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CRISPR/Cas12a-Mediated Promoter Editing for Yield-Enhancing Alleles: Precision Breeding Strategies in Cereal Crops

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Abstract

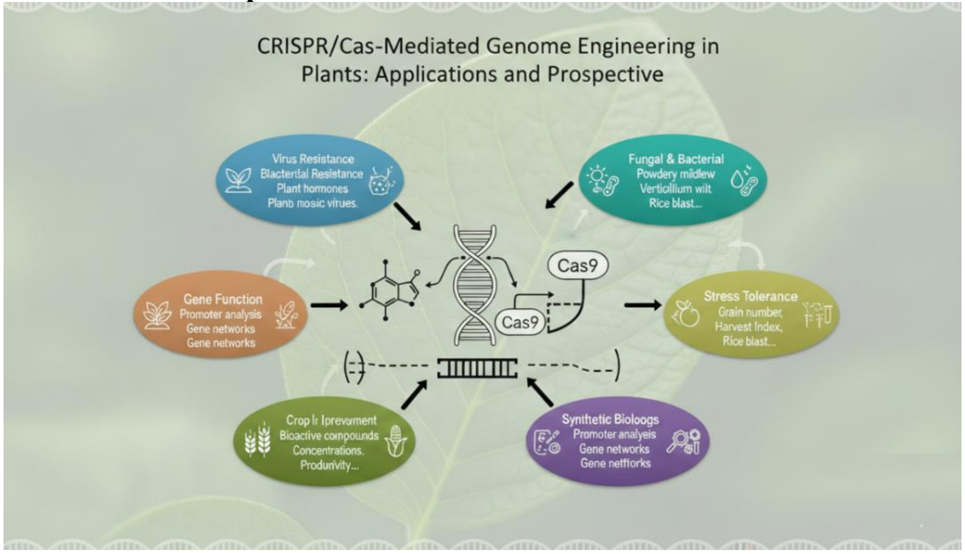
The advent of CRISPR/Cas12a technology has revolutionized precision breeding in cereal crops by enabling targeted editing of promoter regions to generate yield-enhancing alleles. This review explores the molecular advantages of Cas12a over Cas9, including its preference for AT-rich sequences and staggered cleavage patterns, which facilitate tunable gene expression in non-coding regulatory elements. Through case studies in rice, wheat, barley, and maize, we highlight successful applications such as decoupling pleiotropic effects in the IPA1 locus for increased grain yield, optimizing nutrient transporters like OsPHO1;2, and modulating meristem size via the CLAVATA pathway. Technical optimizations, including codon adaptation, intron integration, and multiplexed guides, have achieved mutagenesis efficiencies exceeding 90% in polyploid species. Emerging tools like base and prime editing, coupled with AI-driven promoter design, further enhance regulatory precision and trait stacking. The global market for CRISPR-edited plants is projected to reach $50.1 billion by 2030, driven by evolving regulations favoring non-GMO classifications for simple edits. This paradigm shift toward promoter engineering offers sustainable solutions for food security amid climate challenges, emphasizing quantitative trait modulation over gene knockouts.

Keywords: CRISPR/Cas12a, Promoter Editing, Cereal Crops, Yield Enhancement, Precision Breeding, Cis-Regulatory Elements, Base Editing, Prime Editing, AI in Agriculture, Regulatory Landscape

Introduction

The historical trajectory of agricultural innovation, transitioning from the rudimentary selection of wild grasses to the sophisticated manipulation of genomic sequences, reflects a persistent endeavor to secure global food supplies against the twin pressures of population expansion and environmental instability (Gao, 2021). In this context, the emergence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (CAS) systems has represented a definitive shift from random mutagenesis toward the precise engineering of specific genomic loci (Biswas et al., 2021). While early applications of CRISPR/Cas9 were largely confined to the disruption of protein-coding sequences through targeted knockouts, the field is undergoing a transition toward the modification of non-coding regulatory regions, specifically promoters (Zhou et al., 2024). This shift is predicated on the understanding that agronomically significant traits are often quantitative and controlled by complex regulatory networks rather than single "on/off" genetic switches (Shi et al., 2023). Among the burgeoning toolkit of CRISPR nucleases, Cas12a (formerly Cpf1) has distinguished itself as a uniquely capable effector for promoter engineering in cereal crops (Malzahn et al., 2019). Its distinct biochemical properties most notably a T-rich protospacer adjacent motif (PAM) requirement and a staggered cleavage pattern render it particularly suitable for the AT-rich environments typical of plant promoters (Wang et al., 2021). By precisely editing cis-regulatory elements (CREs) within these promoters, researchers can achieve a "tunable" regulation of gene expression, thereby creating a continuous spectrum of quantitative trait variation (QTV) that circumvents the deleterious pleiotropic effects often associated with total gene disruption (Mackay et al., 2024). This report evaluates the current state of CRISPR/Cas12a-mediated promoter editing in staple cereals, including rice, maize, wheat, and barley, detailing the molecular mechanisms, technical optimizations, and emerging case studies that define this new frontier in precision breeding (Lawrenson et al., 2024).

Figure 1. Diverse Applications of CRISPR/Cas-Mediated Genome Engineering for Crop Improvement and Functional Genomics.



Molecular Architecture and Mechanisms of Cas12a

The adoption of CRISPR/Cas12a as a preferred tool for promoter engineering is fundamentally rooted in its molecular divergence from the archetypal Cas9 system. While Cas9, derived from Streptococcus pyogenes (SpCas9), recognizes a G-rich PAM (5'-NGG-3') and produces blunt-ended double-strand breaks (DSBs), Cas12a effectors require a T-rich PAM (5'-TTTV-3') (Weisbach, 2022). This distinction is of paramount importance in cereal genomics because non-coding regulatory regions, including promoters, introns, and 5' and 3' untranslated regions (UTRs), are often characterized by a high AT content (Bandyopadhyay al., 2020). Consequently, Cas12a provides access to genomic "GC-deserts" that remain largely inaccessible to standard Cas9 systems (Agha et al., 2025). The cleavage mechanism of Cas12a further differentiates its utility for precision breeding. Cas12a induces a staggered DSB at the PAM-distal end of the target sequence, resulting in 5'-overhangs of four to five nucleotides (Mohanraju, 2019). This staggered cut is hypothesized to favor homology-directed repair (HDR) or microhomology-mediated end joining (MMEJ) over the more common, error-prone non-homologous end joining (NHEJ) associated with blunt cuts (Pascual, 2024). Furthermore, because the cleavage occurs distal to the PAM, the PAM sequence itself remains intact following an initial round of cutting and imperfect repair (Santos et al., 2022). This allows the Cas12a enzyme to re-cut the target site multiple times until a more substantial deletion or a specific sequence insertion occurs, often leading to larger and more predictable deletions in the promoter region compared to the small, stochastic indels produced by Cas9 (Jabalera et al., 2025).

Table 1. Technical Comparison of SpCas9 and LbCas12a Editing Platforms.

Feature	**CRISPR/Cas9 (SpCas9)**	**CRISPR/Cas12a (LbCas12a)**
PAM Sequence	5'-NGG-3'	5'-TTTV-3'
Cut Geometry	Blunt-ended	Staggered (5' overhang)
Guide Architecture	sgRNA (crRNA + tracrRNA)	crRNA only
RNA Processing	Host-dependent or exogenous	Autocatalytic (RNase activity)
Target Preference	GC-rich (Coding)	AT-rich (Regulatory)
Multiplexing	Requires individual promoters/linkers	Single array processing

A significant structural advantage of the Cas12a system lies in its simplified RNA requirement. Unlike Cas9, which requires both a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA) often fused into a single guide RNA (sgRNA), Cas12a requires only a short crRNA of approximately 42 nucleotides (Liao et al., 2021). Moreover, Cas12a possesses intrinsic ribonuclease (RNase) activity, allowing it to process its own pre-crRNA arrays into mature functional units (Strezoska et al., 2020). This autocatalytic processing enables highly efficient multiplexed editing, as a single transcript containing multiple crRNAs can be precisely cleaved by the Cas12a protein itself to target several different cis-regulatory elements or genes simultaneously (Chang, 2022). In the context of yield improvement, where multiple quantitative traits must often be optimized in tandem, this multiplexing capability is a transformative feature of the Cas12a platform (Mir et al., 2018).

Theoretical Framework of Promoter Engineering in Cereals

The strategic move toward promoter editing is a response to the inherent limitations of gene knockouts in crop improvement. In many instances, the total disruption of a gene leads to harmful pleiotropy a phenomenon where a single gene affects multiple, often negatively correlated, phenotypic traits (Pelea et al., 2022). For example, a gene that increases grain size might also cause severe dwarfing or reduced fertility when completely silenced (Zhou et al., 2024). Promoter editing provides a mechanism to modulate gene expression intensity, timing, or tissue-specificity, thereby decoupling these restrictive relationships (Ansari et al., 2020).

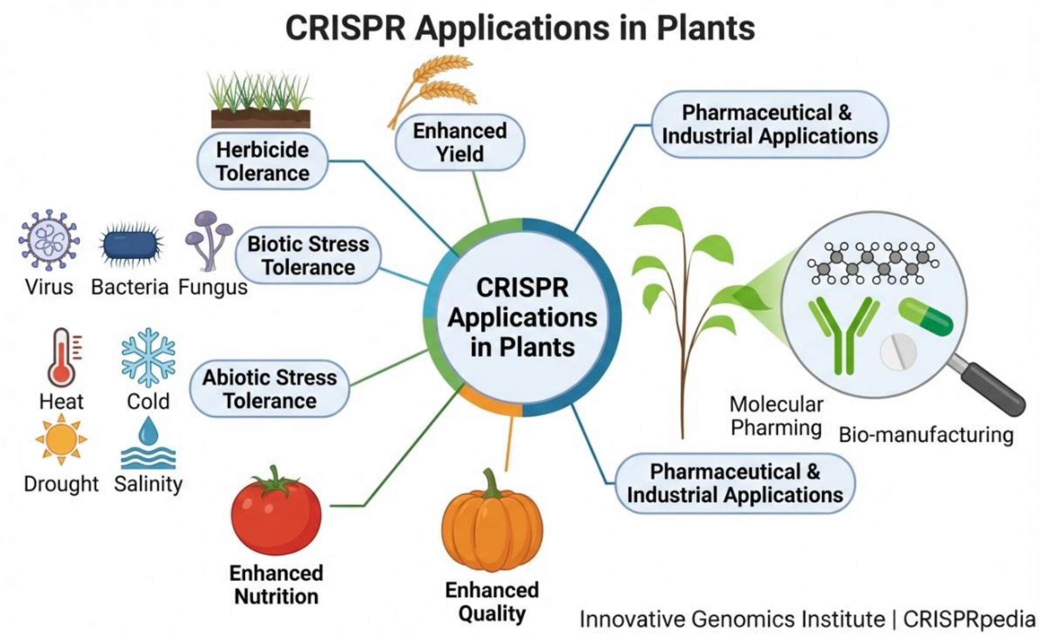


Figure 2 CRISPR Applications in Plants

Architecture of the Regulatory Landscape

Cereal promoters are complex modular units consisting of several functional domains:

1. **Core Promoter:** This region, typically encompassing plus/minus 50 bp around the transcription start site (TSS), is essential for the assembly of the RNA polymerase II preinitiation complex and determines the basal level and direction of transcription (Liu et al., 2021).
2. **Proximal Promoter:** Located upstream of the core promoter, this region contains the primary binding sites for general and tissue-specific transcription factors that regulate the core machinery (Haberle et al., 2018).
3. **Distal Promoter and Enhancers:** These elements can be located several kilobases away from the TSS and are responsible for high-level modulation of gene expression in response to environmental cues or developmental signals (Sloutskin et al., 2021).
4. **5' Untranslated Region (5' UTR) and Upstream ORFs (uORFs):** These regions, located between the TSS and the start codon, play critical roles in translational regulation (Andersson et al., 2020).

By utilizing Cas12a to target specific cis-regulatory elements (CREs) within these modules, researchers can achieve "near-isogenic" trait variation. This approach allows for the creation of a "phenotypic gradient," where different promoter mutations result in varying degrees of gene knockdown or activation (Zhu et al., 2020). This enables breeders to select the "optimal" allele that maximizes yield components while minimizing deleterious side effects (Rehman et al., 2022).

Precision Breeding in Rice: Yield Enhancement through Regulatory Diversification

Rice (Oryza sativa) has served as the primary proving ground for promoter editing due to its genomic accessibility and the identification of numerous genes that regulate its plant architecture and grain development (Tang et al., 2020). The application of CRISPR/Cas12a in rice has transitioned from proof-of-concept experiments to the generation of elite lines with significantly enhanced yield potential (Huang et al., 2021).

Decoupling Pleiotropy in the IPA1 Locus

One of the most definitive examples of yield enhancement through promoter editing involves the Ideal Plant Architecture 1 (IPA1) gene. IPA1 encodes the SPL14 transcription factor, a master regulator that promotes grain number per panicle but negatively regulates the number of tillers (Hou et al., 2022). Because both grain number and tiller number are critical yield components, total knockout or constitutive overexpression of IPA1 fails to maximize yield due to these compensatory effects (Song et al., 2022). Researchers addressed this challenge by using CRISPR/Cas to target the IPA1 promoter, specifically deleting a 54 bp fragment that contained a binding site for the transcriptional repressor An-1 (a motif identified as GCGCGTGT). This targeted deletion resulted in the IPA1-Pro10 line, which exhibited a tissue-specific alteration in IPA1 expression (Zhou et al., 2024). By removing the repressor motif, expression was increased in the panicle but maintained at appropriate levels in the basal regions of the plant (Li et al., 2016). This surgical regulatory adjustment resulted in an 8.5% increase in grain number per panicle and a concurrent 7.2% increase in tiller number, leading to an overall grain yield increase of 15.9% under field conditions (Indibi et al., 2025).

Optimizing Nutrient Use and Grain Quality

Beyond architecture, yield is fundamentally limited by a plant's ability to acquire and transport nutrients. The phosphate transporter gene OsPHO1; 2 is essential for moving phosphate from the roots to the shoots. Traditional overexpression of this gene often leads to phosphate toxicity and stunted growth (Biswas et al., 2021). To fine-tune its expression, promoter editing was used to excise a specific W-box cis-regulatory element, which serves as a binding site for the negative regulator OsWRKY6 (Zulfiqar et al., 2025). The resulting OsPHO1; 2: PE lines showed enhanced root-to-shoot phosphate transport and significant increases in both seed and panicle numbers under varying phosphorus conditions (Heuer et al., 2017). Similarly, biofortification efforts have utilized promoter editing to improve grain quality while maintaining yield. The OsNAS2 promoter was edited using CRISPR to delete an ARR1AT motif, resulting in increased concentrations of Zinc (Zn) and Iron (Fe) in the grain (Ludwig & Slamet-Loedin, 2024). Interestingly, these edits also correlated with an increased spikelet number per main panicle, suggesting that the precise regulation of micronutrient transporters can have secondary beneficial impacts on grain productivity (Liang et al., 2025).

Creating Allelic Gradients in Grain Size and Heading Date

Grain weight is primarily determined by the negative regulators GS3 (Grain Size 3) and GW2 (Grain Width 2). CRISPR/Cas12a-mediated promoter editing of these genes has been used to generate a continuous spectrum of grain size variation (Gasparis et al., 2023). For example, targeting the promoters of GS3 and D18 (involved in gibberellin signaling) has allowed researchers to select alleles that provide the optimal balance between grain weight and panicle density (Rameshrao, 2020). In other studies, targeting a 2.0 kb region in the promoters of the heading date genes Ghd7 and DTH8 created a range of quantitative variations in flowering time and grain number per paniclehou. This "promoter scanning" approach enables the customization of elite varieties for specific agroecological zones where the timing of maturation is critical for yield stability (Xu et al., 2015).

Technical Paradigms for Wheat and Barley Improvement

Wheat (Triticum aestivum) and barley (Hordeum vulgare) pose unique challenges for genome editing, ranging from the massive, polyploid genome of hexaploid bread wheat to the low efficiency of traditional transformation methods (Luo et al., 2025). However, recent efforts have focused on optimizing Cas12a toolkits specifically for these temperate cereals, achieving high-efficiency mutagenesis that was previously impossible (Lawrenson et al., 2024).

Toolkit Optimization for Monocots

To maximize editing efficiency in wheat and barley, researchers have systematically evaluated and modified various components of the Cas12a system (Gaillochet Luo et al., 2023).

Table 2. Summary of Optimization Strategies for Enhanced Mutagenesis in Wheat and Barley.

Optimization Strategy	Description	Impact on Mutagenesis Efficiency
Codon Optimization	Use of Arabidopsis-optimized sequences (ttAtCas12a) rather than human-optimized versions (Lawrenson et al., 2024; Luo et al., 2025)	Superior performance in both barley and wheat protoplasts and stable lines (Lawrenson et al., 2024)
Intron Integration	Incorporation of 8 to 13 introns into the Cas12a coding sequence (Lawrenson et al., 2024; Luo et al., 2025)	Significantly improved mRNA stability and protein expression levels in monocots (Luo et al., 2025)
D156R Mutation	Use of an engineered Cas12a variant with the D156R mutation (Lawrenson et al., 2024; Luo et al., 2025)	Synergistically boosted mutagenesis when combined with intron-optimized sequences (Lawrenson et al., 2024)
tRNA-Based Guides	Utilizing tRNA-based multiguide arrays (V3) for RNA processing (Lawrenson et al., 2024; Luo et al., 2025)	Achieved 90% mutant alleles in multiple genes; 22% improvement at recalcitrant loci (Lawrenson et al., 2024)
GRF-GIF Boosting	Co-expression of GRF-GIF transformation boosting cassettes (Lawrenson et al., 2024; Luo et al., 2025)	Maximized transformation efficiency and workflow speed in recalcitrant wheat varieties (Lawrenson et al., 2024)

In barley, the optimized ttAtCas12a system with 8 introns and tRNA-based guides resulted in a remarkable 90% mutation rate across three simultaneously targeted genes (chr6Hg0653951, chr7Hg0684671, and chr2Hg0138701). In wheat, the same toolkit achieved mutation rates in 86% and 93% of T0 plants for two simultaneously targeted yield genes, GW2 and GW7 (Bandyopadhyay et al., 2020). This level of efficiency is critical for promoter editing projects, as it ensures that nearly every transformed plant carries a potentially useful regulatory mutation, thereby reducing the scale of tissue culture required (Ebrahimi et al., 2024).

Targeted Regulation of Grain Weight in Polyploid Wheat

The hexaploid nature of bread wheat (AABBDD genome) requires the simultaneous editing of three homoeologous copies of a gene to manifest a significant phenotypic change. CRISPR/Cas12a is uniquely suited for this due to the conservation of T-rich PAM sites across subgenomes, which allows a single crRNA to target the A, B, and D homoeologs (Wang et al., 2025). Targeting negative regulators such as TaGW2, TaGS3, and TaGASR7 has led to significant increases in grain weight and thousand-kernel weight (TKW) (Fan et al., 2024). Specifically, knocking out TaGW7-B1 using an engineered LbCas12a-RVR variant (recognizing a TATV PAM) induced heritable changes in grain width and size in regions that were previously inaccessible to standard Cas9 (Nigro et al., 2023). Furthermore, research into TaGS3 has revealed non-additive dosage-dependent effects on grain size. By using Cas12a to create a range of promoter and coding mutations across the three subgenomes, researchers observed that the most substantial increases in grain weight (up to 9.6%) occurred when specifically, one or two homoeologs were edited, providing a non-additive suppressive effect that outperforms total triple-homoeolog disruption in some contexts (Schaart et al., 2021). This granular control over "gene dosage" through multi-genome editing is a cornerstone of current wheat yield enhancement strategies (Lv et al., 2021).

Shoot Apical Meristem (SAM) Delivery

To bypass the bottlenecks of conventional tissue culture, a Shoot Apical Meristem (SAM)-based biolistic delivery method has been developed for genotype-independent editing in wheat. This method targets the sub-epidermal (L2) cells, which have the potential to develop into germline cells, thereby producing stably edited plants in the next generation (Hu et al., 2025). When combined with the high-temperature incubation strategy (30 degrees C for 7 days post-bombardment), this approach has demonstrated a 2-fold increase in GE efficiency, establishing a robust pipeline for introducing yield-enhancing alleles into elite wheat cultivars that are otherwise recalcitrant to Agrobacterium-mediated transformation (Bekalu et al., 2023).

Maize (Zea mays): Meristem Regulation and Targeted Insertion

In maize, promoter editing has been primarily directed toward the regulation of inflorescence meristem size and the development of high-efficiency targeted sequence insertion (TIN) platforms (Dong et al., 2025).

Fine-Tuning the CLAVATA Pathway

Maize yield components, such as ear row number and kernel number per row, are fundamentally limited by the size of the inflorescence meristem. The CLAVATA signaling pathway, including genes like ZmCLE7 and ZmFCP1, acts to restrict meristem size (Wang et al., 2021). Total loss of function in these genes leads to extreme meristem overgrowth and fasciated ears, which are non-productive (Chaudhry et al., 2024). However, CRISPR/Cas-mediated editing of the cis-regulatory regions within these promoters has allowed for a moderate increase in meristem size, leading to significant increases in multiple grain-yield-related traits without the structural abnormalities associated with null mutants (Dresselhaus et al., 2025).

Targeted Insertion (TIN) and Large Payloads

Maize is also at the forefront of Cas12a-mediated targeted sequence insertion. Utilizing homology-directed repair (HDR), researchers have successfully integrated sequences up to 10 kb in size into the maize genome (Barco et al., 2025).

Table 3. Parameters and Outcomes of Targeted Sequence Insertion in Maize.

TIN Parameter	Optimization Result	Impact on "Quality Events"
Cas12a Variant	eMb2Cas12a-Opt1 (E797A, F357W, V921K)	Achieved 69.5% biallelic editing and 2.8% TIN rate (Dong et al., 2025)
Payload Size	Range: 6.1 kb to 10.1 kb	TIN rate decreased from 0.9% to 0.3% with larger inserts (Dong et al., 2025)
Donor Concentration	Increased from 0.38 to 0.76 pmol	3.3-fold increase in double-junction positive TIN events (Dong et al., 2025)
Vector Design	Two T-DNA strategy (separate machinery and donor)	Increased recovery of "clean" marker-free insertion events to 61.5% (Dong et al., 2025)

This capability for large-scale insertion is crucial for "trait stacking," where multiple yield and resistance genes are integrated into a single genomic "safe harbor" under the control of precisely engineered promoters (Wolter et al., 2019). The study of the ZmSH1 locus has further emphasized the need for epigenetic screening, as high DNA methylation levels were found to be the primary cause of gRNA failure in maize regulatory regions (Lee et al., 2019).

Precision Advancements: Base Editing and Prime Editing

The evolution of CRISPR technology has introduced tools that provide even greater precision for promoter engineering: Base Editors (BE) and Prime Editors (PE) (Ni et al., 2023).

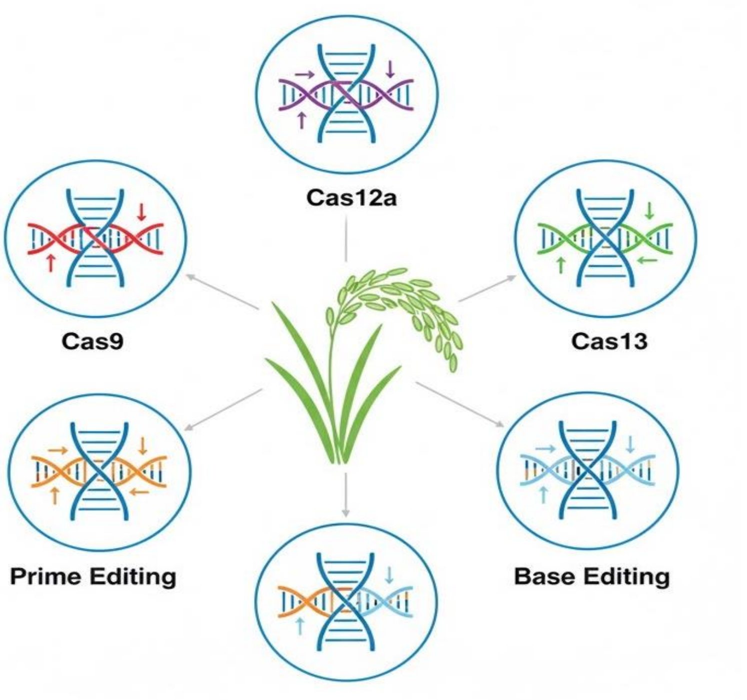


Figure 3. Schematic Representation of the Expanded CRISPR Toolkit, including Cas Nucleases (Cas9, Cas12a, Cas13) and Advanced Precision Tools (Base and Prime Editing).

Multiplexed Base Conversions

Base editors utilize a deactivated or nickase Cas protein fused to a deaminase enzyme to achieve direct nucleotide transitions (e.g., A-to-G or C-to-T) without creating double-strand breaks (Anzalone et al., 2020). Cas12a-based Adenine Base Editors (ABEs) have been specifically optimized for rice, demonstrating high efficiency in converting specific "A" bases within enhancer motifs to "G" (Berman et al., 2025). This is highly advantageous for promoter editing, as it allows for the disruption of specific transcription factor binding sites with single-nucleotide resolution, providing the ultimate level of control over gene activation or repression (Zhou et al., 2024).

Prime Editing for Regulatory Recoding

Prime editing represents the most versatile tool currently available, capable of performing all twelve possible base conversions as well as precise small insertions and deletions (Strezoska et al., 2020). In wheat, the development of the ePPEplus editor, which features a V223A reverse transcriptase mutation, has revolutionized the ability to multiplex-edit up to eight regulatory sites simultaneously (Chang, 2022). This "search-and-replace" capability allows researchers to precisely "recite" a promoter to mimic elite natural haplotypes, such as the high-yielding OsGATA8-H nitrogen-use efficiency haplotype, directly into a commercial cultivar's background (Mir et al., 2018).

Integration of AI and Machine Learning in Promoter Design

The sheer complexity of promoter sequences and the density of cis-regulatory elements make manual prediction of mutation outcomes extremely difficult. To address this, researchers are increasingly employing Artificial Intelligence (AI) and Machine Learning (ML) to direct their editing strategies (Indibi et al., 2025).

Deep Learning for Regulatory Prediction

Models such as EpiBERT and DeepPromoter are trained on vast datasets of DNA sequence and chromatin accessibility to predict the expression levels of potential promoter alleles (Oubounyt et al., 2019). In maize, AI-based key gene prediction has significantly improved yield prediction accuracy, achieving an R-squared of 0.89, which dramatically outperforms traditional genomic selection. These tools allow researchers to "test" thousands of virtual promoter mutations in silico before selecting the most promising candidates for in planta editing (Zulfiqar et al., 2025).

De Novo Synthetic Promoter Design

Beyond modifying existing promoters, AI is now enabling the de novo design of synthetic promoters from scratch. By using "insulated design strategies," researchers can separate host-dependent regulatory effects from the desired transcriptional output (Xu et al., 2015). The Pymaker model, for example, specializes in designing sequences that achieve specific gene expression levels while minimizing the number of required edits (Rameshrao, 2020). These synthetic promoters can be engineered to respond to specific cues (like drought or nutrient deficiency), providing a level of control over yield-enhancing genes that is not present in nature (Song et al., 2022).

Global Market and Regulatory Landscape (2025–2030)

The commercial and regulatory environment for CRISPR-edited cereals is evolving rapidly as these technologies mature toward commercialization (Ahmad et al., 2024)

Table 4. Projected Global Trends and Regulatory Milestones (2025–2030).

Market / Regulatory Factor	Projected Trend (2025–2030)	Key Data Point
Global Market Value	Significant growth driven by food security demands	Projected to reach $50.1 Billion by 2030 (15% CAGR) (Research and Markets, 2025)
China Market Growth	Strong investment in NBTs and rapid approval	Forecasted to reach $7.8 Billion by 2030 (14.4% CAGR) (Research and Markets, 2025)
US Regulatory Stance	Science-based, risk-proportionate, streamlined	2020 USDA revisions treat SDN-1/2 as non-GMO (Ahmad et al., 2024)
EU Regulatory Status	Transitioning toward a tiered approval system	New 202 framework proposed for NGT-1/NGT-2 categories (Ahmad et al., 2024)
African Adoption	Emerging reference for flexible innovation	Kenya and Nigeria lead with distinct GEd guidelines (Akinbo et al., 2025)

The economic impact of CRISPR technology is exacerbated by climate change, with global staple cereal yield failures (such as the 21% loss in Brazilian maize production in 2020/21) driving the urgent adoption of gene-edited resilience. Regulatory harmonization remains a challenge, as divergent rules between regions create complexities for international trade (Akinbo et al., 2025). However, a growing trend toward "product-based" regulation where plants with simple mutations that mimic natural variation are exempt from GMO oversight is significantly accelerating the path to market for high-yield cereal varieties (Tachikawa & Matsuo, 2023)

Conclusions

In summary, CRISPR/Cas12a-mediated promoter editing represents a transformative approach in cereal crop improvement, shifting from disruptive gene knockouts to finely tuned regulatory modifications that optimize yield while mitigating pleiotropic trade-offs. Through detailed mechanistic insights, optimized toolkits, and successful applications across rice, wheat, barley, and maize, this technology has demonstrated substantial gains in grain size, number, nutrient efficiency, and meristem development. Advancements in base and prime editing, integrated with AI for predictive design, promise even greater precision and scalability. As regulatory frameworks evolve toward harmonization and product-based assessments, the path to commercializing high-yielding, resilient varieties accelerate, addressing global food security imperatives in an era of environmental uncertainty. Future research should focus on expanding multiplexed edits and de novo promoter synthesis to fully unlock the potential of this precision breeding frontier.

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