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The Role of Genetic Engineering in Enhancing Sugarcane Resistance to Insect Pests

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Abstract Sugarcane (*Saccharum officinarum*) is a vital crop for sugar production globally, yet it faces significant yield losses due to insect pest attacks. Traditional breeding methods have struggled to enhance pest resistance due to the complex genetic makeup of sugarcane and the absence of inherent resistance genes. Genetic engineering has emerged as a promising alternative, enabling the introduction of genes that confer resistance to pests. This study explores various genetic engineering strategies employed to enhance sugarcane resistance to insect pests. Key approaches include the overexpression of cry proteins, Vegetative Insecticidal Proteins (VIP), lectins, and Proteinase Inhibitors (PI), as well as the application of advanced biotechnological tools such as Host-Induced Gene Silencing (HIGS) and CRISPR/Cas9. This study also discusses the integration of multiple resistance genes, such as Cry1Ab and EPSPS, and their impact on pest resistance and agronomic traits. The findings highlight the potential of genetic engineering to develop transgenic sugarcane lines with robust resistance to insect pests, thereby contributing to sustainable sugarcane production.
Keywords Genetic engineering; Sugarcane; Insect pest resistance; transgenic plants; CRISPR/Cas9

1 Introduction

Sugarcane (*Saccharum* spp.) is a vital crop globally, contributing approximately 80% of the world's sugar and a significant portion of biofuel production (Budeguer et al., 2021). It is cultivated extensively in tropical and subtropical regions, where it serves as a primary source of income for millions of farmers and plays a crucial role in the economies of many countries (Verma et al., 2022). The demand for sugar and its by-products continues to rise, necessitating sustainable improvements in sugarcane yield and quality (Narayan et al., 2020).

Despite its economic importance, sugarcane cultivation faces significant challenges, particularly from insect pests. Insect attacks, such as those from stem borers (e.g., *Diatraea saccharalis*) and the fall armyworm (*Spodoptera frugiperda*), lead to substantial yield losses and reductions in sucrose content (Li et al., 2022). Traditional pest management strategies, including the use of insecticides and integrated Pest Management (IPM) techniques, have had limited success and often pose environmental and health risks (Narayan et al., 2020; Iqbal et al., 2021). The lack of naturally resistant germplasms further complicates efforts to mitigate these pest-related challenges (Zhou et al., 2018).

Given the limitations of conventional breeding and pest management approaches, there is a pressing need for advanced resistance mechanisms to protect sugarcane crops. Genetic engineering offers a promising solution by enabling the introduction of specific resistance genes into sugarcane varieties. Techniques such as the overexpression of cry proteins, Vegetative Insecticidal Proteins (VIP), lectins, and Proteinase Inhibitors (PI) have shown potential in enhancing insect resistance (Wang et al., 2017; Iqbal et al., 2021). Additionally, modern biotechnological tools like host-induced gene silencing (HIGS) and CRISPR/Cas9 provide innovative avenues for developing sustainable pest-resistant sugarcane cultivars.

This study explores the role of genetic engineering in enhancing insect resistance in sugarcane by examining various gene modification strategies and their effectiveness, while providing a comprehensive understanding of the current progress and future prospects in this field. The study also delves into the integration of exogenous genes (such as cry1Ac) and their synergistic effects with endogenous stress-related genes, as well as the practical applications and challenges associated with developing transgenic sugarcane varieties. The aim is to highlight the potential of genetic engineering to revolutionize pest management in sugarcane cultivation, thereby contributing to increased productivity and sustainability in the industry.

2 Traditional Methods of Insect Pest Control in Sugarcane

2.1 Chemical control: pesticides and their limitations

Chemical control through the use of pesticides has been a common method for managing insect pests in sugarcane. Pesticides can provide immediate and effective control of pest populations, reducing the damage caused by insects such as cane borers and grasshoppers. However, the use of chemical pesticides presents several limitations. Firstly, many pesticides are relatively short-lived after application, necessitating frequent reapplication to maintain their effectiveness (Boulter, 1989). Additionally, pesticides can harm non-target organisms, including beneficial insects, humans, and other animals, leading to broader ecological and health concerns. The environmental impact of pesticide use, including contamination of soil and water, further complicates their application in sustainable agriculture (Iqbal et al., 2021).

2.2 Biological control: natural predators and parasitoids

Biological control involves the use of natural predators and parasitoids to manage insect pest populations. This method leverages the natural ecological relationships between pests and their natural enemies. For example, certain species of wasps and beetles are known to parasitize or prey on sugarcane pests, thereby reducing their numbers. Biological control is considered environmentally friendly and sustainable as it minimizes the need for chemical interventions and reduces the risk of pest resistance development (Verma et al., 2018). However, the effectiveness of biological control can be inconsistent due to factors such as environmental conditions and the availability of natural predators (Srikanth et al., 2011). Additionally, the introduction of non-native biological control agents must be carefully managed to avoid unintended ecological consequences.

2.3 Breeding for resistance: conventional approaches

Conventional breeding for resistance involves selecting and cross-breeding sugarcane varieties that exhibit natural resistance to insect pests. This method aims to develop cultivars that possess traits such as physical barriers (e.g., thicker stalks) or biochemical defenses (e.g., production of deterrent compounds) that reduce pest damage (Verma et al., 2018). However, the genetic complexity of sugarcane, coupled with the lack of naturally occurring resistance genes in its germplasm, makes conventional breeding a challenging and time-consuming process (Srikanth et al., 2011; Iqbal et al., 2021). Despite these challenges, conventional breeding remains a valuable tool in integrated pest management strategies, contributing to the development of more resilient sugarcane varieties over time.

In summary, while traditional methods of insect pest control in sugarcane, including chemical control, biological control, and conventional breeding, have their respective advantages, they also present significant limitations. These challenges highlight the need for innovative approaches, such as genetic engineering, to enhance sugarcane resistance to insect pests and ensure sustainable crop production.

3 Genetic Engineering: A Modern Approach to Pest Resistance

3.1 Introduction to genetic engineering techniques

Genetic engineering involves the direct manipulation of an organism's genome using biotechnology. This modern approach allows for the introduction of new traits and the enhancement of existing ones, which is particularly useful in agriculture for developing pest-resistant crops. Techniques such as *Agrobacterium*-mediated transformation, CRISPR/Cas9, and RNA interference (RNAi) are commonly employed to insert or modify genes that confer resistance to pests and diseases (Birch, 1996; Talakayala et al., 2020; Iqbal et al., 2021). These methods have revolutionized the way we approach crop improvement, offering precise and efficient solutions compared to traditional breeding methods.

3.2 Overview of genetic modifications in sugarcane

Sugarcane, a vital crop for sugar production, has been a significant focus of genetic engineering efforts due to its susceptibility to various insect pests and environmental stresses. Researchers have successfully introduced genes such as Cry1Ac, Cry2A, and EPSPS into sugarcane to confer resistance to cane borers and tolerance to glyphosate herbicide (Figure 1) (Qamar et al., 2015; Wang et al., 2017; Qamar et al., 2021). These transgenic sugarcane varieties have shown remarkable resistance to pests like *Chilo infuscatellus*, with up to 100% mortality of the pest larvae in some cases. Additionally, these genetically modified plants have demonstrated high tolerance to glyphosate, ensuring effective weed control without damaging the crop (Wang et al., 2017; Qamar et al., 2021).

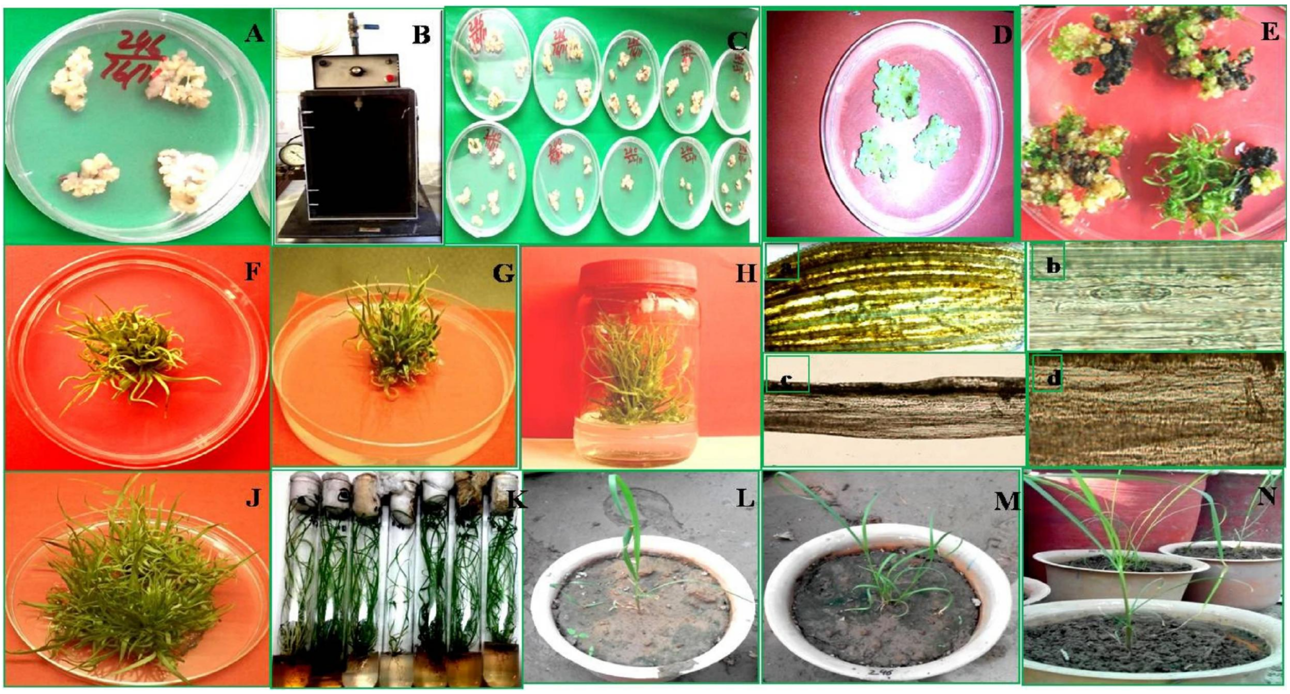

Figure 1 Schematic presentation of all the steps involved in genetic modification of sugarcane (Adopted from Qamar et al., 2021)
Image caption: (A) Callus for Bombardment. (B) Homemade Biolistic machine. (C) Bombarded Callus after bombardment with DNA-coated tungsten particles. (D) Bombarded callus shifted on selection media with Kanamycime (50 mg/L) after 2 days. (E,F) Transformed callus regenerated on double selection (Kanamycine 50 mg/L + Glyphosate 40 mM) media, (G,H) Regenerated sugarcane plantlets on glyphosate selection media (45 mM), shifting on shoot multiplication media with Kanamycime (50 mg/L) and glyphosate (50 mM) selections. (I) Gus Assay for transgenic plant screening (abcd). (J) Transgenic plants for rooting. (K) Shifting on rooting media without any selection drug. (L, M) Acclimatization: Transgenic sugarcane plantlets in soil pots under green house conditions (Adopted from Qamar et al., 2021)

3.3 Benefits of genetic engineering over traditional methods

Genetic engineering offers several advantages over traditional breeding methods. Firstly, it allows for the precise introduction of specific traits, such as pest resistance, without the need for extensive cross-breeding and selection processes (Qamar et al., 2015). This precision reduces the time required to develop new crop varieties. Secondly, genetically engineered crops can incorporate traits that are not naturally present in the species' gene pool, such as the Cry proteins from Bacillus thuringiensis, which provide effective pest resistance (Talakayala et al., 2020; Iqbal et al., 2021). Moreover, these crops often require fewer chemical inputs, such as pesticides and herbicides, leading to reduced environmental impact and lower production costs (Birch, 1996; Verma et al., 2022). The development of transgenic sugarcane with enhanced resistance to pests and herbicides exemplifies the potential of genetic engineering to improve crop resilience and productivity, ultimately benefiting both farmers and the environment (Hilder et al., 1987; Wang et al., 2017; Qamar et al., 2021).

4 Mechanisms of Genetic Engineering for Insect Pest Resistance

4.1 Bt toxin production in sugarcane

The production of *Bacillus thuringiensis* (Bt) toxins in genetically engineered sugarcane has been a significant advancement in pest resistance. Bt toxins, such as Cry proteins, have been successfully expressed in transgenic sugarcane to combat various insect pests. These proteins act by binding to specific receptors in the insect gut, causing cell lysis and death. The introduction of *Bt* genes into sugarcane has shown promising results in both laboratory and field conditions, significantly reducing pest populations and damage (Iqbal et al., 2021). However, the evolution of resistance in some pest species remains a challenge, necessitating the development of strategies to manage and mitigate resistance (Tabashnik et al., 2023).

4.2 RNA interference (RNAi) for targeted pest control

RNA interference (RNAi) is another powerful tool for enhancing insect pest resistance in sugarcane. This technique involves the expression of double-stranded RNA (dsRNA) that targets and silences specific genes essential for pest survival. RNAi offers high target specificity and minimal environmental impact compared to traditional chemical insecticides. Studies have demonstrated the effectiveness of RNAi in downregulating detoxification genes in pests, leading to reduced pest viability and increased susceptibility to plant defenses (Price and Gatehouse, 2008; Eakteiman et al., 2018; Chung et al., 2021). Despite its potential, the commercial application of RNAi in transgenic plants is still limited, and further research is needed to optimize gene targets and delivery methods (Chung et al., 2021; Halder et al., 2022).

4.3 CRISPR-Cas9 and its potential in developing resistant varieties

The CRISPR-Cas9 genome editing system has emerged as a revolutionary tool for developing insect-resistant sugarcane varieties. By precisely targeting and modifying specific genes, CRISPR-Cas9 can create mutations that confer resistance to insect pests. For instance, CRISPR-mediated knockout of ABC transporter genes in pests has been shown to confer high levels of resistance to Bt toxins (Guo et al., 2019; Fabrick et al., 2021). This technology allows for the rapid development of resistant strains and provides insights into the genetic mechanisms underlying pest resistance. The potential of CRISPR-Cas9 in sugarcane genetic engineering is vast, offering a flexible and efficient approach to enhance pest resistance.

4.4 Stacked traits for enhanced resistance

Combining multiple resistance traits, or "stacking," is a strategy to enhance the durability and effectiveness of pest-resistant sugarcane. Stacked traits can include the expression of multiple Bt toxins, RNAi constructs, and other resistance genes within a single plant. This approach aims to provide broad-spectrum resistance and reduce the likelihood of pests developing resistance to any single trait. For example, transgenic sugarcane lines expressing both Bt toxins and herbicide tolerance genes have shown strong resistance to pests and improved agronomic performance under field conditions (Wang et al., 2017; Iqbal et al., 2021). The integration of stacked traits represents a comprehensive strategy to achieve sustainable pest management in sugarcane cultivation.

By leveraging these genetic engineering mechanisms, researchers aim to develop sugarcane varieties with robust and long-lasting resistance to insect pests, thereby improving crop yield and reducing reliance on chemical pesticides.

5 Case Study

5.1 Overview of a specific genetic engineering initiative in sugarcane

One notable genetic engineering initiative aimed at enhancing sugarcane resistance to insect pests involved the introduction of the *cry1Ac* gene, which encodes an insecticidal protein from *Bacillus thuringiensis* (Bt). This initiative was undertaken to address the significant yield losses caused by the sugarcane stem borer (*Diatraea saccharalis*), a prevalent pest in sugarcane cultivation. The *cry1Ac* gene was selected due to its proven efficacy in other crops and its ability to produce a protein that is toxic to certain insect pests but safe for humans and other non-target organisms (Weng et al., 2006; Zhou et al., 2018; Qamar et al., 2021).

5.2 Implementation and outcomes of the case study

The implementation of this genetic engineering initiative involved several key steps. First, the *cry1Ac* gene was synthetically optimized to match the codon usage of sugarcane, enhancing its expression in the plant. This synthetic gene was then introduced into sugarcane varieties using *Agrobacterium*-mediated transformation and microprojectile bombardment techniques (Weng et al., 2006; Dessoky et al., 2020).

The transformed sugarcane lines were subjected to rigorous testing to confirm the integration and expression of the *cry1Ac* gene. Molecular analyses, including PCR and Southern blotting, verified the presence of the gene, while immunoblotting and ELISA assays confirmed the production of the Cry1Ac protein (Weng et al., 2006; Wang et al., 2017; Dessoky et al., 2020).

Field trials demonstrated that the transgenic sugarcane lines exhibited high levels of resistance to the stem borer. In bioassays, larvae fed on transgenic sugarcane showed significantly higher mortality rates compared to those fed on non-transgenic controls. For instance, one study reported up to 100% mortality of *Chilo infuscatellus* larvae when fed on 80-day-old transgenic plants. Additionally, the transgenic lines maintained their resistance over multiple generations, indicating stable gene expression (Weng et al., 2006; Qamar et al., 2021).

5.3 Lessons learned and future directions

The case study of cry1Ac transgenic sugarcane provides several valuable lessons for future genetic engineering initiatives. Firstly, the importance of optimizing gene sequences for the target plant species was highlighted, as this can significantly enhance gene expression and the effectiveness of the transgene. Secondly, the use of multiple transformation techniques and rigorous molecular and field testing are crucial for ensuring the stability and efficacy of the transgenic lines (Weng et al., 2006; Wang et al., 2017; Dessoky et al., 2020). However, the study also revealed some challenges. For instance, some transgenic lines exhibited poor agronomic traits, such as reduced plant height and biomass, which could impact their commercial viability. This underscores the need for a balanced approach that not only focuses on pest resistance but also considers overall plant health and productivity.

Future directions for research could include the development of transgenic lines with stacked traits, combining insect resistance with other desirable characteristics such as herbicide tolerance or drought resistance (Qamar et al., 2021). Additionally, exploring the synergistic effects of combining cry1Ac with other insect resistance genes or stress-related genes could further enhance the durability and effectiveness of the resistance (Zhou et al., 2018).

In conclusion, the genetic engineering of sugarcane for insect resistance using the *cry1Ac* gene has shown promising results, providing a sustainable alternative to chemical pesticides and contributing to improved sugarcane yields. Continued research and development in this area hold the potential to further enhance the resilience and productivity of this vital crop.

6 Environmental and Ecological Implications

6.1 Impact on non-target species and biodiversity

The introduction of Genetically Engineered (GE) sugarcane for pest resistance has raised concerns about its potential impact on non-target species and overall biodiversity. The overexpression of cry proteins, Vegetative Insecticidal Proteins (VIP), lectins, and proteinase inhibitors (PI) in transgenic sugarcane has been shown to effectively control target pests such as cane borers (Iqbal et al., 2021; Qamar et al., 2021). However, these proteins can also affect non-target organisms, including beneficial insects, soil microorganisms, and other wildlife. For instance, the widespread use of Bt crops has been associated with reduced populations of non-target insects, which can disrupt local ecosystems and food webs. Additionally, the potential for gene flow from GE sugarcane to wild relatives or other crops could lead to unintended ecological consequences, such as the development of new pest-resistant weed species (Budeguer et al., 2021).

6.2 Potential risks and safety assessments

The deployment of GE sugarcane necessitates thorough risk assessments to ensure environmental and human safety. Studies have demonstrated that transgenic sugarcane expressing Cry1Ab and EPSPS proteins exhibit strong resistance to pests and herbicides, respectively. However, these modifications can also pose risks, such as the development of resistance in target pest populations and unintended effects on non-target organisms. Safety assessments must include comprehensive evaluations of the potential for allergenicity, toxicity, and environmental persistence of the introduced genes and their products (Wang et al., 2017; Verma et al., 2022). Additionally, long-term field studies are essential to monitor the ecological impacts and effectiveness of GE sugarcane in diverse agricultural settings (Qamar et al., 2021).

6.3 Regulatory frameworks and compliance

The commercialization of GE sugarcane is subject to stringent regulatory frameworks designed to ensure biosafety and environmental protection. Regulatory authorities require detailed molecular studies to assess transgene insertion sites, the number of transgenes, and gene expression levels before approving GE crops for commercial use. In Brazil, for example, only three insect-resistant transgenic sugarcane varieties have been officially approved for commercialization, highlighting the rigorous evaluation process (Budeguer et al., 2021). Compliance with international biosafety standards, such as those outlined by the Cartagena Protocol on Biosafety, is crucial for the global acceptance and trade of GE sugarcane. These regulations aim to mitigate potential risks and ensure that GE crops are safe for cultivation and consumption (Verma et al., 2022).

In conclusion, while genetic engineering offers promising solutions for enhancing sugarcane resistance to insect pests, it is imperative to carefully consider the environmental and ecological implications. Ongoing research, robust safety assessments, and adherence to regulatory frameworks are essential to ensure the sustainable and responsible use of GE sugarcane in agriculture.

7 Challenges and Limitations of Genetic Engineering in Sugarcane

7.1 Technical challenges in gene editing

The genetic complexity of sugarcane presents significant technical challenges for gene editing. Sugarcane is a highly polyploid and aneuploid crop with an extremely large genome, which complicates the development of efficient transformation protocols. Each new genotype requires optimized tissue culture and plant generation procedures, making the process laborious and time-consuming (Budeguer et al., 2021). Additionally, the lack of a complete sequenced reference genome for sugarcane hinders molecular studies necessary for commercial release, such as determining transgene insertion sites and gene expression levels. Transgene silencing is another technical hurdle, as it occurs in a large proportion of genetically modified sugarcane plants, affecting the stability and effectiveness of the introduced traits (Arruda, 2012). Moreover, the advent of CRISPR/Cas9 technology, while promising, is still in its infancy for sugarcane, and challenges such as low transformation efficiency and transgene silencing remain significant obstacles (Mohan, 2016).

7.2 Public perception and acceptance

Public perception and acceptance of Genetically Modified (GM) crops, including sugarcane, are critical factors influencing their adoption. Despite the potential benefits, there is widespread skepticism and resistance to GM crops due to concerns about their safety, environmental impact, and ethical considerations (Tyagi et al., 2020). Regulatory issues further complicate the commercial approval of genetically modified sugarcane, as stringent policies for risk assessment and biosafety must be met (Arruda, 2012). The presence of genome editing cassettes in the genome of edited plants also raises regulatory challenges, which can be mitigated by using transgene-free genome editing techniques (Krishna et al., 2023). However, public acceptance remains a significant barrier, and efforts to educate and engage with the public are essential to address these concerns and facilitate the adoption of genetically engineered sugarcane (Tyagi et al., 2020).

7.3 Economic considerations and market adoption

Economic considerations and market adoption are crucial for the successful commercialization of genetically engineered sugarcane. The development and commercialization of transgenic sugarcane are costly and time-consuming processes, requiring substantial investment in research, development, and regulatory compliance . Despite the potential for improved yield and pest resistance, the economic viability of genetically modified sugarcane must be carefully evaluated. For instance, while transgenic sugarcane lines have shown strong insect resistance and herbicide tolerance, they often present poor agronomic characteristics and industrial traits compared to non-transformed control plants, which can affect their marketability (Wang et al., 2017). Additionally, the limited number of officially approved transgenic sugarcane varieties for commercialization highlights the challenges in achieving market adoption (Figure 2) (Budeguer et al., 2021). Therefore, economic considerations, including cost-benefit analysis and market acceptance, play a pivotal role in determining the feasibility and success of genetically engineered sugarcane.

In summary, while genetic engineering holds promise for enhancing sugarcane resistance to insect pests, several challenges and limitations must be addressed. Technical challenges in gene editing, public perception and acceptance, and economic considerations and market adoption are critical factors that influence the successful implementation and commercialization of genetically engineered sugarcane (Arruda, 2012; Tyagi et al., 2020; Budeguer et al., 2021; Krishna et al., 2023).

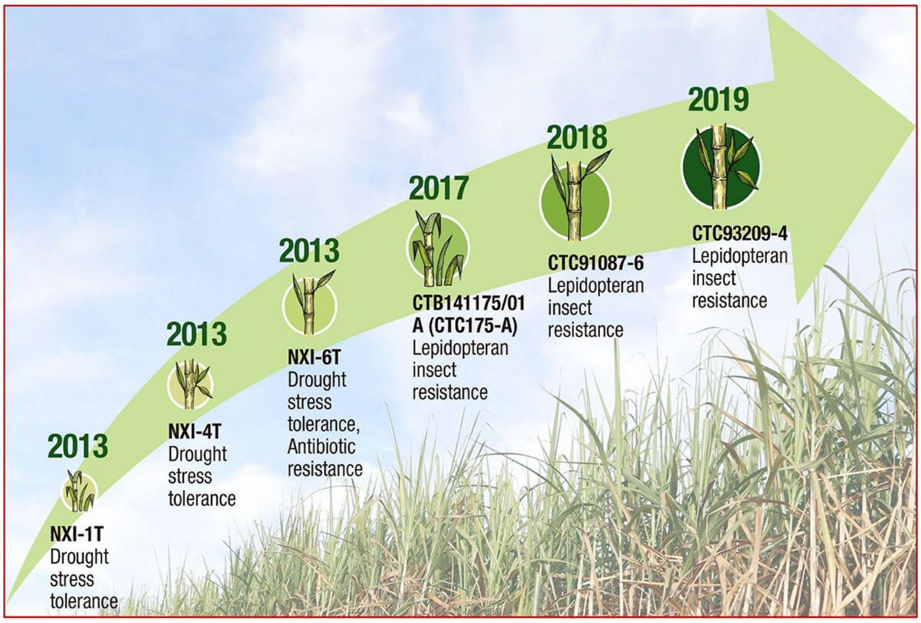

Figure 2 The sugarcane events approved for domestic or non-domestic cultivation (Adopted from Verma et al., 2022)

8 Future Perspectives and Research Directions

8.1 Innovations in genetic engineering techniques

The future of enhancing sugarcane resistance to insect pests lies in the continuous innovation of genetic engineering techniques. Recent advancements such as CRISPR/Cas9 and Host-Induced Gene Silencing (HIGS) have shown promising results in providing sustainable control of insect pests in sugarcane. These techniques allow for precise genetic modifications, enabling the development of sugarcane varieties with enhanced resistance to a broad spectrum of pests. Additionally, the overexpression of cry proteins, vegetative insecticidal proteins (vip), lectins, and proteinase inhibitors (PI) has been successfully implemented in transgenic sugarcane, demonstrating significant resistance to various insect pests (Srikanth et al., 2011; Narayan et al., 2020; Iqbal et al., 2021). Future research should focus on optimizing these techniques to ensure stable and high-level expression of resistance genes, as well as exploring new insecticidal proteins and their combinations to delay resistance development in pests (Riaz et al., 2020).

8.2 Integrating genetic engineering with other pest management strategies

While genetic engineering provides a powerful tool for pest resistance, integrating it with other pest management strategies can enhance its effectiveness and sustainability. Diversified Integrated Pest Management (IPM) plans that combine Genetically Engineered (GE) crops with traditional methods such as crop rotation, biological control, and the use of refuges can help manage resistance development in pest populations (Anderson et al., 2019). For instance, the use of Bt crops has been shown to reduce reliance on chemical insecticides, but the evolution of resistance in some pest species necessitates the integration of other control measures (Tabashnik et al., 2023). By leveraging the strengths of both genetic engineering and conventional pest management strategies, a more robust and sustainable approach to pest control in sugarcane can be achieved (Wang et al., 2017; Qamar et al., 2021).

8.3 Long-term sustainability and monitoring

Ensuring the long-term sustainability of genetically engineered sugarcane requires continuous monitoring and management of resistance development in pest populations. Regular resistance monitoring, as demonstrated in the global analysis of Bt crops, is crucial for detecting early signs of resistance and implementing timely management strategies (Tabashnik et al., 2023). Additionally, the development of transgenic sugarcane lines with multiple resistance genes can provide a more durable defense against pests. However, it is essential to address potential non-target effects and ensure that transgenic lines maintain desirable agronomic traits (Srikanth et al., 2011; Wang et al., 2017). Future research should also focus on understanding the ecological impacts of transgenic sugarcane and developing strategies to mitigate any adverse effects on beneficial insects and the environment (Verma et al., 2018; Verma et al., 2022). By adopting a proactive and integrated approach, the long-term sustainability of genetically engineered sugarcane for pest resistance can be achieved.

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Conflict of Interest Disclosure
The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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