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The Impact of Genetic Engineering on Maize Herbicide Tolerance

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Abstract The adoption of genetically engineered maize for herbicide tolerance has significantly impacted agricultural practices, particularly in weed management. This study examines the development, implementation, and consequences of herbicide-tolerant maize varieties. The introduction of transgenic maize expressing genes such as *dicamba monooxygenase* (*DMO*) and *CP4-EPSPS* has enabled higher tolerance levels to herbicides like dicamba and glyphosate, respectively, leading to improved weed control and reduced crop injury. However, the widespread use of these genetically modified (GM) crops has also led to the emergence of herbicide-resistant weeds, necessitating the development of dual herbicide-tolerant varieties and new herbicide tolerance traits. Meta-analyses and field studies indicate that while GM crops have reduced overall pesticide use and increased crop yields and farmer profits, the long-term sustainability of these benefits is challenged by evolving weed resistance. This study synthesizes findings from multiple studies to provide a comprehensive understanding of the agronomic, economic, and environmental impacts of herbicide-tolerant maize, highlighting both the advantages and the ongoing challenges in this field.

Keywords Genetic engineering; Herbicide tolerance; Maize (*Zea mays*); Weed resistance; Transgenic crops

1 Introduction

Maize (*Zea mays* L.) is a staple crop with significant economic and nutritional importance worldwide. However, weed interference is a major biotic stress that can dramatically reduce maize yields. Effective weed management is crucial for maintaining favorable growing conditions and ensuring high crop productivity. Traditional weed control methods often rely on the application of herbicides, which can be labor-intensive and environmentally challenging. The development of herbicide-tolerant maize varieties through genetic engineering has emerged as a promising solution to enhance weed control efficiency and reduce the environmental impact of herbicide use (Perry et al., 2016; Larue et al., 2019).

Herbicide-tolerant crops allow farmers to use specific herbicides that the crops can withstand, thereby effectively controlling weeds without damaging the crop itself. This technology has led to significant changes in agricultural practices, including reduced tillage, which helps in soil conservation and moisture retention. However, the widespread adoption of herbicide-tolerant crops has also raised concerns about the potential for increased herbicide use and the development of herbicide-resistant weed populations (Perry et al., 2016).

Genetic engineering involves the manipulation of an organism's genome using biotechnology to introduce, enhance, or modify specific traits. In agriculture, genetic engineering has been used to develop crops with improved resistance to pests, diseases, and environmental stresses, as well as enhanced nutritional profiles. The introduction of herbicide tolerance traits in crops is one of the most successful applications of genetic engineering in agriculture (Fu et al., 2021).

Several techniques are employed in the genetic engineering of crops, including the use of recombinant DNA technology to insert specific genes into the plant genome. For instance, the development of glyphosate-tolerant maize involves the insertion of the *CP4-EPSPS* gene, which confers resistance to the herbicide glyphosate. Similarly, the *bar* gene is used to develop glufosinate-tolerant maize. These genes are often introduced into the plant genome using Agrobacterium-mediated transformation or biolistic (gene gun) methods (Fu et al., 2021; Bonny, 2016; Bao et al., 2022).

Recent advancements in genetic engineering have also focused on developing crops with multiple herbicide tolerance traits to address the issue of herbicide-resistant weeds. For example, maize varieties tolerant to both glyphosate and glufosinate have been developed to provide farmers with more flexible and effective weed management options (Fu et al., 2021; Bao et al., 2022).

The primary objective of this study is to evaluate the impact of genetic engineering on herbicide tolerance in maize. Assess the effectiveness of genetically engineered herbicide-tolerant maize in improving weed control and crop yields. Examine the environmental and economic implications of adopting herbicide-tolerant maize varieties. Investigate the potential risks associated with the development of herbicide-resistant weeds and the increased use of herbicides. Explore the advancements in genetic engineering techniques that have contributed to the development of herbicide-tolerant maize.

By synthesizing findings from multiple research studies, this study seeks to provide a comprehensive understanding of the benefits and challenges associated with genetically engineered herbicide-tolerant maize. The insights gained from this study will inform future research and policy decisions aimed at optimizing the use of genetic engineering in agriculture for sustainable crop production.

2 Genetic Engineering Techniques for Herbicide Tolerance

2.1 Genetic engineering techniques for herbicide tolerance

Transgenic approaches involve the introduction of foreign genes into the maize genome to confer herbicide tolerance. One of the most common genes used in this context is the *CP4-EPSPS* gene, which provides resistance to glyphosate, a widely used broad-spectrum herbicide. For instance, the development of transgenic maize expressing both *CP4-EPSPS* and *bar* genes has shown significant tolerance to glyphosate and glufosinate, allowing for effective weed management and reducing the risk of herbicide resistance in weeds (Yu et al., 2023). Additionally, the use of codon-optimized synthetic *CP4-EPSPS* genes has been demonstrated to confer high levels of glyphosate tolerance in transgenic rice, suggesting similar potential applications in maize (Hummel et al., 2018).

Genome editing techniques such as CRISPR/Cas9 and TALENs have revolutionized the field of genetic engineering by enabling precise modifications at specific genomic loci. CRISPR/Cas9, in particular, has been employed to introduce targeted mutations in the *EPSPS* gene, resulting in glyphosate-resistant crops. For example, CRISPR/Cas9-mediated homology-directed repair has been used to introduce amino acid substitutions in the *EPSPS* gene of rice, conferring glyphosate resistance and enhancing grain yield (Figure 1) (Sony et al., 2023). Similarly, the CRISPR/Cas9 system has been utilized to optimize glyphosate tolerance in rapeseed by precise gene replacement, demonstrating the potential for similar applications in maize (Wang et al., 2021).

2.2 Key genes involved in herbicide tolerance

The *EPSPS* (*5-enolpyruvylshikimate-3-phosphate synthase*) gene is a critical target for glyphosate, which inhibits the shikimate pathway essential for the biosynthesis of aromatic amino acids. Mutations in the *EPSPS* gene can render the enzyme less sensitive to glyphosate, thereby conferring resistance. For instance, the introduction of mutant variants of the *EPSPS* gene with specific amino acid substitutions has been shown to provide glyphosate tolerance in rice (Achary et al., 2020). Additionally, the co-expression of *EPSPS* with other genes, such as glyphosate oxidase, has been demonstrated to enhance glyphosate tolerance and reduce herbicide residues in transgenic crops (Wen et al., 2021).

Apart from the *EPSPS* gene, other genes have also been identified to confer herbicide tolerance. For example, the *bar* gene, which encodes phosphinothricin acetyltransferase, provides resistance to glufosinate, another commonly used herbicide. The co-expression of *CP4-EPSPS* and *bar* genes in transgenic maize has been shown to confer dual herbicide tolerance, allowing for more flexible and effective weed management strategies (Yu et al., 2023). Additionally, the *igrA* gene from Pseudomonas, which encodes a glyphosate detoxifying enzyme, has been used in combination with EPSPS to enhance glyphosate tolerance and reduce herbicide residues in transgenic rice (Fartyal et al., 2018).

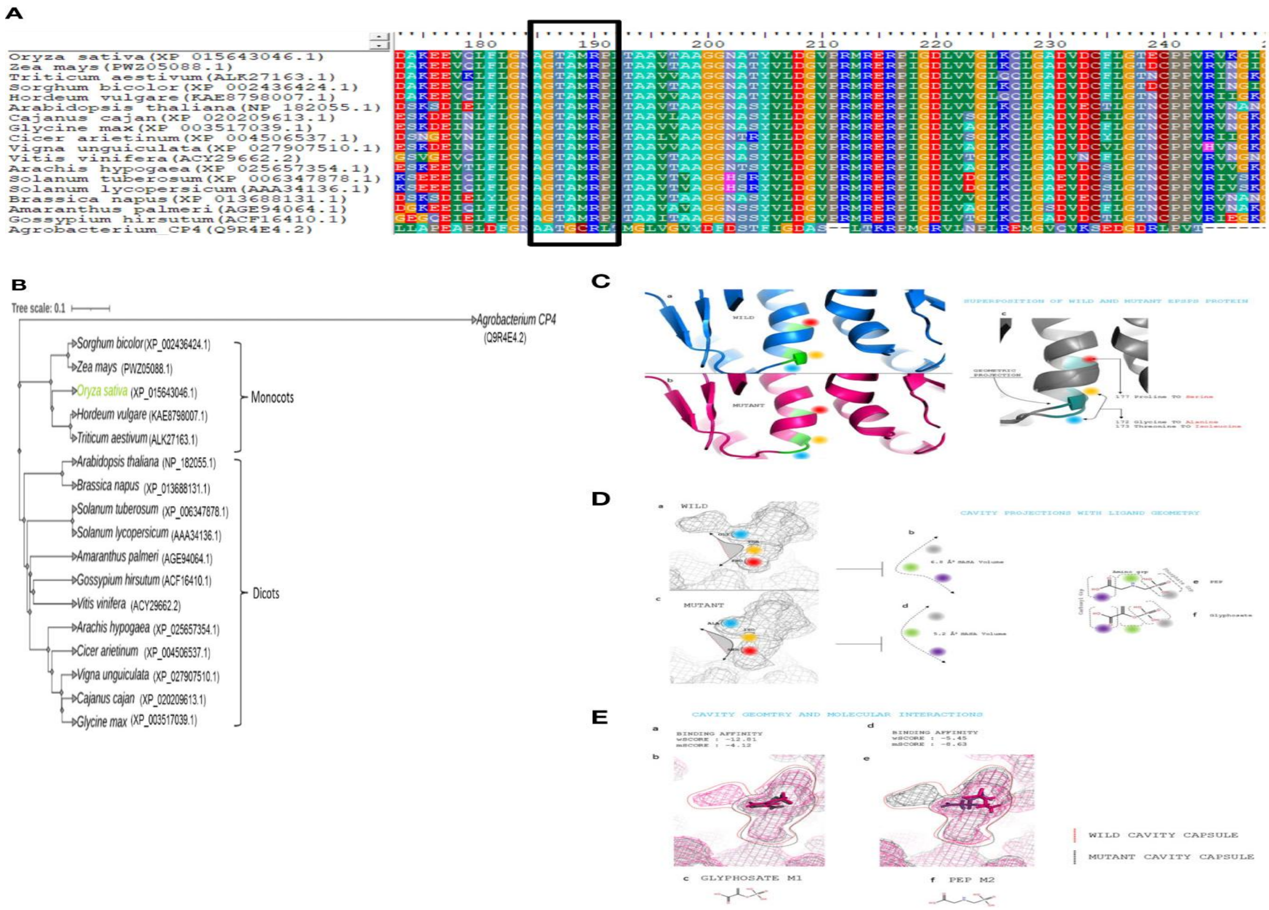

Figure 1 In silico analysis and identification of glyphosate-resistant (GR) mutations site (Adopted from Sony et al., 2023)

Image caption: A: The conserved amino acid (G172, T173, and P177) sites in EPSPS protein of different plants (B) Phylogenetic analysis of EPSPS protein of different plants; C: (a) Wild structure of EPSPS protein in ribbon-like representation with positioning of selected amino acid to edit; (b) Mutant structure of EPSPS protein in ribbon-like representation with positioning of selected amino acid to edit; (c) Superpositioned structure of mutant and WT EPSPS proteins for geometrical projections; Position of 172, 173, and 177 amino acids are highlighted as blue, yellow, and red, respectively; D: (a) Active site volume of wild EPSPS protein, highlighting the selected three amino acids to edit; (b) The area of the volume within the selected amino acids. (c) Active site volume of Mutant EPSPS protein, highlighting the selected three amino acids to edit; (d) The area of the volume within the selected amino acids. (e) Two-dimensional structure of PEP, mentioning the position of phosphate, amino, and carboxyl group; (f) Two-dimensional structure of glyphosate, mentioning the position of phosphate, amino, and carboxyl group; E: (a) Active site volume of wild EPSPS protein, highlighting the selected three amino acids to edit; (b) The area of the volume within the selected amino acids; (c) Active site volume of mutant EPSPS protein, highlighting the selected three amino acids to edit; (d) The area of the volume within the selected amino acids; (e) Two-dimensional structure of PEP, mentioning the position of the phosphate, amino, and carboxyl group; (f) Two-dimensional structure of Glyphosate, mentioning the position of the phosphate, amino, and carboxyl group (Adopted from Sony et al., 2023)

Genetic engineering techniques, including transgenic approaches and genome editing, have significantly advanced the development of herbicide-tolerant maize. Key genes such as *EPSPS* and *bar* play crucial roles in conferring resistance to glyphosate and glufosinate, respectively, while the integration of additional genes like *igrA* can further enhance tolerance and reduce herbicide residues. These advancements hold great promise for improving weed management and crop productivity in maize.

3 The Impact of Genetic Engineering on Maize Herbicide Tolerance

3.1 Historical development of herbicide-tolerant maize

The development of herbicide-tolerant maize has been a significant milestone in agricultural biotechnology. The initial efforts focused on creating maize varieties that could withstand specific herbicides, thereby simplifying weed management and reducing crop damage. Early genetically engineered (GE) maize varieties were primarily designed to tolerate glyphosate, a broad-spectrum herbicide. This innovation allowed farmers to control a wide range of weeds with a single herbicide application, leading to more efficient and cost-effective farming practices (Perry et al., 2016).

3.2 Major commercial herbicide-tolerant maize varieties

Several major commercial herbicide-tolerant maize varieties have been developed and widely adopted. One notable example is the glyphosate-tolerant maize, which has been extensively used in the United States. This variety has shown a reduction in herbicide use by 1.2% compared to non-adopters, highlighting its efficiency in weed management (Perry et al., 2016). Another significant development is the creation of maize varieties tolerant to both glyphosate and glufosinate. The SCB-29 maize event, for instance, expresses both *CP4-EPSPS* and *bar* genes, providing robust tolerance to these herbicides and demonstrating stable integration and expression of the transgenes over multiple generations (Yu et al., 2023).

3.3 Case studies of successful genetic engineering for herbicide tolerance

Several case studies illustrate the success of genetic engineering in developing herbicide-tolerant maize. One such example is the development of maize tolerant to dicamba, a herbicide used for broadleaf weed control. Transgenic maize plants expressing the *dicamba monooxygenase* (*DMO*) gene linked with a chloroplast transit peptide (CTP) have shown enhanced tolerance to dicamba, significantly exceeding the recommended application rates without crop injury (Cao et al., 2011). Another case study involves the engineering of maize with tolerance to aryloxyphenoxypropionate (FOP) and synthetic auxin herbicides like 2,4-D. This was achieved by developing an enzyme with robust and specific activity for these herbicide families, providing farmers with additional tools for effective weed management (Fu et al., 2021) (Figure 2).

These advancements underscore the potential of genetic engineering to enhance herbicide tolerance in maize, offering significant benefits in terms of weed control, crop yield, and environmental sustainability. However, the continuous evolution of herbicide-resistant weeds remains a challenge, necessitating ongoing research and development to sustain the effectiveness of these genetically engineered crops (Bonny, 2016).

4 Agronomic and Environmental Impacts

4.1 Agronomic benefits of herbicide-tolerant maize

Herbicide-tolerant maize has significantly improved weed management and crop yield. The adoption of genetically modified (GM) crops, including herbicide-tolerant maize, has led to a reduction in chemical pesticide use by 37% and an increase in crop yields by 22% on average (Klümper and Qaim, 2014). Specifically, transgenic maize plants expressing genes for herbicide tolerance, such as those tolerant to dicamba, have shown enhanced weed control and increased crop yield potential (Cao et al., 2011). Additionally, the development of maize tolerant to both glyphosate and glufosinate has provided a robust weed management system, delaying the development of herbicide resistance in weeds and maintaining high crop yields (Yu et al., 2023).

4.2 Environmental concerns and considerations

While herbicide-tolerant maize offers agronomic benefits, there are concerns regarding its impact on biodiversity. The widespread adoption of GM crops has led to homogenization of agricultural practices, which can affect non-target biota and soil food web properties (Rizzo et al., 2022). Studies have shown that the herbicide systems associated with GM crops can alter the fungal-to-bacterial biomass ratios in soil, potentially impacting soil health and biodiversity (Rizzo et al., 2022). Additionally, the potential for indirect harmful effects on farmland biodiversity through losses in food resources and shelter has been highlighted, although these effects have not been consistently demonstrated (Devos et al., 2008).

The introduction of herbicide-tolerant maize has also contributed to reduced tillage practices, which are beneficial for soil conservation. The Farm Scale Evaluations in the UK demonstrated that GM herbicide-tolerant crops generally required fewer herbicide applications and less active ingredient compared to conventional crops, which can reduce soil disturbance and erosion (Champion et al., 2003). Moreover, the use of broad-spectrum herbicides like glyphosate and glufosinate in herbicide-tolerant maize systems has been associated with lower environmental impact and reduced need for mechanical weed control, further promoting soil conservation (Devos et al., 2008).

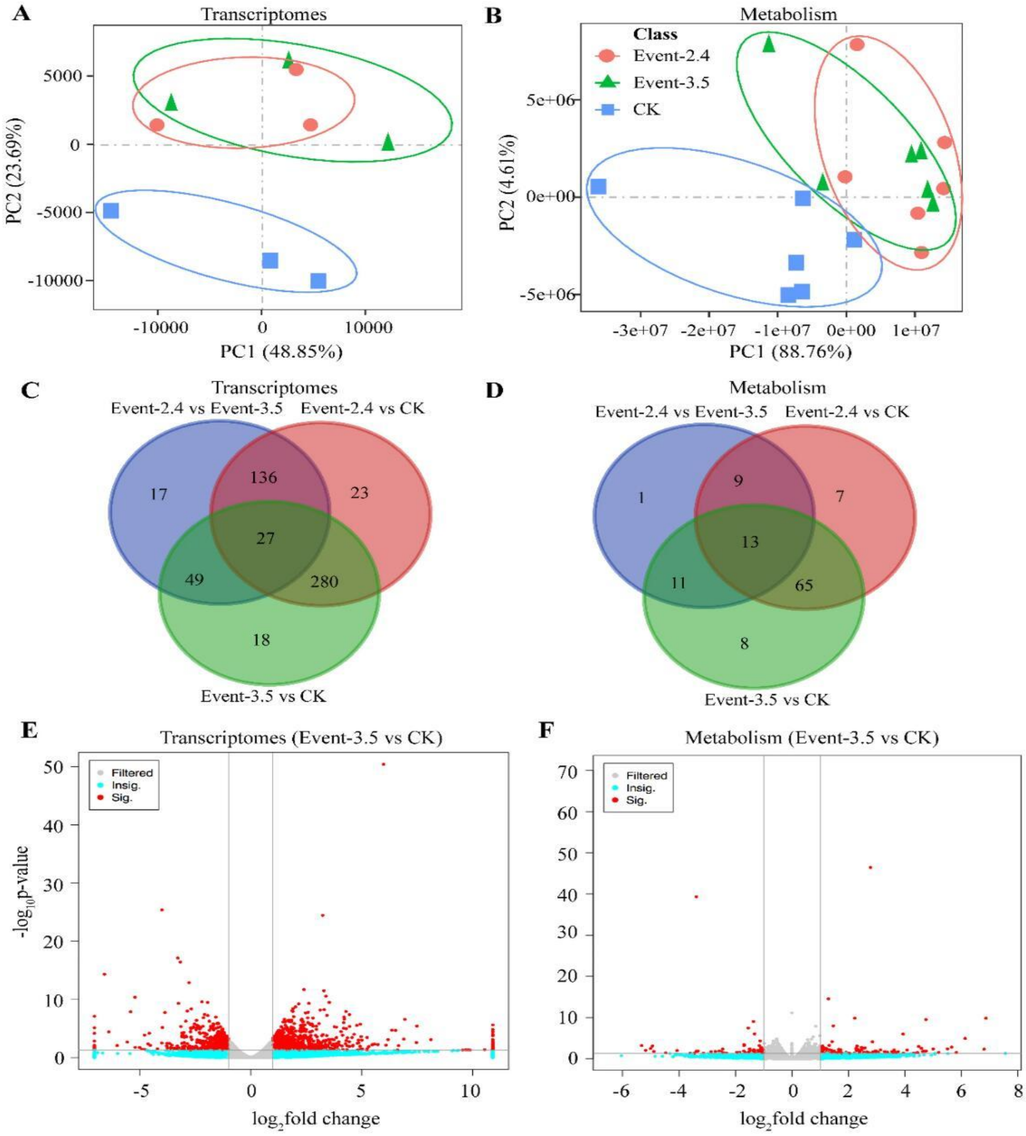

Figure 2 PCA analysis on differently abundant genes and metabolites in ZD958 maize transgenic lines grown in Beijing (Adopted from Fu et al., 2021)

Image caption: (A): transcriptomes analysis on different experimental sites across different transgenic lines of transgenic event-2.4 (Event-2.4), transgenic event-3.5 (Event-3.5) and CK; n = 3; (B): Metabolomic analysis on different experimental sites across different transgenic lines of 2.4, 3.5 and ck. n = 6; (C), overlapping genes between different ZD958 transgenic events and non-GM ZD958 (CK) grown in Beijing. The detailed overlapping genes are referred to Supplementary Table S2; (D), overlapping metabolites. The detailed overlapping metabolites are referred to Supplementary Table S3. (E,F), differential abundant genes and metabolites between event-3.5 and CK; The differential abundant genes (DAGs) and metabolites (DAMs) were indicated in red scatters (Adopted from Fu et al., 2021)

One of the major environmental concerns associated with herbicide-tolerant maize is the development of herbicide-resistant weeds. The repeated use of glyphosate-tolerant crops and glyphosate applications has led to the emergence of glyphosate-resistant weed populations, necessitating the use of additional herbicides and increasing overall herbicide use (Perry et al., 2016). This resistance development poses a significant challenge to sustainable weed management and highlights the need for integrated weed management strategies that include herbicide rotation and diversification (Devos et al., 2008).

While herbicide-tolerant maize provides substantial agronomic benefits, including improved weed management, increased crop yields, and reduced tillage, it also raises important environmental concerns. These include potential impacts on biodiversity and the development of herbicide-resistant weeds, which require careful management and further research to ensure sustainable agricultural practices.

5 Socioeconomic and Regulatory Aspects

5.1 Economic impact on farmers and the agricultural industry

The adoption of genetically engineered (GE) herbicide-tolerant (HT) maize has shown varied economic impacts on farmers. Studies indicate that GE crops can lead to both increased and decreased herbicide use, depending on the crop and the specific genetic modification. For instance, adopters of GE glyphosate-tolerant (GT) maize used 1.2% less herbicide than non-adopters, which can translate into cost savings for farmers (Perry et al., 2016). However, the emergence of glyphosate-resistant weeds has led to increased herbicide use over time, potentially offsetting initial cost benefits (Bonny, 2016). Additionally, the development of dual herbicide-tolerant maize, such as the SCB-29 event, which is tolerant to both glyphosate and glufosinate, offers a promising solution to manage herbicide resistance and maintain economic benefits for farmers (Yu et al., 2023).

The market dynamics surrounding GE HT crops are influenced by several factors, including regulatory approvals, market acceptance, and the prevalence of herbicide-resistant weeds. The rapid adoption of GMHT crops, particularly in the USA, highlights their perceived benefits in terms of weed management and crop yield (Bonny, 2016). However, the spread of glyphosate-resistant weeds has necessitated changes in herbicide application strategies, impacting adoption rates and market dynamics (Bonny, 2016). The Farm Scale Evaluations in the UK demonstrated that GMHT crops generally required fewer herbicide applications compared to conventional crops, which could influence adoption rates positively (Yu et al., 2023).

5.2 Regulatory frameworks and biosafety considerations

International regulations and guidelines play a crucial role in the adoption and management of GE HT crops. Regulatory frameworks vary by country, with some regions adopting more stringent biosafety measures than others. For example, the European Union has implemented rigorous risk assessment protocols for GM crops, which can delay or restrict their approval and commercialization (Yu et al., 2023). In contrast, countries like the USA have more streamlined regulatory processes, facilitating quicker adoption of GE crops (Bonny, 2016). The development of dual herbicide-tolerant maize in China underscores the importance of regulatory frameworks in addressing herbicide resistance and ensuring biosafety (Zilberman et al., 2010).

Risk assessment and management are critical components of the regulatory process for GE HT crops. These processes involve evaluating the potential environmental and health impacts of GE crops, including the development of herbicide-resistant weeds and the effects on non-target organisms. Studies have shown that the use of GE HT crops can lead to changes in herbicide use patterns, which must be carefully monitored and managed to mitigate risks (Perry et al., 2016; Bonny, 2016). The integration of herbicide resistance genes, such as the *NPK1* gene for drought and salt tolerance, further complicates risk assessment, necessitating comprehensive evaluation of both agronomic and environmental impacts (Brookes, 2019).

The socioeconomic and regulatory aspects of GE HT maize are multifaceted, involving cost-benefit analyses for farmers, market dynamics, and stringent regulatory frameworks. Effective risk assessment and management are essential to ensure the sustainable adoption and use of these crops, balancing economic benefits with environmental and biosafety considerations.

6 Future Perspectives and Research Directions

6.1 Advancements in genetic engineering technologies

Recent advancements in genetic engineering technologies have significantly enhanced the development of herbicide-tolerant maize varieties. Techniques such as CRISPR/Cas9 and other genome editing tools have enabled precise modifications in the maize genome, leading to the creation of crops with improved herbicide tolerance and other desirable traits (Bao et al., 2022). The integration of morphogenic regulators has also increased transformation efficiency and genotype independence, facilitating the development of genetically modified maize varieties with multiple stacked traits (Bao et al., 2022). These advancements are expected to continue driving innovation in maize biotechnology, allowing for the development of more robust and versatile herbicide-tolerant crops.

6.2 Emerging herbicide tolerance traits and genes

The discovery and incorporation of new herbicide tolerance traits and genes are crucial for addressing the evolving challenges in weed management. For instance, the development of maize varieties tolerant to both glyphosate and glufosinate has shown promise in delaying the development of weed resistance (Yu et al., 2023). Additionally, the identification of genes such as *ZmGHT1*, which confers glufosinate tolerance, provides new avenues for breeding herbicide-resistant maize (Guo et al., 2023). The engineering of enzymes with robust activity against multiple herbicide families, such as aryloxyphenoxypropionate and synthetic auxins, further expands the toolkit available for effective weed control (Larue et al., 2019).

6.3 Integrating herbicide tolerance with other agronomic traits

Integrating herbicide tolerance with other agronomic traits, such as insect resistance and improved yield, is essential for developing comprehensive solutions for crop management. Transgenic maize varieties harboring multiple genes, such as *cry2Ab*, *vip3A*, and *CP4-EPSPS*, have demonstrated strong insect resistance and herbicide tolerance, providing valuable germplasm for future breeding programs (Liu et al., 2023). Moreover, the elimination of trade-offs between resistance, tolerance, and growth in certain maize genotypes highlights the potential for breeding varieties that perform well under complex field conditions. This integrated approach ensures that herbicide-tolerant maize varieties also possess other beneficial traits, enhancing their overall utility and performance.

6.4 Addressing challenges and mitigating risks

Despite the progress made, several challenges and risks associated with herbicide-tolerant maize need to be addressed. The emergence of herbicide-resistant weeds due to prolonged use of specific herbicides remains a significant concern (Perry et al., 2016). Strategies such as deploying dual herbicide-tolerant crops from the outset and rotating herbicides with different modes of action can help mitigate this risk (Yu et al., 2023). Additionally, ensuring the safety and stability of genetically modified maize through rigorous testing, such as 90-day feeding studies, is crucial for gaining public trust and regulatory approval (Zhu et al., 2013). Ongoing research should also focus on understanding the genetic and environmental factors influencing herbicide tolerance to develop more resilient and sustainable crop management practices.

The future of herbicide-tolerant maize lies in leveraging advanced genetic engineering technologies, discovering new tolerance traits, integrating multiple agronomic traits, and addressing the associated challenges and risks. Continued research and innovation in these areas will be pivotal in enhancing the effectiveness and sustainability of herbicide-tolerant maize varieties.

7 Concluding Remarks

The adoption of genetically engineered (GE) maize has led to varied changes in herbicide use. While GE glyphosate-tolerant (GT) maize showed a slight reduction in herbicide use compared to non-GE maize, the environmental impact quotient indicated a more significant reduction in herbicide use. Additionally, the development of dual herbicide-tolerant maize, such as those tolerant to both glyphosate and glufosinate, has shown promise in delaying weed resistance.

Advances in genetic engineering have enabled the development of maize varieties with robust tolerance to multiple herbicide families, such as aryloxyphenoxypropionate and synthetic auxin herbicides. This diversification in herbicide tolerance traits is crucial for effective weed management and mitigating the risk of herbicide resistance. Studies on the safety of GE herbicide-tolerant maize, such as those involving glyphosate-tolerant maize with the *G2-aroA* gene, have demonstrated that these crops are as safe and nutritious as their non-GE counterparts. This is supported by comprehensive toxicological assessments in animal models.

Genetic modifications, such as the overexpression of the *zmm28* gene, have not only enhanced herbicide tolerance but also significantly increased maize grain yield and yield stability under various environmental conditions. This highlights the dual benefits of genetic engineering in improving both crop protection and productivity. The emergence of glyphosate-resistant weeds due to the repetitive use of glyphosate-tolerant crops has necessitated the development of new strategies and herbicide combinations to manage resistant weed populations effectively. This includes the use of transgenic maize with high-level tolerance to herbicides like dicamba.

The future of genetically engineered herbicide-tolerant maize appears promising, with significant potential to enhance agricultural productivity and sustainability. The continuous development of new herbicide tolerance traits and the integration of multiple herbicide resistances in a single crop variety are likely to provide farmers with more effective tools for weed management. This will be crucial in addressing the challenges posed by herbicide-resistant weeds and ensuring long-term crop protection.

Moreover, the safety and nutritional equivalence of GE herbicide-tolerant maize, as demonstrated by rigorous scientific studies, support its continued adoption and acceptance. The positive impact on yield and agronomic performance further underscores the value of genetic engineering in meeting the growing global demand for food. However, it is essential to remain vigilant about the potential risks and unintended consequences associated with genetic modifications. Ongoing research and monitoring are necessary to ensure that these technologies are used responsibly and sustainably. Collaborative efforts between scientists, industry stakeholders, and regulatory bodies will be key to maximizing the benefits of genetically engineered herbicide-tolerant maize while minimizing any adverse impacts.

Genetically engineered herbicide-tolerant maize holds great promise for the future of agriculture. By leveraging advanced genetic engineering techniques, we can develop crop varieties that are not only more resilient to herbicides but also capable of delivering higher yields and improved environmental outcomes. This will be instrumental in achieving sustainable agricultural practices and ensuring food security for future generations.

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Conflict of Interest Disclosure
The authors affirm 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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