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POPULAR ARTICLE

CRISPR-Cas and precision editing technologies for crop improvement: Mechanisms, translational applications, and the road to climate-resilient agriculture

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Intricacies of the subject

Genome editing has fundamentally transformed plant biotechnology by enabling targeted, nucleotide-level rewriting of crop genomes. The evolution from first-generation nucleases (ZFNs, TALENs) to CRISPR-Cas9, Cas12a, Cas13, base editors (CBEs, ABEs), and prime editors (PEs) has decoupled sequence modification from random transgene insertion and collateral double-strand break (DSB) damage. This review synthesizes the molecular mechanics governing RNA-guided endonucleases, examines engineered plant prime editors (ePPEs, twinPE), and evaluates delivery systems including ribonucleoproteins (RNPs) and nanocarriers. We highlight high-impact agronomic traits spanning yield, biotic/abiotic resilience, nutritional enrichment, and de novo domestication, featuring three milestone translational case studies: multiplex knockout of the soybean allergen GmP34 family, the high-yielding Indian rice variety DRR Dhan 100 ('Kamala'), and the drought/salinity-tolerant Pusa DST Rice 1. Finally, we dissect global product-based regulatory convergence (India's SDN-1/SDN-2 exemption, US SECURE rule, EU NGT frameworks) and outline strategic solutions to overcome persistent bottlenecks in transformation recalcitrance, editing specificity, and equitable smallholder access.

Introduction

Global agri-food systems face compounded pressures: feeding an estimated 9.7 billion people by 2050 necessitates a 50-70% increase in food production, whereas climate change is projected to decrease staple cereal yields (rice, wheat, maize) by 10-25% per 1°C of global warming (Zhao *et al.*, 2017). Conventional breeding cycles (8-12 years) remain too protracted to address emerging climate extremes and virulent pathogen pressures. While classical transgenesis delivered pivotal successes (e.g., Bt cotton, herbicide-tolerant crops), random transgene integration has encountered severe regulatory and public hurdles. Genome editing using site-directed nucleases (SDNs) provides a transformative paradigm, generating predictable single-nucleotide substitutions, small indels, or precise allele swaps at native loci. When freed of foreign DNA cassettes, these edits are classified in mulltiple progressive

jurisdictions under streamlined conventional frameworks, accelerating the bench-to-field delivery of climate-resilient crops.

Mechanistically, SpCas9 interrogates genomic DNA for a 5′-NGG-3′ protospacer-adjacent motif (PAM). Upon PAM recognition, Cas9 unwinds the target duplex to form an R-loop; subsequently, its HNH and RuvC endonuclease domains cleave the target and non-target strands, respectively, yielding a blunt double-strand break (DSB) 3 bp upstream of the PAM. Cellular repair occurs primarily via non-homologous end joining (NHEJ)-an error-prone pathway active across all somatic stages yielding frameshift indels and gene knockouts-or homology-directed repair (HDR), which permits precise sequence replacement via homologous donor templates but remains heavily outcompeted by NHEJ (<1-10% efficiency in somatic plant cells) (Chen *et al.,* 2019). Beyond SpCas9, the toolbox has expanded to: (i) Cas12a (Cpf1), which recognizes T-rich PAMs (5′-TTTV-3′), generates 5′-staggered cohesive ends distal to the seed region, and processes its own crRNA arrays for seamless multiplexing (Zetsche *et al.,* 2015; Schindele *et al.,* 2025; Tayalkar, 2026); (ii) Cas13 (Cas13a/b/d), an RNA-targeting ribonuclease enabling transient viral RNA interference without genomic alterations (Aman *et al.,* 2018; Mahas *et al.,* 2019); (iii) High-fidelity Cas9 variants (eSpCas9, SpCas9-HF1, HiFi Cas9) and pre-assembled ribonucleoprotein (RNP) complexes that suppress off-target cleavage (Kleinstiver *et al.,* 2016; Svitashev *et al.,* 2016); and (iv) Catalytically dead Cas9 (dCas9) fused to transcriptional activators/repressors (CRISPRa/CRISPRi) or epigenomic modifiers (e.g., TET1 demethylase) to fine-tune dosage-sensitive quantitative traits (Lowder *et al.,* 2018; Gallego-Bartolomé *et al.,* 2018).

Next-generation precision editing: Base and prime editing

To bypass the collateral insertions, deletions, and chromosomal translocations inherent to DSB repair, DSB-free precision rewriting architectures have been established:

Base editing systems: Cytosine Base Editors (CBEs) and Adenine Base Editors (ABEs) tether a nickase Cas9 (nCas9-D10A) to single-stranded cytidine deaminases (e.g., APOBEC1, PmCDA1) or evolved tRNA adenosine deaminases (TadA-8e), respectively (Komor *et al.,* 2016; Gaudelli *et al.,* 2017). Guided to target loci, CBEs convert C•G to T•A transitions within an editing window (~4-8 nt), while ABEs catalyze A•T to G•C conversions with superior product purity. Advanced C-to-G base editors (CGBEs) and adenine transversion editors (AYBEs) further broaden mutation space to transversions (Molla *et al.,* 2024).

Prime editing architecture and enhancements: Prime Editing (PE) enables all 12 base substitutions, insertions (up to ~50 bp), and deletions without DSBs or donor DNA (Anzalone *et al.,* 2019). The system pairs nCas9(H840A) fused to Moloney murine leukemia virus reverse transcriptase (M-MLV RT) with a prime editing guide RNA (pegRNA) containing a primer-binding site (PBS) and reverse transcription template (RTT). Second-generation plant optimizations-including engineered plant prime editors (ePPEs with RNase H-truncated RT and viral nucleocapsid fusion; Zong *et al.,* 2022), PEmax, engineered pegRNAs (epegRNAs with 3′ structured motifs), and twinPE strategies-have enhanced editing efficiencies from <5% to >40% in monocots (Xu *et al.,* 2025; Nguyen *et al.,* 2025).

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Table 1: Comprehensive comparison of precision genome editing platforms in crop plants

Platform	DSB	Donor DNA	Installed edits	Typical plant efficiency	Key strengths	Key limitations
Cas9 + NHEJ	Yes	No	Indels / Knockouts	High (30–90%)	Simple, robust, highly multiplexable	Unpredictable indel spectrum; no precision
Cas9 + HDR	Yes	Yes	Substitutions, insertions	Low (<1–10%)	Precise allele replacement / insertion	Template delivery dependent; low in somatic cells
CBE (C→T)	No (Nick)	No	C•G → T•A transitions	Moderate–High (10–70%)	DSB-free; high efficiency	Bystander editing within window; transitions only
ABE (A→G)	No (Nick)	No	A•T → G•C transitions	Moderate (5–60%)	DSB-free; clean product purity	Restricted to transition mutations
CGBE (C→G)	No (Nick)	No	C•G → G•C transversions	Low–Moderate (5–30%)	Enables base transversions	Lower editing purity; bystander effects
Prime Editing	No (Nick)	No (pegRNA)	All 12 substitutions, indels	Low–Moderate (1–50%)	Universal versatility; DSB-free	Design complexity; lower efficiency in dicots
Cas12a (Cpf1)	Yes	Optional	Indels; cohesive-end HDR	Moderate–High (20–70%)	T-rich PAM (TTTV); crRNA processing	Temperature sensitivity (≤25 °C) in some wildtypes
Cas13 (a/b/d)	No	No	RNA cleavage / knockdown	Variable (20–80%)	Transient; direct RNA viral resistance	Collateral trans-cleavage activity in plant cells

Delivery platforms, specificity profiling and multi-omics integration

Conventional *Agrobacterium tumefaciens*-mediated T-DNA delivery and biolistic bombardment commonly require protracted tissue culture and null-segregation over sexual generations. State-of-the-art delivery modalities include: (i) Biolistic delivery of pre-assembled Cas9/sgRNA RNPs to immature embryos or protoplasts, which ensures rapid clearance within hours, preventing genomic integration and slashing off-target frequency (Woo *et al.*, 2015; Svitashev *et al.*, 2016); (ii) Nanoparticle-mediated carriers (carbon nanotubes, layered double hydroxides) for non-transgenic cargo translocation across cell walls (Tashima, 2025); (iii) Geminivirus/tobacco rattle virus replicon systems for systemic cargo amplification; and (iv) Haploid-inducer-mediated editing (HI-Edit/IMGE) to introduce edits into elite inbred backgrounds without direct transformation (Kelliher *et al.*, 2019). Rigorous specificity evaluation is essential for publication and regulatory filings. Contemporary protocols integrate in silico prediction tools (Cas-OFFinder, CRISscan) with genome-wide empirical cleavage assays (CIRCLE-seq, SITE-seq, DISCOVER-Seq) and deep whole-genome sequencing (WGS) of lead events (Tsai *et al.*, 2017; Wienert *et al.*, 2019). Concurrently, coupling CRISPR with multi-omics layers (pan-genomics, single-cell transcriptomics, and metabolomics) drives causal target discovery, haplotype-specific guide design, and metabolic pathway re-engineering (Borah *et al.*, 2024).

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Trait applications and milestone translational case studies

CRISPR precision editing has advanced across diverse agronomic domains: (i) Yield and Architecture: Disruption of negative regulators (Gn1a/OsCKX2, DEP1, GS3, GW2) enhances panicle branching and grain size; promoter editing creates continuous quantitative expression variation (Rodríguez-Leal *et al.*, 2017); (ii) Biotic Resistance: Targeted knockout of susceptibility (S) genes (OsSWEET11/13/14 for bacterial blight, TaMLO for wheat powdery mildew, eIF4E for cassava virus resistance; Wang *et al.*, 2014); (iii) Abiotic Tolerance: Modulation of transcription factors (DST, OsRR22, DREB, NAC) and insertion of stress-responsive elements (e.g., heat-shock element into CWIN promoter; Lou *et al.*, 2025); (iv) Quality and Industrial Biofactories: Tuning amylose via Wx editing, generating low-gluten wheat, high-oleic oils (FAD2 knockout), humanized therapeutic glycoprotein production in Nicotiana benthamiana (Jansing *et al.*, 2019), and de novo domestication of wild tomato (Solanum pimpinellifolium) and orphan groundcherry (Li *et al.*, 2018b; Zsögön et al., 2018).

Case study 1: Multiplex knockout of gmp34 allergen family in soybean

Soybean (*Glycine max*) contains the immunodominant P34 protein (Gly m Bd 30 K), a papain-like cysteine protease. Baek et al. (2025) leveraged multiplex CRISPR-Cas9 to simultaneously target GmP34 (Glyma.08G116300) and its redundant paleopolyploid paralogs GmP34h1 (Glyma.08G116400) and GmP34h2 (Glyma.05G158600). Targeted deep sequencing identified frameshift indels (-7 bp, +1 bp, -2 bp) across all three loci, and western blotting confirmed complete protein elimination in T-DNA-free triple mutants. Crucially, the authors scientifically classified the line as having 'reduced allergenic potential' rather than declaring absolute 'hypoallergenicity', acknowledging remaining allergens (Gly m 5/6/8).

Case study 2: DRR Dhan 100 ('Kamala') - Ultra-high-yielding Indian rice

Officially released by ICAR–IIRR on 4 May 2025, DRR Dhan 100 ('Kamala') represents one of the world's first officially commercialized genome-edited rice varieties (ICAR, 2025). Developed in the elite Samba Mahsuri (BPT 5204) background, it carries an SDN-1 knockout of OsCKX2 (Gn1a locus). Cytokinin oxidase disruption elevates active cytokinin in inflorescence meristems, promoting rachis branching and increasing grain number per panicle (Ashikari *et al.*, 2005). Kamala delivers a ~19% average yield increase (5.37 t ha^{-1} vs 4.50 t ha^{-1}; potential up to 9 t ha^{-1}) and matures ~20 days earlier (~130 days), significantly curbing irrigation demands and paddy methane emissions.

Case study 3: Pusa DST Rice 1 - Multi-stress resilient saline/drought rice

Developed by ICAR–IARI from elite mega-variety MTU 1010, Pusa DST Rice 1 harbors an SDN-1 loss-of-function mutation in DST (Drought and Salt Tolerance), a C2H2 zinc-finger repressor regulating stomatal H_2O_2 homeostasis (Huang *et al.*, 2009; ICAR, 2025). Loss of DST represses stomatal conductance and boosts ROS scavenging under osmotic stress. In national coordinated trials, Pusa DST Rice 1 demonstrated marked yield advantages under stress: +9.66% under inland salinity, +14.66% in alkaline soils, and up to +30.4% under coastal salinity, while maintaining parental yield under non-stress conditions. Cultivation of Kamala and Pusa DST Rice 1 over 5 million hectares is projected to add 4.5 Mt paddy annually while cutting GHG emissions by ~20%.

Global regulatory landscapes and harmonization

Regulatory models governing genome-edited crops have historically diverged into process-based (EU) and product-based (USA, Americas) architectures. A global convergence toward tiered product-based regulation is currently accelerating:

- **India:** In 2022, the Ministry of Environment, Forest and Climate Change (MoEF and CC) and Department of Biotechnology (DBT) exempted SDN-1 and SDN-2 edits from the stringent biosafety provisions of Rules 7–11 of the Environment (Protection) Act, 1986. Following Institutional Biosafety Committee (IBSC) and Review Committee on Genetic Manipulation (RCGM) clearance, transgene-free varieties are handled as conventional crops, directly enabling the 2025 releases of DRR Dhan 100 and Pusa DST Rice 1.
- **Americas and Asia-Pacific:** The USDA-APHIS SECURE rule (2020) exempts modifications achievable via conventional breeding. Latin American nations (Argentina, Brazil, Chile, Colombia, Paraguay) employ streamlined case-by-case determinations establishing non-GMO status for SDN-1/SDN-2 products. Japan and Australia similarly exempt SDN-1 edits lacking foreign DNA.
- **European Union:** The 2025 European Parliament and Council agreement on New Genomic Techniques (NGTs) establishes Category 1 NGTs (equivalent to natural/conventional variants), exempting them from GMO risk assessment while requiring public database listing and seed traceability, narrowing historic international regulatory asymmetries.

Persistent challenges and future perspectives

Realizing the full translational potential of precision crop editing requires resolving key bottlenecks: (i) Transformation recalcitrance: Overcoming tissue-culture dependency via morphogenic regulators (WUS2, BBM, GRF-GIF chimeras), de novo meristem induction, and germline-targeting nanoparticles; (ii) Polygenic trait editing: Deploying multiplexed base and prime editing arrays guided by machine learning and pan-genome variation; (iii) High-fidelity validation: Integrating long-read sequencing and transcriptomic profiling to confirm zero structural variations; (iv) Organellar editing: Expanding mitoTALENs and DddA-derived cytosine base editors for plastid and mitochondrial engineering; and (v) Technology equity: Establishing open-access licensing, regional training consortia, and CGIAR public-good pipelines to ensure smallholder farmers benefit globally.

Conclusion

Precision genome editing has evolved from simple double-strand cleavage to base- and prime-editing architectures capable of programmable, single-nucleotide rewriting. The release of landmark varieties such as DRR Dhan 100 and Pusa DST Rice 1 provides definitive proof that precision editing has transitioned from laboratory proof-of-concept to farmers' fields. Supported by science-based regulatory frameworks and integrated multi-omics pipelines, genome editing represents an indispensable pillar for securing climate-resilient agriculture and global food security through 2050 and beyond.

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