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CRISPR/Cas9 and Modern Breeding Tools for Genetic Improvement of Maize Seeds

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Abstract: *Maize (Zea mays) is a cornerstone of global food security, yet its production faces intensifying pressures from climate variability, biotic stresses, and rising nutritional demands. Traditional breeding methodologies, while historically successful, now face stagnation due to long generational cycles and linkage drag, rendering them insufficient to meet the 2030 zero hunger goals. This review explores the paradigm shift toward genomics-assisted precision breeding, highlighting the integration of Genomic Selection (GS) and CRISPR/Cas9 genome editing. We examine the evolution of breeding tools, detailing how GS utilizes whole-genome prediction to accelerate polygenic trait improvement, while CRISPR/Cas9 offers unprecedented precision for creating novel alleles to address monogenic deficiencies, such as Provitamin A availability and drought tolerance. The review further analyzes technical bottlenecks, including genotype-dependent transformation and off-target effects, and proposes a synergistic "Triple-A" framework combining Allele Creation (CRISPR), Assessment (GS), and Acceleration (Speed Breeding) to maximize genetic gain. Finally, we discuss the divergent global regulatory landscapes, contrasting process-based restrictions with product-based "regulatory escape" pathways, and their implications for the commercial deployment of climate-resilient maize.*

Keywords: *Maize (Zea mays) Breeding, CRISPR/Cas9, Genomic Selection (GS), Precision Agriculture, Abiotic Stress Tolerance, Biofortification, Regulatory Frameworks*

1. Introduction

Maize (*Zea mays*) stands as a cornerstone of the global agricultural economy, having been domesticated approximately 9,000 years ago in southern Mexico. Today, the total annual production of maize surpasses that of both wheat and rice, making it the highest produced grain globally, with world production reaching 1.1 billion tonnes in 2020 (Zhou etal., 2024). Its uses are diverse and critical, encompassing animal feed, industrial feedstocks such as ethanol, and human consumption, including sweet corn, cornmeal, and corn oil (Yang et al., 2024). Furthermore, maize is indispensable as a staple food for over 1.2 billion people, particularly across sub-Saharan Africa (SSA) and Latin America. For instance, Africa's maize production was approximately 75 million tons in 2018, harvested from an estimated 40 million hectares (IITA, 2018).

The continuous demand for increased yield and resilience is driven by intersecting global crises. Economic development, demographic expansion, and shifts in dietary habits have placed relentless pressure on existing food systems (Jankauskiene et al., 2022). Simultaneously,

the intensifying frequency and severity of climate variability and extremes, alongside biotic constraints (like infections, which cause 20–40% of global output losses) and abiotic stresses (like drought and salinity, which can reduce crop yield by up to 50%), necessitate unprecedented genetic adaptation (Karki et al., 2022; Wang et al., 2018). Current global efforts are acknowledged to be "not on track to meet our commitments to end world hunger and malnutrition in all its forms by 2030" (Jubily et al., 2025). In this context, advanced breeding technologies are not merely luxurious enhancements for efficiency but represent strategic necessities for achieving fundamental climate resilience and ensuring global food stability.

Traditional breeding methodologies, despite achieving groundbreaking successes over nearly a century, have reached a point of stagnation due to inherent limitations (Sinha et al., 2023). These constraints are primarily defined by the time and resource complexity involved in trait introgression and the restricted scope of achievable genetic variation.

Classical breeding requires long generational cycles, making the development of new, adapted varieties a tedious and time-intensive endeavor (Wang et al., 2023). Furthermore, conventional techniques, such as backcrossing, are typically best suited for qualitative traits but struggle significantly when dealing with the pervasive issue of linkage drag . Linkage drag occurs when the beneficial gene of interest is tightly associated with undesirable, unwanted genes in the parental genome. The removal of these deleterious or unessential genes becomes a challenging phenomenon through conventional means, slowing the purification of new lines (Muntean et al., 2022). Moreover, classical biparental breeding populations offer only a limited ability to capture the existing natural variation present in the maize gene pool. This limitation hinders the dissection of complex traits, which often involve several quantitative trait loci (QTLs) for traits like pest resistance or yield, making them inherently less efficient than modern multiparental breeding lines that capture a higher degree of polymorphisms (Wang et al., 2023).

2. Modern Breeding Tools: A Spectrum of Precision and Prediction

The limitations of classical breeding have spurred the development of biotechnology-based molecular breeding approaches, often termed predictive breeding, which utilize genomics and advanced phenomics to expand genetic resources indefinitely and accelerate genetic gain (Li et al., 2025).

2.1. Genomic Selection (GS) and Accelerated Breeding Cycles

Genomic Selection (GS) represents a paradigm shift in plant improvement, moving from phenotypic selection to predictive breeding based on genotypic information (Ahmad et al., 2023). The core mechanism of GS exploits genome-wide molecular genetic markers, frequently Single Nucleotide Polymorphisms (SNPs) generated via next-generation sequencing (NGS)-based platforms such as Genotyping by Sequencing (GBS) (Abo-Elyousr, 2016). GS utilizes these markers to predict the Genomic-Estimated Breeding Value (GEBV) of untested genotypes (Ahmad et al., 2023). This capacity significantly reduces the necessity for extensive and costly field phenotyping, which is a major constraint in conventional breeding, thereby enhancing genetic gain per unit time and cost (Ahmad et al., 2023; Abo-Elyousr, 2016).

The implementation of NGS-based genotyping has been instrumental in making GS a routine practice, particularly by increasing the accuracy of GEBV predictions over previous marker platforms (Abo-Elyousr, 2016). This approach is particularly valuable for complex traits—those governed by numerous small-effect loci (Abo-Elyousr, 2016). Research has demonstrated that Whole-Genome Prediction (WGP), the mechanism underlying GS, is remarkably robust across diverse maize inbred lines, irrespective of the precise number and effect of QTLs, often due to the high level of linkage disequilibrium prevailing in elite germplasm (Schulthess et al., 2013).

When coupled with Speed Breeding (SB) a revolutionary method involving raising crops in controlled, optimal simulated environments to accelerate their life cycle—GS facilitates the creation of multiple generations within a single calendar year (Wang et al., 2023; Khangura et al., 2024; Pook et al., 2024). This synergistic combination, termed Rapid Cycling Genomic Selection, dramatically shortens the generation interval and is crucial for maximizing the rate of genetic gain (Wang et al., 2023; Khangura et al., 2024; Dar, 2025).

2.2. Targeting Induced Local Lesions in Genomes (TILLING)

TILLING (Targeting Induced Local Lesions in Genomes) provides a powerful, non-transgenic method for reverse genetics (Till et al., 2003). This technique involves chemically induced mutagenesis, commonly using ethyl methanesulfonate (EMS), to generate random point

mutations across the genome (Liu et al., 2020). High mutation density is key to the efficiency of TILLING (Till et al., 2003). Following mutagenesis, high-throughput screening methods, such as the LI-COR method using the CEL I enzyme or high-resolution melting (HRM), are employed to detect beneficial nucleotide and amino acid changes in genes with known functions (Jana & Das, 2017; Liu et al., 2020).

TILLING has been successfully applied to a broad array of cereal crops, including maize (Liu et al., 2020; Jana & Das, 2017). It effectively extends the use of spontaneous and induced mutants in plant breeding, allowing for the rapid characterization and use of new allelic variants as genetic markers for selection (Liu et al., 2020; Jana & Das, 2017).

2.3. The Emergence of Genome Editing

The development of genome editing technologies marks a significant progression toward molecular precision. Historically, earlier precise targeting methods such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) were developed (Jiang et al., 2024). While precise, these tools proved to be time-consuming and cost-ineffective, limiting their widespread application in large-scale crop breeding programs (Jiang et al., 2024).

A technological watershed moment occurred in 2012 with the description of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system (Charpentier & Doudna, 2012). This technology, recognized with the 2020 Nobel Prize, uses a programmable RNA molecule to guide the Cas9 enzyme to cut specific DNA sequences (Charpentier & Doudna, 2012). Due to its simplicity, cost-effectiveness, and high efficiency, the CRISPR-Cas system has superseded earlier nucleases and revolutionized the potential for directed genetic improvement (Gao et al., 2023).

Table 2.1 Comparative Analysis of Key Modern Maize Breeding Tools

Tool	Core Mechanism	Target Type	Precision Level	Time/Cycle	Cost & Complexity

				Acceleration	
Traditional Breeding	Phenotypic Selection, Crossing	All Traits	Low (Susceptible to Linkage Drag)	Slow (Long cycles)	Low initial cost, high labor/time cost
TILLING	Random Mutagenesis (EMS) & Screening	Reverse Genetics (Known Gene Function)	Low-Moderate (Random Point Mutations)	Medium (Requires mutant pool creation)	Low initial cost, high screening throughput need
Genomic Selection (GS)	Whole-genome marker prediction (GEBVs)	Complex/Polygenic Traits	High (Prediction Accuracy)	High (Accelerated cycles via reduced phenotyping)	Requires NGS genotyping (GBS)
CRISPR/Cas9	RNA-guided DNA Double-Strand Break (DSB)	Monogenic/Oligogenic Fixes	Very High (Site-specific Indels/KOs)	High (Rapid allele creation)	Low/Moderate, but high transformation skill required

3. The CRISPR/Cas9 System: Mechanism, Delivery, and Evolution in Maize

3.1. Fundamental Mechanisms of Cas9 Action

The fundamental utility of the CRISPR/Cas9 system stems from its ability to introduce precise modifications (*in vivo*) at specific DNA sequences (Subburayalu et al., 2020). The Cas protein is directed to a target genomic locus by a designed guide RNA (gRNA), where it generates a DNA Double-Strand Break (DSB) (Subburayalu et al., 2020).

Once a DSB is induced, the cell attempts to repair the damage using endogenous pathways. The primary repair mechanism in somatic cells is Non-Homologous End Joining (NHEJ), which is error-prone and often leads to the insertion or deletion of nucleotides (indels) at the cleavage site. These indels typically result in a frameshift mutation and a subsequent gene knockout (Gao et al., 2023). The alternative, less frequent repair pathway is Homology-Directed Repair (HDR). By providing an exogenous donor DNA template, HDR can be leveraged to introduce precise gene replacements or insertions, though achieving high HDR efficiency remains a significant challenge in crop species (Li et al., 2025).

3.2. Overcoming Transformation Barriers in Maize

A primary technical hurdle for deploying CRISPR/Cas9 in maize is efficient delivery of the editing components into the target cells. While gene-editing tools can theoretically produce genomic changes without requiring DNA vector carriers, stable integration of DNA coding for these tools (transgenesis) remains the most widely used approach (Subburayalu et al., 2020). However, transgenesis presents inherent risks, including the generation of unintended transgenic integrations and prolonged expression of the Cas9 enzyme, which may increase cleavage at off-target sites . Furthermore, selecting the few genetically modified cells from millions of treated cells is particularly challenging in plant systems (Krishna et al.., 2023).

To circumvent these issues, researchers have focused on DNA-free delivery methods. The introduction of pre-assembled Ribonucleoprotein complexes (RNPs), composed of purified recombinant Cas9 enzyme and *in vitro* transcribed guide RNA (gRNA), has emerged as a superior strategy (Subburayalu et al., 2020). In maize, this RNP strategy has been successfully employed by delivering the complexes into protoplasts using polyethylene glycol (PEG) 4000 (Subburayalu et al., 2020). This protocol achieved effective editing with efficiency rates ranging from 0.85% up to 5.85%—comparable to DNA-free protocols used in other plant species (Subburayalu et al., 2020). This approach eliminates the potential pitfalls associated

with stable integration, serving as an efficient and easy assay method for screening gRNAs suitable for editing genes in the large and GC-rich maize genome (Subburayalu et al., 2020). The RNP strategy also limits Cas9 activity to a transient period, mitigating collateral effects attributed to persistent Cas9 expression and vector integration (Shi et al., 2020).

3.3. Next-Generation Precision: Base and Prime Editing Systems

The CRISPR/Cas system is continuously evolving, leading to next-generation tools that prioritize precise single-base modification without requiring the creation of DSBs (Jiang et al., 2022). This is particularly relevant given that many important agronomic traits are controlled by mutations of a single or a few bases (Jiang et al., 2022).

Base Editing (BE): Base editors are derived from CRISPR/Cas technology and allow for the precise generation of single DNA base changes (Jiang et al., 2022). Cytosine Base Editors (CBEs) facilitate C-G to T-A changes, while Adenine Base Editors (ABEs) induce A-T to G-C changes (Jiang et al., 2022). Although these editors have been successfully applied in crops such as rice and wheat, evidence suggests that **only CBEs have been successfully reported in maize** to date, indicating ongoing efforts to fully deploy the BE platform in this crop (Jiang et al., 2022).

Prime Editing (PE): Prime editing represents a highly advanced genome manipulation technique based on a "search and replace" approach (Gao et al., 2023). PE systems utilize a Cas9 H840A nickase fused to a reverse transcriptase (RT) and a prime editing guide RNA (pegRNA) (Jiang et al., 2022). This system is capable of mediating all 12 possible base-to-base conversions, as well as small targeted insertions and deletions, without requiring exogenous donor DNA or DSBs (Gao et al., 2023). While PE holds immense potential for expanding the scope of genomic editing and has been successfully used in rice and wheat, a significant hurdle remains: **the editing efficiency of Prime Editors is currently very low in plants**, necessitating considerable optimization before they can be routinely implemented in practical maize breeding (Jiang et al., 2022).

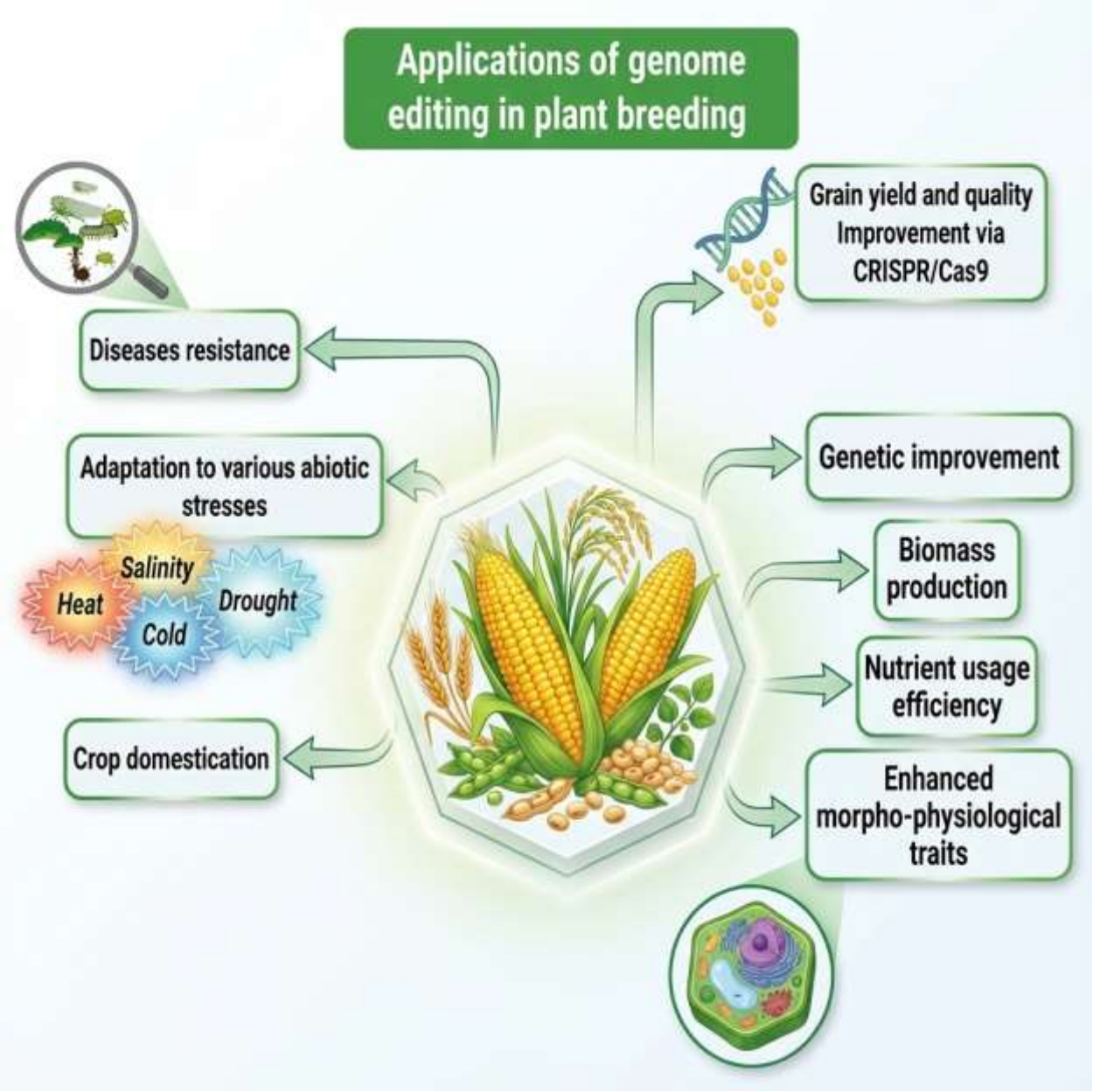
The adoption of delivery methods and editing platforms reveals a crucial trade-off between efficiency and specificity. While stable integration of the Cas9 vector often yields higher

transformation efficiency, transient expression systems like RNP delivery offer enhanced genetic stability by avoiding prolonged Cas9 exposure, which reduces the risk of unintended off-target activity (Guo et al., 2023). This tactical preference for transient systems, even with moderately lower efficiency, underscores the scientific community's emphasis on minimizing genomic instability in the final edited product.

4. Advancements in Maize Seed and Crop Traits via Genome Editing

Since the successful application of the CRISPR/Cas9 system to maize genome editing in 2014, the technology has driven revolutionary changes in generating new germplasm (Jiang et al., 2022; Wang et al., 2022). Applications span functional gene confirmation, boosting yield, improving quality, creating male-sterility mutants, and enhancing stress resistance (Jiang et al., 2022; Wang et al., 2022).

Figure 4.1 Schematic Overview of CRISPR/Cas9 Applications for Agronomic Trait Improvement in Maize



4.1. Enhancing Nutritional Quality (Seed Biofortification)

Genome editing offers unprecedented control over kernel composition, leading to biofortified maize varieties.

Starch Profile Modification: The starch components, amylose and amylopectin, are key determinants of quality, particularly for human health. Researchers have utilized CRISPR/Cas9 to manipulate genes encoding related enzymes in starch biosynthesis to influence the content

and ratio of amylose and resistant starch (RS) (Zhang et al., 2023). Specifically, producing mutants of starch branching enzymes *SBEI* and *SBEIIb* allowed researchers to analyze apparent amylose content (AAC) and resistant starch content (RSC) (Maheshwari et al., 2022). Knocking out the function of *SBEIIb* resulted in maize varieties with higher AAC, demonstrating a path toward creating functional crops with high nutritional quality potentially beneficial for diabetics (Maheshwari et al., 2022; Zhang et al., 2023).

Provitamin A (Beta-Carotene) Biofortification: Maize is a vital food source in developing nations but often lacks sufficient Provitamin A. The *PSY1* gene is a limiting factor in beta-carotene synthesis in maize (Gaikwad et al., 2020). CRISPR-Cas-mediated editing successfully targeted and modified the *PSY1* gene, leading to a significant increase in the levels of beta-carotene and Vitamin A in the resulting maize plants (Shah et al., 2024). This application demonstrates the precision and efficiency of genome editing in biofortifying crops to combat widespread nutritional deficiencies, such as Vitamin A Deficiency (VAD) (Shah et al., 2024).

4.2. Improving Yield and Plant Architecture

Genome editing has enabled specific, beneficial changes to complex agronomic traits that were difficult to target using traditional methods.

Stress Resilience (Drought): Climate change mandates varieties capable of sustained yield under stress. Precise editing was used to target and modify the promoter sequence of the *ARGOS8* gene. This modification resulted in the upregulated expression of *ARGOS8*, which subsequently enhanced maize grain yield under drought stress conditions (Yunus et al., 2025). This case exemplifies how precision editing can be utilized to fine-tune gene expression rather than simply knocking out function, leading to adaptive gains.

Lodging Resistance: Optimizing plant architecture is crucial for high-density planting and preventing yield loss from lodging (stem breakage). Researchers successfully generated semidwarf maize plants by editing the *ZmGA20ox3* gene using CRISPR/Cas9 technology. These semidwarf varieties offer superior lodging resistance, making them highly suitable for intensive cultivation systems (Wang et al., 2022).

Accelerated Breeding Tools: Beyond trait enhancement, CRISPR/Cas9 facilitates the breeding pipeline itself. The technology has been used to create male sterile lines and generate genome-edited haploids in elite lines (Jiang et al., 2022;). Haploid-inducer mediated genome editing greatly accelerates maize breeding by enabling the rapid creation of pure lines with desirable traits (Wang et al., 2022).

4.3. Engineering Resistance to Biotic and Abiotic Stresses

The ability of genome editing to confer specific resistances is vital for mitigating global crop losses.

General Stress Tolerance: Abiotic stresses—including salinity, drought, heavy metals, and extreme temperatures are major factors causing crop yield reduction (Karki et al., 2022). Genome editing provides molecular options to enhance plant tolerance mechanisms against these environmental challenges. Similarly, biotic stresses, ranging from viral to fungal and bacterial infections, account for substantial agricultural losses globally (Karki et al., 2022; Wang et al., 2018). CRISPR/Cas systems are instrumental in developing novel germplasm sources with enhanced resistance to both categories of stress (Singh et al., 2023).

Herbicide Tolerance: Advanced CRISPR systems have been leveraged to develop herbicide-tolerant maize. Optimized prime editing systems, such as ePE5max, have been used to generate heritable mutations in genes encoding key enzymes like EPSPS, ALS, and ACCase (Alam et al., 2024). Base editing has also been instrumental in achieving herbicide resistance via point mutation introduction in genes like *ALS* (Base Editing in Maize, 2024). These precise mutations significantly improve the resilience of maize to herbicide stress, offering farmers greater flexibility in weed management (Alam et al., 2024; Chen, 2024).

Table 4.1 Key Maize Traits Improved by CRISPR/Cas9 Genome Editing

Trait Category	Target Gene/Locus	Editing Outcome/Effect	Significance for Seed/Crop

Nutritional Quality	*SBEI, SBEIIb*	Modified starch composition (higher apparent amylose content)	Biofortification for healthier functional foods, beneficial for diabetics.
Nutritional Quality	*PSY1*	Increased beta-carotene synthesis in kernels	Addressing Vitamin A Deficiency (VAD) through targeted biofortification.
Abiotic Stress	*ARGOS8* Promoter	Upregulated gene expression	Enhanced grain yield and resilience under drought stress.
Plant Architecture	*ZmGA20ox3*	Generated semidwarf phenotype	Improved lodging resistance for high-density planting.
Breeding Efficiency	Various (Haploid Inducers, Sterility)	Creation of male sterile lines, edited haploids	Greatly accelerating the creation of new pure lines.

5. Technical Bottlenecks and Optimization Strategies in Maize GE

Despite the successes of CRISPR/Cas9, its application in maize remains constrained by technical challenges related to genotype dependency, off-target specificity, and scaling for complex traits.

5.1. Genotype Dependency and Transformation Efficiency

A critical limitation in maize genetic engineering is the genotype-dependent nature of transformation protocols. Many agronomically crucial maize lines, particularly tropical germplasm, are recalcitrant to standard *Agrobacterium*-mediated transformation, which restricts the application of genome editing to a narrow range of amenable genotypes (Dahlem et al., 2023).

To address this, researchers have pioneered the use of Morphogenic Regulators (MRs), which are plant transcription factors. The ectopic overexpression of specific MRs, such as *BABY BOOM* (*BBM*) and *WUSCHEL* (*WUS*), has emerged as a promising strategy to overcome low efficiency and genotype dependency (Dahlem et al., 2023). Recent work has further explored novel delivery methods for these regulators, including the use of viral vectors (Yu et al., 2024). By including MR genes within the T-DNA cassette for stable integration, protocols have successfully enabled Genome Editing (GE) in previously challenging agronomically relevant tropical maize lines, achieving transformation efficiencies up to 6.63% (Zhou et al., 2024).

5.2. Specificity and Off-Target Activity

Although CRISPR offers high precision, concerns regarding potential off-target editing activity remain prevalent within the scientific community (Wang et al., 2024). The strategy of using MRs to boost transformation efficiency, while effective, introduces a layer of complexity regarding editing specificity.

In one study utilizing an improved MR-based transformation protocol for tropical maize, researchers observed off-target activity of the CRISPR construct, specifically targeting the *VYL* paralog, *VYL-MODIFIER* (Dahlem et al., 2023). The unexpected frequency of this off-target event led the authors to suggest it could be partly due to the stable expression of the *WUSCHEL* (WUS) MR . Previous reports have noted that using *WUS* to facilitate transformation can increase GE efficiency as a side-effect, potentially by altering cellular repair kinetics (Dahlem et al., 2023). The correlation between the process optimization (using WUS to enhance efficiency) and the integrity of the genetic product (increased off-target events) presents a significant optimization paradox that demands further investigation (Dahlem et al., 2023). If the use of MRs is causally linked to increased unspecific GE, future protocol design must shift toward transient MR expression or optimized Cas variants that mitigate this side effect while maintaining high transformation rates in recalcitrant lines (Yang et al., 2024).

Furthermore, the detection of off-target activity is itself a challenge. While Whole-Genome Sequencing (WGS) provides the most comprehensive evaluation of on- and off-target effects, the large size and complexity of the maize genome mean that WGS remains cost-prohibitive

for routine use across large populations, leaving a substantial gap in the full genome-wide assessment of editing specificity (Beying et al., 2020).

5.3. Scaling for Complex and Polygenic Traits

Genome editing is extremely powerful for targeting monogenic or oligogenic traits, such as those related to nutritional quality or specific stress resistances (Jiang et al., 2022). However, the most critical agronomic traits, such as overall yield stability and performance, are highly polygenic, governed by numerous genes with small cumulative effects (Schulthess et al., 2013).

For these complex traits, Whole-Genome Prediction (WGP), the foundation of Genomic Selection, is the superior methodological choice. WGP models, regardless of the underlying assumptions about genetic effect distribution, have proven robust in predicting line and hybrid performance in diverse elite maize germplasm, demonstrating high prediction accuracies (Mishra et al., 2025). While CRISPR/Cas9 can precisely fix single known deficiencies or introduce novel alleles, it is GS that provides the necessary infrastructure to manage the majority of the genetic variance associated with quantitative trait improvement and overall population genetic gain (Abo-Elyousr, 2016; Ahmad et al., 2023).

6. Integration of Tools: Synergistic Breeding Frameworks

Future breakthroughs in maize improvement will hinge on moving beyond isolated tool deployment towards synergistic breeding frameworks that intelligently combine the strengths of prediction and precision.

6.1. Integrating CRISPR/Cas9 and GS for Optimal Genetic Gain

The rational integration of CRISPR/Cas9 and Genomic Selection overcomes fundamental weaknesses in traditional breeding. CRISPR/Cas9 serves as the rapid Allele Creation platform, enabling the swift generation of precise, beneficial alleles—such as the edited promoter for *ARGOS8* to confer drought tolerance (Guo et al., 2025).

Once this high-value allele is created, Genomic Selection assumes the role of Assessment and Acceleration. GS utilizes GEBVs to rapidly predict the performance of progeny and accelerate the introgression of the new allele into multiple elite genetic backgrounds (Ahmad et al., 2023;

Abo-Elyousr, 2016). This approach allows breeders to simultaneously optimize the polygenic background traits (general yield, stability) that are critical for overall performance while incorporating the specific gain from the precise edit.

Crucially, the use of molecular markers, intrinsic to both Marker-Assisted Selection (MAS) and GS, helps researchers identify and subsequently purge unwanted genes closely linked to the beneficial trait. This capability effectively mitigates linkage drag—the historical limitation of classical backcrossing—thereby accelerating the creation of enhanced varieties without compromising genetic purity (Gaikwad et al., 2020).

6.2. Accelerated Cycle Breeding

The most sophisticated framework for modern maize improvement involves the convergence of all three critical methodologies: Allele Creation (CRISPR/Cas9), Assessment and Prediction (Genomic Selection), and Acceleration (Speed Breeding). This Triple-A Framework (GE + GS + SB) dramatically shortens the development timeline (Wang et al., 2023; Khangura et al., 2024; Dar, 2025; Pook et al., 2024). By combining high-precision allele insertion, genome-wide predictive selection, and shortened generation cycles through optimized environmental conditions, this synergistic strategy provides the capacity to rapidly respond to evolving environmental constraints and achieve significant genetic gain faster than any previous system (Khangura et al., 2024; Dar, 2025; Pook et al., 2024).

7. Regulatory Divergence and Societal Acceptance of GE Maize

The ultimate commercialization and global deployment of genetically engineered maize is profoundly influenced by regulatory classification, which varies dramatically across jurisdictions.

7.1. Global Regulatory Frameworks: Product vs. Process

Process-Based Regulation: Jurisdictions like the European Union (EU), India, and China regulate crops based on the method used to create them (Jankauskiene et al., 2022). Under this framework, genome editing, regardless of the resulting change, is defined as a genetic modification process (Jankauskiene et al., 2022). Consequently, CRISPR-edited crops are classified as Genetically Modified Organisms (GMOs), subjecting them to stringent regulation,

mandatory labeling, and high approval barriers (Jankauskiene et al., 2022; Wang et al., 2021; Arrúa et al., 2024). If classified and regulated identically to transgenic GM crops, their future cultivation and public acceptance, particularly in the EU, face substantial limitations (Wang et al., 2021).

Product-Based Regulation: Countries including the United States and Canada regulate crops based solely on the characteristics of the final product (Jankauskiene et al., 2022). If the genetic modification could have theoretically been achieved through conventional breeding methods (i.e., the edited product does not contain novel, stably integrated foreign DNA), the regulatory burden is substantially lighter (Jankauskiene et al., 2022).

7.2. The Significance of Transgene-Free Edited Crops (Regulatory Escape)

The ability to produce a transgene-free edited plant is a defining factor in commercial viability within product-based regulatory systems (Shi et al., 2020). Transgene-free mutants can be generated through sexual reproduction, where the Cas9/gRNA DNA cassette segregates away from the beneficial edited allele, or through DNA-free delivery methods like RNP transfection (Shi et al., 2020).

The United States Department of Agriculture (USDA) has set a crucial precedent by confirming that CRISPR-Cas9 modified corn—such as a variety engineered by DuPont Pioneer to knock out the *Wx1* gene—"escapes" the agency's GMO regulations, provided that the DNA encoding the editing system has been successfully removed from the final plant (Shi et al., 2020; Doudna & Charpentier, 2021). This rationale dictates that if the endpoint product is identical to one achievable by traditional means, it should not be subject to the costly, time-intensive regulatory process intended for traditional GMOs (Shi et al., 2020). This phenomenon, often termed "regulatory escape," provides the fastest route to market for CRISPR-edited maize in product-based regulatory environments (GLP, 2024). By late 2024, the SECURE rule was noted to have exempted many gene-edited plants from pre-market review (Gene Editing on the Farm, 2025). Specific CRISPR-edited maize products, such as waxy corn with high starch content, have also gained market approval in other product-based or evolving regulatory environments, such as Japan in 2024 (GLP, 2024).

The current regulatory landscape creates a geopolitical feedback loop. Product-based systems incentivize researchers to optimize DNA-free delivery (RNP) or subsequent segregation methods to achieve regulatory escape (Subburayalu et al., 2020; Shi et al., 2020). This technical solution accelerates commercial adoption and investment in the US. Conversely, process-based regulation reinforces public concern over the "process" of modification, maintaining high barriers to entry and potentially widening the gap in agricultural technology deployment between global economic regions (Wang et al., 2021).

Table 7.1 Global Regulatory Frameworks and Commercial Impact on GE Maize

Region	Regulatory Approach	Classification of CRISPR-Edited Crops	Impact of Transgene-Free Status	Commercial Implication
United States (US)	Product-Based	Non-regulated (if DNA cassette is removed)	Enables "Regulatory Escape" from USDA oversight.	Fastest route to market entry and commercialization.
European Union (EU)	Process-Based	Defined as a Genetically Modified Organism (GMO)	Remains regulated as a GMO due to the process used.	High market barrier; discourages EU research investment in commercial GE crops.
Japan	Product-Based (Evolving)	Case-by-case approval	Approved specific GE products (e.g., CRISPR Waxy Corn).	Market access possible but requires specific governmental review.

7.3. Public Perception and Familiarity

Public perception is an influential factor, particularly in jurisdictions with process-based regulation. While surveys show that the public in North America and Europe is generally more familiar with the concepts of genome editing and GMOs than in other regions, this familiarity does not necessarily translate to universal acceptance (Jankauskiene et al., 2022). Persistent concerns regarding off-target effects, environmental impacts, and the difficulty of controlling CRISPR-edited crops after release contribute to lower public acceptance, particularly where stringent regulatory classification reinforces these reservations (Wang et al., 2021).

8. Conclusion and Future Perspectives

8.1. Synthesis of Current Impact and Remaining Gaps

Genome editing, spearheaded by the CRISPR/Cas9 system, represents a transformational advancement in maize breeding. It has facilitated the precise modification of monogenic traits, achieving critical breakthroughs in nutritional quality (increased Provitamin A and modified starch profiles) and enhancing resilience to specific stresses (drought tolerance via *ARGOS8* promoter editing) (Shah et al., 2024; Wang et al., 2022).

However, the current generation of breeding tools demonstrates that the maximum genetic gain will be achieved not through precision editing alone, but through the synergistic integration of high-throughput prediction (GS) and high-precision mutation (CRISPR/Cas9), coupled with acceleration (SB). GS efficiently manages the complex, polygenic basis of yield, while CRISPR/Cas9 rapidly generates the novel alleles needed to address specific genetic and environmental limitations, simultaneously mitigating the challenge of linkage drag associated with traditional introgression (Wang et al., 2023; Abo-Elyousr, 2016; Khangura et al., 2024).

Critical technical gaps remain. The most advanced systems, such as Prime Editing, still suffer from low efficiency in complex maize systems, requiring intensive protocol optimization (Jiang et al., 2022). Furthermore, efforts to optimize transformation efficiency in recalcitrant, agronomically superior tropical maize lines using Morphogenic Regulators introduce a potential trade-off, where the enhanced transformation rate may correlate with an increased risk of unintended off-target edits (Dahlem et al., 2023).

8.2. Future Research Directions

Future research and development efforts must be directed toward four strategic areas to realize the full potential of precision breeding in maize:

1. **Universal Transformation Systems:** Developing robust, genotype-independent transformation protocols for all elite maize germplasm, particularly tropical lines, while minimizing or eliminating the use of stably integrated MRs to mitigate the potential risk of increased off-target activity. New approaches like viral vector delivery of MRs offer promising avenues for reducing this risk (Yu et al., 2024).

2. **Refining Next-Generation Tools:** Focused optimization of protocols for Prime Editing to achieve high-efficiency, reliable sequence replacement across the complex maize genome structure, thereby unlocking the capacity for highly nuanced base-level modifications.

3. **High-Throughput Multiplex Editing:** Advancing CRISPR platforms to enable the simultaneous and efficient modification of multiple genes involved in complex, polygenic traits, moving beyond the current focus on single-gene or functional gene identification.

4. **Harmonizing Regulation:** Addressing the global regulatory fragmentation between process-based and product-based systems. Clear, science-driven definitions for genome-edited crops that align across major economic blocs are essential to permit the rapid and safe deployment of climate-resilient and nutritionally enhanced maize varieties required to meet global food security goals (Arrúa et al., 2024).

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