. Only A genome species can generate spinnable fibres, while D genome species exhibit traits that are significant for agronomy. *G. barbadense* was found to be extensively dispersed over much of South America, southern Mesoamerica, and the Caribbean basin, according to historical sources. *G. hirsutum* was distributed across Mesoamerica, the Central Caribbean islands, and the Gulf of Mexico coast. *G. mustelinum* is another allopolyploid that is rare and has an island-like distribution since it is only found in a very limited area of Northeastern Brazil [10].

3. TRANSGENIC COTTON'S INCONSISTENT INSECTICIDAL EFFECTIVENESS AS A RESULT OF SALT STRESS

Cotton can tolerate salt levels up to 7.7 dS/m, which is a middling range for a crop [11]. The fruiting and blooming seasons of cotton plants are impacted by salt stress. Saline soil has a significant impact on cotton output [12]. A hindrance in the field of agriculture, soil salinity limits the appropriate output and development of Bt cotton crops. Saline soil has consistently been highlighted as one of the main obstacles to sustainable agricultural growth [13]. Drought and water logging conditions are additional

environmental variables that seem to reduce the Bt protein levels. Humidity and hot air currents are also connected with the reduced unstable performance of Bt transgenic cotton [14]. Numerous research studies have been conducted to examine the impact of salt on the lower yield of conventional cotton crops. Regarding the links between Na^+ and K^+ stress for the propagation of Bt cotton, several ideas and experiments have been offered [15].

4. CROP PEST ATTACK DUE TO MOISTURE CONTENT OF THE SOIL

An important investigation on the association between weather and insect susceptibility in cotton was carried out in the Rajasthan area of India [16]. According to the study's findings, insect attacks on cotton crops rose sharply on rainy days when the humidity was too high for larval survival. In addition, this experiment revealed temperature variations. The sustained resistance of the Bt Cotton crop to several insect species is significantly impacted by humidity stress. It was claimed that the Bt crop's unstable protein content towards *H. armigera* resulted from the Cry1Ac gene's reduced expression [17]. The lower effectiveness and unstable resistance were mostly noted and seemed to have a greater effect on cotton at the peak boll stage. The effects of (RH) and (HT) stress on Bt cotton were examined in this study [18]. The insecticidal efficacy of the delta endotoxin Cry1Ac under humidity stress was investigated. Six cultivars were treated with relative humidity ranging from 50 to 95%; three cultivars received a temperature treatment of 18 degrees Celsius, and three cultivars received a temperature treatment of 37 degrees. Accordingly, excessive air temperature and HR stress were associated. China's summer temperatures hover around 35 to 40 degrees Celsius, and the high 95% humidity stress causes cotton yields to drop from around 40 to 55 percent [19]. Two transgenic cotton cultivars with RH treatments of 50, 70, and 90% at 37 degrees were examined at 37 degrees during the 2009–2010 growth season. During the boll phase, the insecticidal proteins in leaves decreased [20]. The combination of high temperatures and extreme humidity led to a decrease in yield. Protease activity is examined for a more thorough analysis in all of these tests, which are conducted in greenhouse settings. Soil moisture content affects cotton lint production and fibre quality [21].

5. LINKS BETWEEN BT PROTEIN CONCENTRATION AND PLANT NITROGEN METABOLISM

Abiotic factors such as waterlogging also significantly lower the protein content of Bt cotton plants by inhibiting the activities, proteases, which in turn lowers the plant's amino acid content [22]. When plants were given remedies for water stress, their defence systems against pest attacks were less effective. The waterlogging stress has a major impact on the expression of the insecticidal gene Cry1Ac [23]. A seminal study also documented the effects of low soil water content on Bt gene expression and insecticidal protein concentration. Bt cotton plants' protein (insecticidal / Bt) concentration decreased when they were subjected to lengthy (96-hour) treatments of water deficit. Researchers have put out a variety of theories as to why cotton under abiotic stress has a lower protein content [24]. Thus far, some reasonable theories include decreased protein synthesis as a result of stress and gene silencing or inactivation. The results were similar to those who spoke about the relationship between boll size and Bt protein concentration [25]. Protein breakdown was more pronounced in N-deficient soil as an abiotic stressor because of the lower soluble protein concentration and reduced GOT and GPT activities in nitrogen metabolism [26]. A crucial component of soil that contributes to the decrease in insecticidal protein content is a nitrogen shortage that inhibits the production of the Cry1Ac gene [27].

6. FEEDING HABIT OF PEST'S IN RESPONSE TO CHANGE IN TEMPERATURE

The eating habits of *A. argillacea* were observed, but with variations in temperature conditions. The first explanation provided by the data was that the proportion of larvae on Bt plants was consistently lower than that of non-Bt plants, regardless of the temperature and duration of the cultivars' exposure [28]. First, this argument effectively illustrates the effectiveness and insecticidal capacity of Bt plants. Furthermore, the study focuses on how temperature stress and variations alter the Bt plant's defense systems against certain pests. Similar to other studies on *D. saccharalis*, it was shown that as temperatures rose over 34°C, larval development and attacks on Bt plants increased. Conversely, non-Bt plants were subjected to insect assault

shortly after reaching 28°C [29]. The manufacture of insecticidal protein, or cry protein that kills pests, is influenced by environmental conditions, including temperature, which is the subject of the present research. Following the temperature's crossing of the optimal threshold, *A. argillacea* began to act on the Bt cotton plants [30]. Bt cotton's whole effectiveness of maximal resistance was lost as a result of insecticidal protein breakdown. Numerous studies have observed how bollworm larvae (*H. armigera*) eat in order to determine how Bt plants respond to salt stress. State that various pests (such cotton bollworms) exhibit various eating habits for various transgenic plants [31]. When compared to non-transgenic types, the feeding rate of bollworm larvae under NaCl stress falls, according to *H. armigera*'s feeding likelihood. However, as seen in other studies on N [32]. The pest behaviour of crawling, twirling, and resting behaviours did not substantially vary in both transgenic and non-transgenic cotton plants. Comparably, while examining the nutritional characteristics of bollworm larvae, it is seen that under saline stress, the intake of food by the larvae in Bt types fluctuates, increasing and decreasing at different times [33].

7. THE EFFECTS OF ABIOTIC STRESSES AND PROTEIN CONTENTS

The expression levels of Bt cotton endotoxins were negatively impacted by both high temperatures and waterlogging [34]. Thus, in this case, the pests start attacking the crop, and the crop eventually loses its maximum ability to defend itself against pests during the flowering and boll-forming periods [35]. On the other hand, the results of an alternative research that was published in 2021 were discussed in terms of the combined effects of drought and high temperatures. This research focused primarily on the relationship between the Bt protein content and different protein expression. In reaction to unfavourable abiotic circumstances, such as high temperatures and droughts, the transgenic cotton crop displays varied protein expression, which in turn influences the plant's overall cellular metabolism. A bioinformatics method called gene ontology (GO) analysis entails systematically assessing gene sets to identify the underlying biological processes, molecular roles, and cellular components they are linked to. Large-scale genomic or transcriptome data is frequently interpreted using GO analysis in genomics and functional genomics research.

A standardised and organised language called the Gene Ontology explains gene products in terms of the biological processes, molecular roles, and cellular components that they are connected with. It offers a standardised and regulated lexicon for cross-species annotation of genes and their products. More specialised concepts are layered underneath larger, more generic ones in the ontology's hierarchical structure. Utilising GO analysis, three essential protein synthesis pathways and the differential expression of up to 217 proteins were analysed in order to explore the metabolic changes caused by abiotic stresses. Previous studies on nitrogen metabolism were carried out in connection with the breakdown of Bt protein content, which will be discussed in more depth in a later section of this paper [36].

8. CONCLUSIONS AND FUTURE ASPECTS

The endotoxin Cry1Ac gene from the soil-dwelling bacteria *Bacillus thuringiensis* is inserted into Bt cotton, a genetically engineered crop, to provide insecticidal qualities that prevent cotton pests (bollworms). For the last 20 years, genetically modified cotton (Bt cotton) has been a major agricultural success. Bt crops have been commercially successfully cultivated in several nations. Global food scarcity has been resolved and agricultural yields have positively grown thanks to transgenic Bt crops. The Bt cotton crop exhibits varying degrees of insect resistance when subjected to various environmental abiotic stressors, such as high temperatures, salt in the soil, waterlogging and scarcity, nitrogen shortage, and humidity. The transgenic crop's endotoxin protein content has been shown to be degraded by these variables, which in turn lowers the crop's insecticidal effectiveness. Plants that are exposed to severe weather have altered nitrogen metabolism and physiological system distress. The main pressures that Bt cotton faces have been covered in this review, along with the underlying processes that explain its inconsistent insecticidal efficiency when faced with both single and multiple stresses. Nitrogen metabolism is the most significant part of this domain that needs investigation, although other major reasons mentioned in this review include protein breakdown, post transcriptional modifications, and gene silencing. As an overview of the many important studies conducted to look into the effects of stress, a thorough meta-analysis table has been created. To get further into the physiological and

metabolic alterations that transgenic cotton crops undergo under extreme stress conditions, a thorough study methodology is required. More study is required to solve the unstable insect protection provided by Bt crops in order to improve the ability and performance of the cotton crop globally, since there are now inadequate evidences and ways to address this issue. The problems associated with the transgenic cotton crop both burden the world economy and provide new avenues for research into insecticidal technology and tactics. Various tactics have been used to increase the insecticidal efficacies by the use of enhancers, such as chaperones, which may elevate protein expressions and endotoxin contents, potentially enhancing Bt cotton's defence mechanisms against pests. In addition to increasing plant resistance to certain pests and conditions, nitrogen fertilisation and soil replenishment may also enhance the production of toxin proteins in transgenic crops. Around the globe, biotech labs are actively working on variants that are more water-efficient and drought-tolerant. Transgenic cotton that can withstand salt and drought guarantees its spread into previously uncultivated areas and soils deemed unsuitable, as well as into areas that were previously only heavily fertilised and irrigated. In many parts of the globe in the future, agricultural activities will be determined by the availability of water. Low water requirements and enhanced drought tolerance are critical for the production of cotton, an annual crop that is grown in Africa and many other regions of the globe with erratic rainfall. When using drought-tolerant cultivars, yield improvements of 5–10% are anticipated. Biotech businesses acknowledge this as the next uncharted territory. In a similar vein, less reliance on nitrogen fertilisers will result from improvements in physiological systems for nitrogen absorption. The use of transgenic crops to control insect and weed pests will become safer when additional varieties expressing different genes than those now on the market are adopted. Insect resistance management and insect control will benefit greatly from new stacked genes, new Bt proteins, or proteins from other sources like protease inhibitors. As was previously mentioned, different modes of action are needed to sustain susceptibility in insects targeted by the proteins expressed in the plants. Similarly, new herbicide-tolerant cotton types will provide alternatives for selecting herbicides to control weed resistance. Herbicide-tolerant cultivars now available have made cultivation techniques conceivable that were not before feasible on the current scale. In

addition to enhancing the agronomic features of cotton farming, new cultivars with unique characteristics created by many biotech businesses will provide farmers with more choices that are more appropriate for their specific field conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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