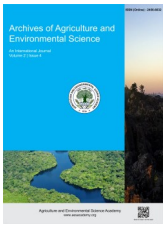
Archives of Agriculture and Environmental Science 10(4): 702-711 (2025)
https://doi.org/10.26832/24566632.2025.1004020

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes



REVIEW ARTICLE

A review on determinants and optimization strategies in prime editing in cereal and non-cereal crops

Bipasha Pandit and Chitra Bahadur Bohara*

Agriculture and Forestry University, Rampur, Chitwan, Nepal
*Corresponding author' E-mail: boharachitra9807@gmail.com

ARTICLE HISTORY

Received: 24 September 2025
Revised received: 30 November 2025
Accepted: 09 December 2025

Keywords

DNA repair
Optimization
pegRNA design
Plant genome editing
Prime editing

ABSTRACT

Prime editing provides precise base changes, minute insertions (Small insertions ≤3 bp showed efficiencies of 2–8%) or deletions, and more defined substitutions without cutting both DNA strands or finding a donor. This is clearly better for safety and control. Plants have quickly taken on, but not in identical way. Changing editor backbones, reshaping pegRNAs, and evaluating out different delivery methods have often made things more efficient, but these improvements don't always work for all species or tissues. Simple design choices like PBS length, RTT layout, adding a 3′ structural tail, or employing paired pegRNAs can have greater implications on results than the editor itself. Editing efficiencies in rice protoplasts ranged from 0.26% to 2.2% for different targets. Rice showed that it was possible, as subsequent initiatives certain of which turned out far more successful than others—propelled into wheat, several dicots, and even some trees. While improvements in editor engineering, more advanced promoters, and computational design all got better, functionality still varies from locus to locus and genotype to genotype. In the real world, the transformation techniques and the local target context often define the outcome. This review summarizes collectively the greatest developments about plant prime editing, focusing on how it can be deployed for specific crops, how procedures can be strengthened, and design guidelines. The degree to which prime editing has been utilized in breeding and functional genomics will depend on further study on pegRNA stabilization, backbone variations, and various methods of delivering it.

Citation of this article: Pandit, B., & Bohara, C. B. (2025). A review on determinants and optimization strategies in prime editing in cereal and non-cereal crops. *Archives of Agriculture and Environmental Science*, *10*(4), 702-711, https://dx.doi.org/10.26832/24566632.2025.1004020

INTRODUCTION

CRISPR-Cas has rendered targeted editing popular among plants by leading to double-strand breaks that cells could fix. The reason is that non-homologous end joining is cluttered. Tools like CRISPResso exhibit the fact it typically causes unexpected by-products, specifically in plants, and that insertions, deletions, and rearrangements occurs a lot (Pinello *et al*., 2016). The technique of homology-directed repair appears to be more straightforward, it seldom demonstrated efficacy in somatic plant cells due to S/G2 timing, donor scarcity, and primary end-joining pathways, which leads to diminished HDR efficiencies and more complicated repair consequences (Li *et al*., 2020; Huang & Liu, 2023). Prime editing relies on a Cas9 nickase–RT fusion guided by a structurally extended pegRNA. Prime editing was developed to prevent these issues. It makes use of a reverse transcriptase and a Cas9 nickase, coupled with a pegRNA which recognizes the site and encodes the edit, to write changes without using donor DNA or DSBs (Anzalone *et al*., 2019). There is solid empirical evidence for all 12 base substitutions and even for small insertions and deletions. as opposed to base editors, which typically generate transitions, prime editors can set up both transitions and transversions, and they often lessen undesired edits when compared to DSB-based methodologies and base editing (Pinello *et al*., 2016; Lee *et al*., 2025). In eukaryotic cells, the repair of DNA double-strand breaks mainly occurs

through four routes: homologous recombination (HR), classical non-homologous end joining (c-NHEJ), microhomology-mediated end joining (MMEJ or alternative end joining), and single-strand annealing (SSA). Each pathway involves different proteins and works at a distinct pace. The rapid c-NHEJ pathway can fix around 70–80 percent of breaks within just a few hours, while HR usually takes about a day to complete. Because c-NHEJ and MMEJ act more quickly, they often compete with HDR and reduce its accuracy. Blocking or slowing these competing pathways has therefore been shown to make genome editing more precise (Mentani *et al*., 2025).

Prime editing in plants involved an adjustment of the mammalian architectures. Early studies have shown that PE2 and PE3 work well in rice and wheat (Lin *et al*., 2020). Similarly, the enpPE2 prime-editing system works efficiently in plants that allows precise and heritable changes in multiple genes. This makes it a useful tool for improving crop traits through accurate genome modification (Li *et al*., 2022). Rice's plant-adaptive pPE2 varied from 0 to 31%, depending on the objective (Xu *et al*., 2020). Rates elevated across more loci with more effective pegRNA designs and later backbones like PEmax (Zhong *et al*., 2024). Other beneficial properties of enpPE2, which reported up to ~70% in T_0 rice for particular targets, comprised promoter substitutions, reverse transcriptase modifications, and pegRNA stability (Lin *et al*., 2022). Delivery is essential: PE3/PE3b performance could be hindered by asynchronous nicking, yet agrobacterium can be beneficial (Xu *et al*., 2020). Researchers are exploring particle bombardment, protoplast transfection, and direct RNA or protein delivery to get past genotype-specific constraints (Laforest & Nadakuduti, 2022). Design tools for example PlantPegDesigner, which originally aimed at monocots, are currently compatible with plant targets (Lin *et al*., 2021). Modern advancement has resulted in a growing version of the toolbox. Numerous reports claim that pegRNA modifications including dual pegRNAs, 3′ structural tails, and careful PBS and RTT length adjustments have improved outcomes (Yu *et al*., 2023). A systematic review of studies concluded that pairing forward and reverse pegRNAs enhanced efficiency by an average roughly 4.2 times, with a maximum of approximately 24.5% in the study set (Tian *et al*., 2025). Prime editing meets breeding needs like modifying regulatory elements or converting alleles because it writes precise changes without DSBs. It should also work well with future multiplex trait stacking (Lee *et al*., 2025). Despite notable advances in optimizing prime editing (PE) for plants, editing efficiency still varied widely across genomic targets and species and remained substantially lower than that achieved with base editors (Li *et al*., 2022). Liu *et al*. (2021) were able to improve prime-editing efficiency in *Oryza sativa* by temporarily reducing the activity of *OsMLH1* (a key gene in the DNA mismatch-repair pathway) by using an RNA interference method built into the ePE5c system. However, several important limitations still exist.

METHODOLOGY

This review was conducted through a structured synthesis of peer-reviewed publications, with particular focus on studies that investigated the architecture, mechanistic principles, optimization strategies, and crop-specific applications of prime editing (PE) systems in plants. To guide the methodological framework, Figures 1 and 2 were used as conceptual anchors representing the core components and mechanistic sequence of PE activity. These figures were referenced throughout the literature screening and analysis process to maintain coherence between molecular mechanisms and applied case studies.

Conceptual framework: Prime editing components

Figure 1 illustrates the essential molecular components of prime editing, which formed the basis of how studies were evaluated and compared in this review. Prime editing relies on three principal elements:

1) a Cas9 nickase (nCas9) bearing the H840A mutation,
2) a reverse transcriptase (RT) fused to the nCas9 protein, and
3) a prime-editing guide RNA (pegRNA).

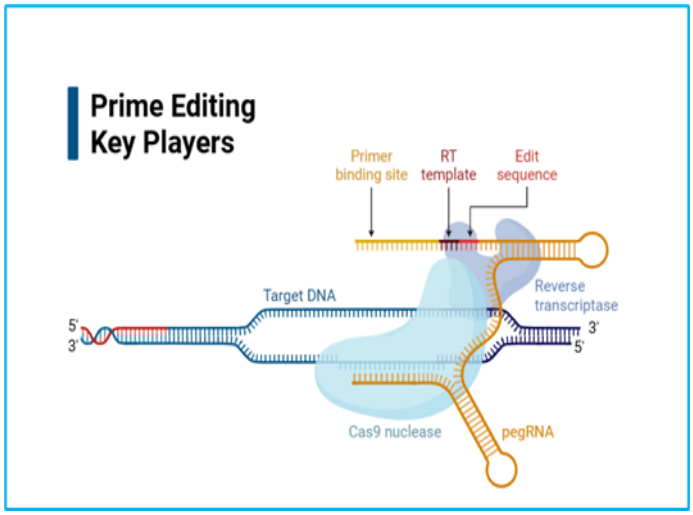


Figure 1. *Prime editing key players.*

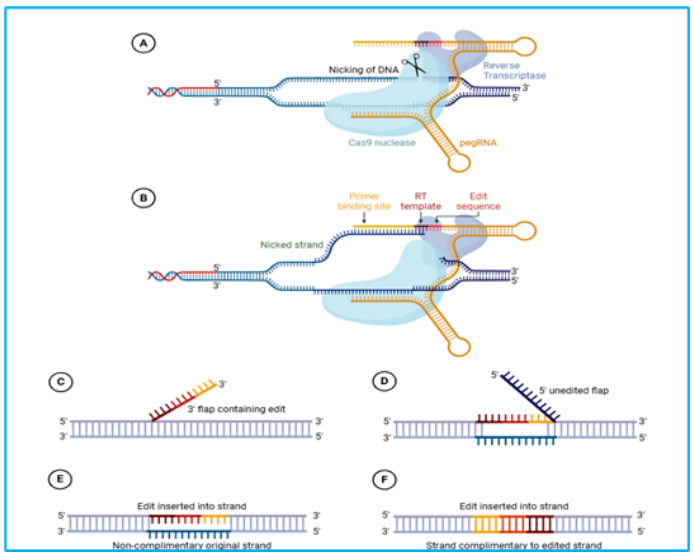


Figure 2. *Mechanism of prime editing.*

The pegRNA includes a conventional CRISPR targeting spacer and two additional modules—the primer binding site (PBS) and reverse transcription template (RTT)—which together encode the desired sequence change (Anzalone *et al.*, 2019; Lin *et al.*, 2020). Because pegRNA structural stability, PBS/RTT length, and backbone modifications influence editing outcomes, all included studies were assessed based on how they engineered or optimized one or more of these components (Nelson *et al.*, 2022; Li *et al.*, 2022). This figure therefore served as a reference point for categorizing determinants of efficiency in crop species such as *Oryza sativa*, *Triticum aestivum*, tomato, and poplar.

Mechanistic sequence of prime editing

To ensure consistency in comparing study designs, Figure 2 was used to define the mechanistic sequence of prime editing, including:

- Target recognition and nicking of a single DNA strand by nCas9(H840A).
- Primer binding as the PBS hybridizes with the nicked strand.
- Reverse transcription, during which the RT synthesizes the edited DNA flap using the RTT region of the pegRNA.
- Flap equilibration, where edited (3′) and unedited (5′) flaps compete for incorporation.
- DNA repair integration, during which endogenous repair pathways insert the edited flap and restore a stable genome configuration (Chen *et al.*, 2021; Shuto *et al.*, 2024).

Because mismatch repair (MMR) strongly influences these steps, papers were included if they addressed MMR inhibition strategies (e.g., MLH1dn expression, MLH1 knockdown) or repair-pathway modulation that improved flap stability (Ferreira da Silva *et al.*, 2022; Ni *et al.*, 2023).

Literature search and selection criteria

A comprehensive literature survey was conducted using databases including Web of Science, Scopus, PubMed, and Google Scholar. The search used combinations of the terms: prime editing, pegRNA, PBS/RTT,

- prime editor plant,
- prime editing rice/wheat/tomato/poplar,
- MMR inhibition,
- delivery systems plant genome editing.

Inclusion criteria

Studies were included if they met the following criteria:

- Reported experimental prime editing results in plants.
- Provided quantitative efficiency data (e.g., PE2, PE3, PE3b, PE5max, PE6 variants).
- Described pegRNA parameters (PBS, RTT, 3' motifs) or editor backbone engineering.
- Evaluated delivery systems (Agrobacterium, particle bombardment, protoplast transfection).
- Explained mechanistic or species-specific editing constraints

Exclusion criteria

Excluded materials included review-only papers unless they contributed mechanistic frameworks relevant to method interpretation.

Data extraction and categorization

Each paper was coded according to:

- Editor system (PE2/PE3/PE3b, PEmax, ePE, PE5max, PE6c, ePPEplus).
- pegRNA features (PBS/RTT length, epegRNA motifs such as evopreQ1 or xrRNA).
- Delivery strategy (Agrobacterium, protoplasts, nanoparticle-based, replicons).
- Genomic context (target locus, monocot/dicot differences).
- Observed editing efficiencies and associated variables.
- Mechanistic explanations mapped to the stages in Figure 2.

These categories were chosen to reflect the process shown in Figure 2, allowing comparison between pegRNA design decisions, nicking strategies, repair pathway interactions, and crop-specific responses.

Integration of figures into methodological analysis

Throughout the review process, Figure 1 informed how editor components were grouped and compared, while Figure 2 guided categorization of mechanistic bottlenecks such as:

- pegRNA folding and degradation (Zhang et *al.*, 2024),
- spacer-PBS complementarity issues (Ponnienselvan *et al.*, 2023),
- MMR interference (Chen *et al.*, 2021),
- asynchronous nicking in PE3/PE3b (Xu *et al.*, 2020),
- reverse transcriptase performance limitations (Ni *et al.*, 2023).

These mechanistic considerations helped interpret why efficiencies varied between loci, species, and delivery routes.

COMPARATIVE OVERVIEW OF PRIME EDITING STRATEGIES AND APPLICATIONS

The consolidated overview of prime editing strategies, methodological variations, and crop-specific applications, as summarized in Tables 1–4, to support a clearer understanding of how these approaches have been optimized across different plant systems. This review discusses the latest developments related to plant prime editing including core architectures, implementations specific to a specified crop, applications that emphasize traits, challenges with technology, and future directions. The primary points of discussion are wheat, rice, and major dicots. Examples of Oryza sativa are listed by locus and trait, along with information on pegRNA parameters, editing strategy, delivery, assay, and observed efficiencies. The patterns we observe—

Table 1. Comparison between base editing and prime editing technologies.

Technology	Capabilities	Strengths	Weaknesses	Approx. age	References
Base Editing	Performs four nucleotide transitions (C→T, G→A, A→G, T→C). Utilizes cytidine or adenine deaminase fused to Cas9 nickase. Avoids double-strand breaks and donor templates.	High precision for point mutations. Simple and efficient delivery. Widely validated in animal and plant systems. Applied in translational and clinical research.	Limited to transition mutations only. Risk of bystander edits and Cas-independent off-target deamination. Ineffective for large insertions or deletions.	7 years (introduced in 2016–2017).	(Komor *et al.*, 2016; Gaudelli *et al.*, 2017; Anzalone *et al.*, 2019)
Prime Editing	Performs all 12 possible base substitutions. Capable of small to large insertions/deletions (up to kb-scale). Uses Cas9 nickase fused to reverse transcriptase guided by pegRNA (PBS + RTT).	Highly versatile—supports substitutions, insertions, deletions. Fewer bystander and off-target edits. High accuracy without DSBs or donor DNA. Applicable across species.	Larger construct size and mechanistic complexity. Requires optimization for efficiency, especially in plants. Delivery challenges in some species.	4 years (introduced in 2019).	(Anzalone *et al.*, 2019; Lin *et al.*, 2020; Zhong *et al.*, 2024)

Table 2. Representative prime editing approaches in *Oryza sativa*: loci, traits, and efficiencies.

Organism / Tissue	PE System	ngRNA	PBS (nt)	RTT (nt)	Delivery	Assay	Target / Trait	Key outcome	Reference
Rice (protoplast, callus)	pPE2, PE3, PE3b	Yes	13	15	Agrobacterium-mediated	Reporter (HPT-ATG), genotyping	HPT-ATG restoration	Editing frequencies 0–31.3 %; PE3/3b comparable or lower; efficiency strongly locus-dependent	(Xu *et al.*, 2020)
Rice (protoplast, callus)	NEPE	Yes	8–10	varying	Agrobacterium-mediated	NGS, reporter	SNPs, MNVs, insertions, deletions, replacements	High efficiency across four edit classes; optimized PBS length and epegRNAs improved outcomes	(Zhong *et al.*, 2024)
Rice (callus, T_0)	epegRNA + high temperature	Yes	8–10	13–15	Agrobacterium-mediated	Genotyping, NGS	OsALS, OsPDS	Elevated editing efficiencies at otherwise recalcitrant loci through combined epegRNA design and temperature treatment	(Zou *et al.*, 2022)
Rice (T_0 plants)	PE5max	Yes	13	15	Agrobacterium-mediated	Resequencing, pathogen challenge	Xa23 (EBE insertion), xa5 (V39E)	Precise Xa23SW14 knock-in (47.2 %; 18 % biallelic) and xa5 editing (88.5 %); broad-spectrum bacterial blight resistance; no off-targets	(Gupta *et al.*, 2023)
Rice (T_0 plants)	Modular multiplex PE (DPE/TPE/QPE)	Yes	13	15	Agrobacterium-mediated	Genotyping, pathogen challenge, herbicide assay	xa5 + Xa23SW11, EPSPS, OsSWEET 11a, OsSPL13	Duplex co-editing 46.1 %; quadruplex 43.5 %; trait stacking; ngRNA choice was decisive for efficiency	(Gupta *et al.*, 2024)

Notes: PE2 / PE3 / PE3b = original plant prime editors; PE3b introduces a nick on the non-edited strand after reverse transcription. NEPE = next-generation plant prime editor with enhanced architecture. epegRNA = engineered pegRNA with 3′ structural motifs to increase stability and efficiency. DPE / TPE / QPE = duplex, triplex, and quadruplex multiplex pegRNA–ngRNA assemblies. BB resistance = bacterial blight resistance achieved through Xa23 promoter editing (Xa23SW14) and xa5 recreation (V39E). PBS = primer binding site; RTT = reverse transcription template; ngRNA = nicking sgRNA.

Table 3. Prime editing strategies in *Triticum aestivum*: Editor backbones, pegRNA designs, and efficiency determinants.

Organism / Tissue	PE System	Key Engineering	Improvement	Edit Types	Delivery	Assay	Application Focus	Reference
Wheat (hexaploid, Triticum aestivum)	ePPEplus	V223A substitution in RT domain within ePPEmax*	33.0× higher than PPE; 6.4× higher than ePPE	SNPs, short insertions, deletions	Agrobacterium-mediated	Targeted amplicon sequencing	Functional genomics and trait improvement in polyploid wheat	(Ni *et al.*, 2023)

Notes: ePPEplus = engineered plant prime editor incorporating V223A substitution in the reverse transcriptase domain within the ePPEmax* framework. Earlier PE2/PE3 systems in wheat exhibited low or negligible editing efficiencies, underscoring the impact of ePPEplus. PBS = primer binding site; RTT = reverse transcription template; ngRNA = nicking sgRNA.

Table 4. Prime editing in dicot models and non-cereal crops: Strategies, delivery methods, and contextual determinants.

Organism / Tissue	PE System	Key Engineering / Design	Efficiency	Target / Trait	Delivery	Assay	Reference
Poplar (hybrid 84K)	PE3	AtU6-26 promoter; 2×35S editor cassette; PlantPegDesigner for pegRNA design	0.1–3.6 % (callus); 3.6–22.2 % (T_0 plants)	Multiple loci tested; 7/9 successfully edited	Agrobacterium-mediated	Genotyping of callus and T_0 plants	(Zou *et al.*, 2024)
Tomato (Solanum lycopersicum)	Early PE2	Cas9–RT fusion inefficiency; pegRNA self-complementarity; PBS shortening and mismatches tested	Inefficient	Reporter and endogenous loci	Agrobacterium-mediated	Reporter assays and genotyping	(Vu *et al.*, 2022)
Tomato / Arabidopsis (Arabidopsis thaliana)	Optimized PE	Vector redesign; promoter optimization; pegRNA refinements for heritable edits	edits achieved (qualitative)	Proof-of-concept; stable T_1 transmission	Agrobacterium-mediated	T_1 heritability analysis	(Song *et al.*, 2021)

Notes: PE3 = prime editor introducing a nick on the non-edited strand after reverse transcription. epegRNA = engineered pegRNA with structural motifs for stability. Poplar efficiencies were measured in callus and regenerated plants using genotyping assays. Tomato early trials demonstrated structural limitations of Cas9–RT fusions. PBS = primer binding site; RTT = reverse transcription template; ngRNA = nicking sgRNA.

such as dependency on pegRNA stability, nick location, and local repair activity—are covered in this article a brief description covering the primary editing methods applied to *Triticum aestivum*, with special emphasis on nicking strategies, editor structural modifications, and pegRNA configuration changes. Differences in RTT length, nick orientation, and backbone engineering are related to observed output distinctions. The fundamental editing mechanisms of other crop and dicot models, additionally including context-specific restrictions that correlate with the sequence-context and delivery determinants listed in table 4. The main editing efforts in dicot species and non-cereal crops, which include Arabidopsis, tomato, and poplar. Heritable prime editing in Arabidopsis and tomato has been made accessible by latest developments in pegRNA and vector technology, while Poplar is the first stable tree system to support PE3. The prime editing reaction proceeds through nicking, DNA priming, RT-mediated extension and flap equilibration (Figure 2). Prime editing requires use of one specific guide RNA that not only directs the editing apparatus to the desired point but also additionally delivers the necessary template for rewriting the DNA. A primer binding site that allows the reverse transcriptase to start synthesis and a reverse transcription template that encodes the desired change are two major extra features of this guide RNA. Once the complex meets the target sequence, reverse transcription directly writes the modified DNA onto the target strand, and the cell's own repair mechanisms incorporate this new flap into the genome. According to early research conducted in rice, prime editing can be productive in plant cells; however, the editing rates were low and highly dependent on the genomic locus (Lin *et al.*, 2020). The nCas9(H840A) enzyme fused with Moloney murine leukemia virus reverse transcriptase (M-MLV RT) has been introduced alongside pegRNAs and expressed under plant promoters through Agrobacterium-mediated transformation into rice callus and wheat tissues. The instability of pegRNAs within plant cells and interference from the mismatch repair pathway were a few of the technical issues that reduced efficiency, according to these prior observations. Work in tomatoes revealed additional species-specific problems, which include repair pathway biases and improper pegRNA processing, which minimized the effect of prime editing (Vu *et al.*, 2022). The fact that different plant species respond differently to the same system is illustrated by the surprising fact that the Cas9(H840A)–RT fusion that worked well for rice did not execute properly for tomatoes. Moss, a popular model for studying specific genome changes in non-seed plants, showed a few of these fundamental differences in DNA repair behavior. The complicated nature of the genome in polyploid wheat caused stable edits harder to accomplish. Recent studies have redesigned the system to overcome these challenges, resulting in more reliable editing outcomes (Ni *et al.*, 2023). Improvement in the M-MLV RT domain's interaction with the primer and template was the key goal of this redesign, as it was discovered to be a major factor limiting editing effectiveness in complex plant genomes (Ni *et al.*, 2023).

TECHNOLOGICAL DISCOVERIES IN DRIVING PRIME EDITING EFFICIENCY

Prime editing systems developed rapid advancements as soon as researchers begin to identify their flaws. Several early issues were brought on by the pegRNA itself. Small changes in primer binding site (PBS) length or reverse transcription template (RTT) size had a large effect on editing efficiency. As an instance, when these parameters modified in combination with RT module engineering—more especially, the implementation of a reverse transcriptase variant derived from Tf1 and dual-RT configurations—performance improvements were noticeable in rice PE6c (Cao *et al*., 2024). Lin *et al*. (2021) concluded that PegRNAs worked best at a melting temperature of about 30 °C in PBS. Similarly, scientists found that silent (same-sense) mutations in the RTT may aid in the removal of unwanted secondary structures, improving pegRNA stability and editing efficiency (Li *et al*., 2022). PegRNAs were more fully stabilized and their ends prevented from degrading by the addition of 3′ structural motifs such as evopreQ1 or xrRNA (Nelson *et al*., 2022; Zhang *et al*., 2024). Additional ingenious changes produced chopped transcripts by cleaning up RNA ends with a F+E scaffold and Csy4 processing modules (Liu *et al*., 2021). By decreasing complementarity between the spacer and PBS, pegRNAs without internal inhibitory features were designed to enhance target binding and R-loop formation while preventing self-folding (Ponnienselvan *et al*., 2023). The use of paired pegRNAs, which enabled alterations at challenging or previously inaccessible locations, was another important development (Lin *et al*., 2021).

On the side of the enzyme, optimization proceeded at the same pace. A single amino acid modification (V223A) in the wheat reverse transcriptase boosted the editing efficiency of the ePPEplus system by over thirty times (Ni *et al*., 2023). The hyPE2 variant, which was generated by Rad51-RT fusions, stabilized the flap intermediates and improved editing in mammalian cells (Song *et al*., 2021). Although research on its usage in plants is still ongoing, this concept has created new prospects.

The DNA mismatch repair (MMR) system, which can reverse prime editing and produce unwanted indels, was another major challenge. Depending on the system, inhibiting MMR activity by either knocking down genes like MLH1 and MSH2 or by expressing a dominant-negative MLH1 mutant tipped the balances in favor of the intended edits and enhanced efficiency by up to 17 times (Chen *et al*., 2021; Ferreira da Silva *et al*., 2022). Similarly, histone deacetylase (HDACi) inhibition improved editing outcomes by increasing chromatin openness (Liu *et al*., 2022). At the same time, delivery methods also improved. By encouraging higher expression of the primary editor cassette during piggyBac -mediated integration, the use of CAG promoters rather than CMV in piggyBac vectors resulted in significant alterations, including up to 17-fold increases (Mu *et al*., 2025). Edits may accumulate and sometimes push efficiency above 80% as a result of using lentiviral systems to prolong the pegRNA expression duration (Mu *et al*., 2025).

These were all little yet beneficial changes. These minor enhancements such as modifications to linkers, codon use, RT variations, nuclear localization signals, and RNA designs—were aggregated with every new generation of prime editors. These modifications collectively revolutionized the field and led to the transition from the original PE2 to the far more active PE6 variations (Doman *et al*., 2023). Figure 3 summarizes the main factors that influence prime-editing efficiency in plants. It highlights how pegRNA stability, reverse-transcription performance, Cas-nickase positioning, and local genomic context collectively determine editing outcomes. It also shows why optimizing pegRNA design—such as PBS/RTT length or adding stabilizing motifs—is crucial for achieving higher editing efficiency (Nelson *et al*., 2022; Zhang *et al*., 2022; Zhao *et al*., 2023). Figure 4 illustrates the structure of a pegRNA, showing its key components: the spacer, PBS, RTT, and optional 3′ stabilizing elements. It explains how these parts work together to guide the nickase and serve as a template for reverse transcription during prime editing. It also shows how improved pegRNA architectures and paired pegRNAs can enhance editing frequencies in plants (Anzalone *et al*., 2019; Lin *et al*., 2021).

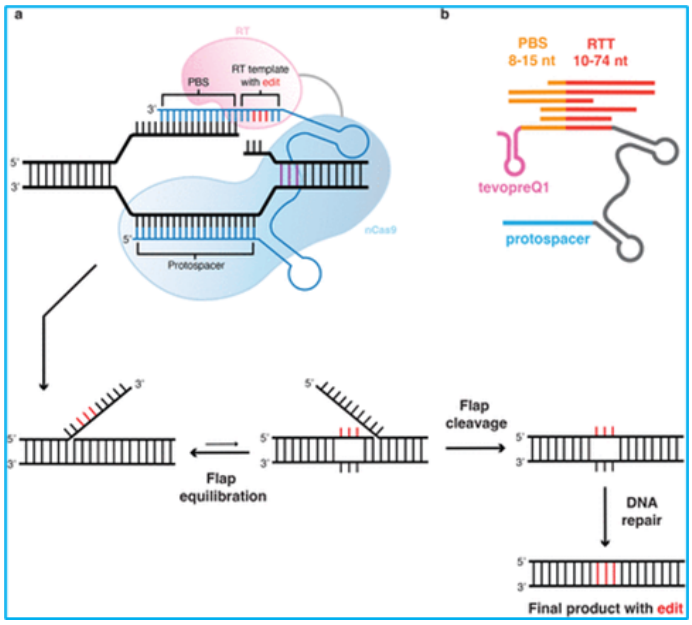


Figure 3. *Structural layout of PBS, RTT, flanking elements.*

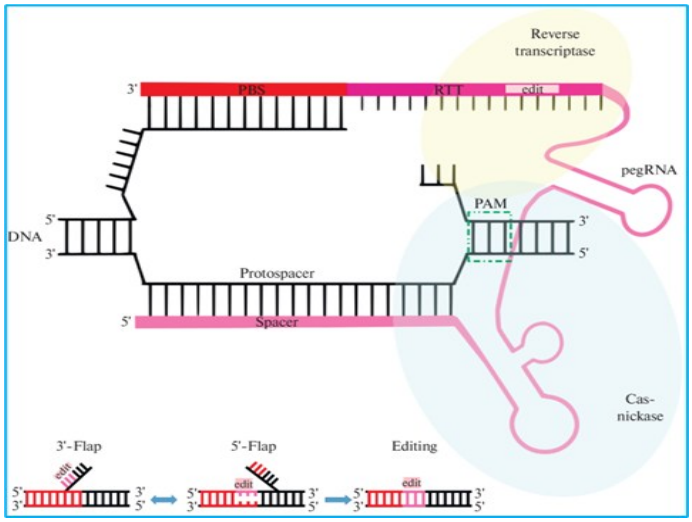


Figure 4. *Schematic of pegRNA with PBS/RTT/spacer.*

DESIGN PRINCIPLES

In this, Zhong *et al.* (2024) showed that adjusting pegRNA features carefully can significantly alter the editing outcome. In rice, pegRNAs with primer binding sites (PBS) of 8–13 nucleotides and reverse transcription templates (RTT) of roughly 15 nucleotides consistently produced higher editing efficiencies across a range of edit types, but locus and editing mode had different effects on performance (Table 2). Shuto *et al.* (2024) elaborated on this and showed how even minor structural changes can have significant functional effects. Structured motifs, such as evopreQ1, were added to the 3′ end to stabilize pegRNAs and reduce their degradation (Nelson *et al.*, 2022). According to Xu *et al.* (2020), the system can be quite sensitive; altering a single base in the RTT or PBS can increase editing efficiency from nearly nil to over thirty percent. There were also repercussions from yet another structural change. In several human cell lines (HeLa, U2OS, K562, and fibroblasts), editing performance was increased three to four times without increasing off-target activity by merely changing stem-loops, interfering with self-complementarity, or enhancing G-quadruplex formation. The use of engineered pegRNAs, or epegRNAs, produced those benefits (Nelson *et al.*, 2022; Li *et al.*, 2022; Ponnienselvan *et al.*, 2023). PegRNA architecture particularly PBS and RTT length critically determines editing activity (Figure 3) and also a functional pegRNA includes a target-binding spacer followed by a PBS and RTT that encode the desired edit (Figure 4). Paired pegRNA techniques represented a significant advancement. By encoding the same edit with two pegRNAs in trans, Lin *et al.* (2021) showed seventeen-fold gains in rice efficiency, thus enabling the system to cooperate. In dicots such as tomato and Arabidopsis, Song *et al.* (2021) used a replicon-based delivery system that coupled Pol II and Pol III promoters for pegRNA production to achieve extremely effective and heritable edits. In addition to enabling multiplex editing while maintaining low levels of by-products, this hybrid promoter approach overcomes the low pegRNA expression barrier that is typical of dicots. Furthermore, entirely novel editing modes were produced by paired pegRNAs. To accurately and silently eliminate particular sequences, the PRIME-Del method use flap annealing and nick coordination rather than double-strand breaks (Choi *et al.*, 2021). Twin prime editing enables the introduction of huge insertions, segment swaps, or inversion of entire regions, hence removing the necessity for harsh cuts (Anzalone *et al.*, 2022). Instead of requiring the genome to be hacked apart, these methods turn it into a document that can be deliberately and meticulously altered. Sometimes the most effective edits are the smallest ones: small changes to promoters or upstream open reading frames (uORFs) can fine-tune gene expression in a way that's comparable to turning off a light, offering a complex way to regulate gene expression without altering the coding sequence itself (Zhang *et al.*, 2018). Repair pathways also influence the outcomes. Prime editing may be hampered by mismatch repair (MMR), which concentrates on altered flaps. Chen *et al.* (2021) showed that producing a dominant-negative MLH1 (MLH1dn) improved efficiency and product purity by protecting these flaps from excision. Another suggested strategy is to fuse exonucleases to the editor to help with difficult edits and 5′ flap trimming. Ni *et al.* (2023) improved the ePPEplus system in plants by adding a V223A mutation into the RT domain, which led to tens of times higher wheat editing efficiency than the original system.

The possibility for pegRNA activity and the nicking step to fall out of sync can reduce the expected efficiency benefits from PE3 and PE3b techniques in Agrobacterium-mediated systems. In order to overcome this, Xu *et al.* (2020) created a surrogate co-selection method that used reporter systems to enrich edited Calli, helping to recover high-efficiency events even when timing was not optimal. For multiplex editing, the selection of nicking guides turned out to be crucial. To overcome this, Gupta *et al.* (2024) created a modular cloning framework that makes it possible to assemble a large number of pegRNAs and nicking guides into multi-cassette arrays that are stable. These constructs were stable after cloning and Agrobacterium delivery, and they were effective in targeting several loci in rice at once. The team's direct evaluation of editing findings in T_0 plants is remarkable since it allowed for rapid confirmation of construct effectiveness without waiting for further generations (Table 1).

EMERGING SYSTEMS AND SPECIAL CONTEXTS: DICOTS AND EXPANSION IN OTHER CROPS

When it comes to primary editing, dicot species have different structural and expression hurdles than monocots. For instance, Vu *et al.* (2022) showed that the low editing effectiveness in tomatoes (at loci notably SlMBP21, SlALC, and SlALS1) is caused by the Cas9–RT fusion-introduced barriers. This only appears to affect nuclease activity and pegRNA self-complementarity. Vu *et al.* (2022) concluded that further improvements to expression systems or enrichment tactics (such promoters, vector copy number, and replicons) are necessary to tip the scales. A transferable design that later served as a foundation for dicot species was created in rice using an improved framework, high-temperature treatment, and modified pegRNAs (Zou *et al.*, 2022). Zou *et al.* (2024) used a 2×35S promoter to drive the editor and AtU6-26 promoters for epegRNAs to create a PE3 system in poplar 84K. Changes were made to PagPDS, PagYUC4, and PagSHR. Callus assays showed editing at most sites, and T□ plants confirmed the intended modifications with little byproducts. The technique stabilized pegRNA activity in a hybrid genome while exhibiting chimerism and low Type III efficiency, as observed in other dicots.

Mechanistic refinements

Recently, Zhang *et al.* (2024) reported that pegRNAs often fold inward, and the PBS forms duplexes with the spacer to block Cas9 and editing. A quick heat phase followed by a slow cooling process unfurled these structures, increasing efficiency by as much as twenty times. A few mismatches were added to further loosen the PBS–spacer grip. Since longer, GC-rich PBS sections were more likely to misfold, correcting this improved RNP as-

sembly and editing, especially for insertions. The protection of edited intermediates was provided by linker optimization to reduce inhibitory structures, MLH1dn-mediated inhibition of mismatch repair, and appropriate attention to PBS–spacer folding regulation (Lee *et al*., 2025). The optimization of every molecular component of the primary editing system was thoroughly investigated by Murray *et al.* (2025). They used pegRNA libraries (MOSAIC) to examine combinations of PBS and RTT lengths in order to identify configurations that offered the highest editing rates for different targets. In this, Perroud *et al.* (2023) developed an improved prime editing approach that reliably works in *Physcomitrium patens*. By evaluating split editor proteins, employing a plant retrotransposon reverse transcriptase, and optimizing pegRNA designs, they were able to boost editing efficiency without compromising accuracy. They also showed that genes other than reporters can be altered by successfully generating a Ppdek10 mutant through indirect selection. Their work serves as an example of how meticulous preparation may simplify the editing process. An efficient delivery is especially crucial because prime editing uses more components and larger structures than conventional CRISPR. Regeneration problems, species-specific obstacles, and the rigid cell wall of plants make it difficult to introduce pegRNA and prime editor proteins effectively. New technologies like viral vectors and nanoparticles are continually being developed, and while methods like protoplast transfection, particle bombardment, and Agrobacterium transformation have been used, they all have unique drawbacks. According to Laforest and Nadakuduti (2022), a robust method should be efficient, DNA-free, genotype-independent, and suitable for stable editing across a range of crops. Cas9 mutations (R221K and N394K) are introduced to improve folding and nuclear delivery, and different linker sequences and nuclear localization signals are examined (Murray *et al*., 2025). According to Petrova & Smirnikhina (2023), twinPE and integrase coupling (e.g. with serine integrases) are mechanistic expansions that allow for the insertion of bigger DNA pieces through attB/attP site recombination. Furthermore, eliminating the RNase H domain from the reverse transcriptase can lessen the breakdown of RNA strands in the RNA–DNA hybrid (Petrova & Smirnikhina, 2023). Mu *et al.* (2025) demonstrated the substantial influence that stable expression and optimized delivery can have by testing the system in a number of cell lines (HeLa, T47D, and MCF7) as well as human pluripotent stem cells (hPSCs). They were able to achieve up to 80% editing in 293T cells and approximately 50% in human stem cells. Changing the promoter from CMV to CAG and attaching a mCherry reporter (via a 2A peptide) enabled the integrated editor to track expression precisely (Mu *et al*., 2025). Sousa *et al.* (2024) fine-tuned PBS/RTT lengths, adjusted nick-guide positions, and produced pegRNAs with stable 3′ tails to enhance prime editing in CFTR F508del. They used MLH1-dn to impede mismatch repair, dead sgRNAs positioned near the target, silent mutations, and PE6 variations to bias repair at the CFTR gene.

CHALLENGES AND BOTTLENECKS

There are notable differences in prime editing effectiveness between species, loci, and edit types. The authors note that the frequent occurrence of undesired indels is one of the primary mechanical problems with prime editing. The main cause of this is competition during flap equilibration between the unedited 5′ flap and the modified 3′ flap. The 5′ flap may be re-ligated if it remains stable in conventional PE systems, which could lead to little additions, deletions, or shoddy edits. This rivalry is one of the primary sources of genetic mistakes (Chauhan *et al*., 2025). PegRNA stability is another issue; misfolding, degradation, or the insertion of an unwanted scaffold during reverse transcription are possible risks. Another issue is pegRNA stability, which is covered in Zhao *et al.* (2023) in their analysis of mechanistic hurdles. Misfolding, degradation, or unwanted scaffold insertion during reverse transcription are examples of mechanistic hazards. They continued by providing more details. Mismatch repair (MMR) is a mechanistic barrier that can recognize heteroduplex DNA and remove alterations. By introducing silent mutations or preventing mismatch repair (for instance, through MLH1-dn), this can be thwarted. High frequencies are produced by structures at some places and very little activity at others. QPE in multiplex assemblies, PE5max in rice, and ePPEplus in wheat serve as examples of this disparity (Gupta *et al*., 2024; Ni *et al*., 2023). The RTT sequence, secondary structure, genomic context, nicking location, and repair activity all affect the outcome. No forecasting model can accurately account for these circumstances. For frequent edits, each parameter needs to be manually changed. OsSPL13 editing rose from 0% to 30% after nicking sgRNA replacement (Gupta *et al*., 2024). For every locus, this type of change is often required. According to Jiang *et al.* (2022), a large number of edited lines showed mosaicism or heterozygosity, indicating poor editing rates or inadequate repair in the early phases of development. Since many genotypes still fail to regenerate or transform, Agrobacterium ultimately does the majority of the work. Clear rules for higher-order co-editing are still lacking, stubborn cultivars remain out of reach, and multiplex editing often breaks down after QPE. Dicot systems lag behind cereals; poplar, tomato, and Arabidopsis all exhibit poor prime-editing rates and weak promoter–pegRNA couplings. Since most articles only provide T_0 results, we don't know much about inheritance, segregation, or transmission of multiplex/regulatory changes. Limited delivery alternatives and an unknown regulatory environment complicate commercial scheduling (Song *et al*., 2021; Vu *et al*., 2022; Zou *et al*., 2024).

FUTURE PROSPECTS

Design at scale: Use big epegRNA libraries with simple reporter readouts and lean on ML to predict which sites (and nicks) will work best in each locus (Ni *et al*., 2023; Gupta *et al*., 2024; Murray *et al*., 2025).

Editor upgrades: Build temperature-tolerant RTs, try split/mini editors, and use cleaner flap control (e.g., vPE) to lift efficiency and cut indels (Petrova & Smirnikhina, 2023; Chauhan *et al.*, 2025).

Smarter expression: Drive pegRNA/editor with tissue- or stage-specific promoters and add replicon support to boost levels in tough tissues (Song *et al.*, 2021; Vu *et al.*, 2024).

Broader delivery: Bring in viral vectors, nanocarriers, and physical methods so we're not stuck with Agrobacterium and can reach recalcitrant genotypes (Laforest & Nadakuduti, 2022; Vu *et al.*, 2024).

Breeding pipelines: Combine prime editing with speed breeding and multiplex trait stacks, and push into legumes, tubers, and minor cereals with clear inheritance tracking (Ni *et al.*, 2023; Gupta *et al.*, 2024).

Conclusion

As prime editing is almost ready for use, there are still several important requirements, such as stable pegRNA design, accurate nick placement, temperature-tolerant or split/mini editors, and delivery strategies that are compatible with different tissues and genotypes. Using tissue- or stage-specific promoters and, when useful, replicons, expression control is essential to maintaining editor and pegRNA levels long enough to finish edits. Dicots lag behind cereals, and many genotypes in plants are still blocked by delivery and regeneration; too many studies stop at T□ instead of following inheritance. Early studies showed that single-base edits and small insertions can work, but the results often shifted depending on the plant species, the target sequence and how the pegRNA was designed. The balance between pegRNA structure, the Cas9 nickase and the reverse transcriptase still decides how well the system performs. Researchers are now trying to push efficiency higher, cut down unwanted indels and handle larger edits with better stability. Prime editing is becoming an important option for crop improvement, functional studies and broader genome engineering work. Work on temperature tuning, plant-optimized RT domains and multiplex pegRNA systems point toward a maturing tool that is steadily gaining precision, stability and practical utility.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Agriculture and Forestry University, Chitwan, Nepal for their support and academic environment that facilitated this work.

DECLARATIONS

Author contribution statement: Conceptualization: B.P; Methodology: B.P; Software and validation: B.P and C.B.B.; Formal analysis and investigation: B.P and C.B.B; Resources: B.P and C.B.B; Data curation: B.P and C.B.B; Writing—original draft preparation: B.P and C.B.B; Writing—review and editing: B.P and C.B.B; Visualization: B.P; Supervision: B.P and C.B.B.; Project administration: B.P and C.B.B.; Funding acquisition: B.P and C.B.B. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

Consent for publication: All co-authors gave their consent to publish this paper in AAES.

Data availability: The data that supports the findings of this study are available on request from the corresponding author.

Supplementary data: No supplementary data is available for the paper.

Funding statement: No external funding is received for this study.

Additional information: No additional information is available for this paper.

REFERENCES

Anzalone, A. V., Gao, X. D., Podracky, C. J., Nelson, A. T., Koblan, L. W., Raguram, A., Levy, J. M., Mercer, J. A. M., & Liu, D. R. (2022). Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. *Nature Biotechnology*, *40*(5), 731–740. https://doi.org/10.1038/s41587-021-01133-w

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, *576*(7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4

Cao, Z., Sun, W., Qiao, D., Wang, J., Li, S., Liu, X., Xin, C., Lu, Y., Gul, S. L., Wang, X., & Chen, Q. (2024). PE6c greatly enhances prime editing in transgenic rice plants. *Journal of Integrative Plant Biology*, *66*(9), 1864–1870. https://doi.org/10.1111/jipb.13738

Chauhan, V. P., Sharp, P. A., & Langer, R. (2025). Engineered prime editors with minimal genomic errors. *Nature*. https://doi.org/10.1038/s41586-025-09537-3

Chen, P. J., Hussmann, J. A., Yan, J., Knipping, F., Ravisankar, P., Chen, P.-F., Chen, C., Nelson, J. W., Newby, G. A., Sahin, M., Osborn, M. J., Weissman, J. S., Adamson, B., & Liu, D. R. (2021). Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. *Cell*, *184*(22), 5635–5652.e29. https://doi.org/10.1016/j.cell.2021.09.018

Choi, J., Chen, W., Suiter, C. C., Lee, C., Chardon, F. M., Yang, W., Leith, A., Daza, R. M., Martin, B., & Shendure, J. (2022). Precise genomic deletions using paired prime editing. *Nature Biotechnology*, *40*(2), 218–226. https://doi.org/10.1038/s41587-021-01025-z

Doman, J. L., Pandey, S., Neugebauer, M. E., An, M., Davis, J. R., Randolph, P. B., McElroy, A., Gao, X. D., Raguram, A., Richter, M. F., Everette, K. A., Banskota, S., Tian, K., Tao, Y. A., Tolar, J., Osborn, M. J., & Liu, D. R. (2023). Phage-assisted evolution and protein engineering yield compact, efficient prime editors. *Cell*, *186*(18), 3983–4002.e26. https://doi.org/10.1016/j.cell.2023.07.039

Ferreira da Silva, J., Oliveira, G. P., Arasa-Verge, E. A., Kagiou, C., Moretton, A., Timelthaler, G., Jiricny, J., & Loizou, J. I. (2022). Prime editing efficiency and fidelity are enhanced in the absence of mismatch repair. *Nature Communications*, *13*, 760. https://doi.org/10.1038/s41467-022-28442-1

Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., & Liu, D. R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, *551*(7681), 464–471. https://doi.org/10.1038/nature24644

Gupta, A., Liu, B., Chen, Q., & Yang, B. (2023). High-efficiency prime editing enables new strategies for broad-spectrum resistance to bacterial blight of rice. *Plant Biotechnology Journal*, *21*(7), 1454–1464. https://doi.org/10.1111/pbi.14049

Gupta, A., Liu, B., Raza, S., Chen, Q.-J., & Yang, B. (2024). Modularly assembled multiplex prime editors for simultaneous editing of agronomically important genes in rice. *Plant Communications*, *5*(2), 100741. https://doi.org/10.1016/j.xplc.2023.100741

Huang, Z., & Liu, G. (2023). Current advancement in the application of prime editing. *Frontiers in Bioengineering and Biotechnology*, *11*, 1039315. https://doi.org/10.3389/fbioe.2023.1039315

Jiang, Y., Chai, Y., Qiao, D., Wang, J., Xin, C., Sun, W., Cao, Z., Zhang, Y., Zhou, Y., Wang, X.-C., & Chen, Q.-J. (2022). Optimized prime editing efficiently generates glyphosate-resistant rice plants carrying homozygous TAP-IVS mutation in EPSPS. *Molecular Plant*, *15*(11), 1646–1649. https://doi.org/10.1016/j.molp.2022.09.006

Laforest, L. C., & Nadakuduti, S. S. (2022). Advances in delivery mechanisms of CRISPR gene-editing reagents in plants. *Frontiers in Genome Editing*, *4*, 830178. https://doi.org/10.3389/fgeed.2022.830178

Lee, J., Kweon, J., & Kim, Y. (2025). Emerging trends in prime editing for precision genome editing. *Experimental & Molecular Medicine*, *57*(7), 1381–1391. https://doi.org/10.1038/s12276-025-01463-8

Li, J., Li, H., Chen, J., Yan, L., & Xia, L. (2020). Toward precision genome editing in crop plants. *Molecular Plant*, *13*(6), 811–813. https://doi.org/10.1016/j.molp.2020.04.008

Li, J., Chen, L., Liang, J., Xu, R., Jiang, Y., Li, Y., Ding, J., Li, M., Qin, R., & Wei, P. (2022). Development of a highly efficient prime editor 2 system in plants. *Genome Biology*, *23*, 161. https://doi.org/10.1186/s13059-022-02730-x

Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A. V., Raguram, A., Doman, J. L., Liu, D. R., & Gao, C. (2020). Prime genome editing in rice and wheat. *Nature Biotechnology*, *38*(5), 582–585. https://doi.org/10.1038/s41587-020-0455-x

Lin, Q., Jin, S., Zong, Y., Yu, H., Zhu, Z., Liu, G., Kou, L., Wang, Y., Qiu, J.-L., Li, J., & Gao, C. (2021). High-efficiency prime editing with optimized, paired pegRNAs in plants. *Nature Biotechnology*, *39*(8), 923–927. https://doi.org/10.1038/s41587-021-00868-w

Liu, Y., Yang, G., Huang, S., Li, X., Wang, X., Li, G., Chi, T., Chen, Y., Huang, X., & Wang, X. (2021). Enhancing prime editing by Csy4-mediated processing of pegRNA. *Cell Research*, *31*(10), 1134–1136. https://doi.org/10.1038/s41422-021-00520-x

Liu, N., Zhou, L., Lin, G., Hu, Y., Jiao, Y., Wang, Y., Liu, J., Yang, S., & Yao, S. (2022). HDAC inhibitors improve CRISPR-Cas9 mediated prime editing and base editing. *Molecular Therapy – Nucleic Acids*, *29*, 36–46. https://doi.org/10.1016/j.omtn.2022.05.036

Mentani, A., Maresca, M., & Shiriaeva, A. (2025). Prime editing: Mechanistic insights and DNA repair modulation. *Cells*, *14*(4), 277. https://doi.org/10.3390/cells14040277

Mu, H., Liu, Y., Chi, Y., Wang, F., Meng, S., Zhang, Y., Wang, X., & Zhao, D. (2025). Systematic optimization of prime editing for enhanced efficiency and versatility in genome engineering across diverse cell types. *Frontiers in Cell and Developmental Biology*, *13*, 1589034. https://doi.org/10.3389/fcell.2025.1589034

Murray, J. B., Harrison, P. T., & Scholefield, J. (2025). Prime editing: Therapeutic advances and mechanistic insights. *Gene Therapy*, *32*(2), 83–92. https://doi.org/10.1038/s41434-024-00499-1

Nelson, J. W., Randolph, P. B., Shen, S. P., Everette, K. A., Chen, P. J., Anzalone, A. V., An, M., Newby, G. A., Chen, J. C., Hsu, A., & Liu, D. R. (2022). Engineered pegRNAs improve prime editing efficiency. *Nature Biotechnology*, *40*(3), 402–410. https://doi.org/10.1038/s41587-021-01039-7

Ni, P., Zhao, Y., Zhou, X., Liu, Z., Huang, Z., Ni, Z., Sun, Q., & Zong, Y. (2023). Efficient and versatile multiplex prime editing in hexaploid wheat. *Genome Biology*, *24*, 156. https://doi.org/10.1186/s13059-023-02990-1

Perroud, P.-F., Guyon-Debast, A., Casacuberta, J. M., Paul, W., Pichon, J.-P., Comeau, D., & Nogué, F. (2023). Improved prime editing allows for routine predictable gene editing in *Physcomitrium patens*. *Journal of Experimental Botany*, *74*(19), 6176–6187. https://doi.org/10.1093/jxb/erad189

Petrova, I. O., & Smirnikhina, S. A. (2023). The development, optimization and future of prime editing. *International Journal of Molecular Sciences*, *24*(23), 17045. https://doi.org/10.3390/ijms242317045

Pinello, L., Canver, M. C., Hoban, M. D., Orkin, S. H., Kohn, D. B., Bauer, D. E., & Yuan, G.-C. (2016). Analyzing CRISPR genome-editing experiments with CRISPResso. *Nature Biotechnology*, *34*(7), 695–697. https://doi.org/10.1038/nbt.3583

Ponnienselvan, K., Liu, P., Nyalile, T., Oikemus, S., Maitland, S. A., Lawson, N. D., Luban, J., & Wolfe, S. A. (2023). Reducing the inherent auto-inhibitory interaction within the pegRNA enhances prime editing efficiency. *Nucleic Acids Research*, *51*(13), 6966–6980. https://doi.org/10.1093/nar/gkad456

Shuto, Y., Nakagawa, R., Zhu, S., Hoki, M., Omura, S. N., Hirano, H., Itoh, Y., Zhang, F., & Nureki, O. (2024). Structural basis for pegRNA-guided reverse transcription by a prime editor. *Nature*, *631*(8019), 224–231. https://doi.org/10.1038/s41586-024-07497-8

Song, M., Lim, J. M., Min, S., Oh, J.-S., Kim, D. Y., Woo, J.-S., Nishimasu, H., Cho, S.-R., Yoon, S., & Kim, H. H. (2021). Generation of a more efficient prime editor 2 by addition of the Rad51 DNA-binding domain. *Nature Communications*, *12*, 5617. https://doi.org/10.1038/s41467-021-25928-2

Sousa, A. A., Hemez, C., Lei, L., Traore, S., Kulhankova, K., Newby, G. A., Doman, J. L., Oye, K., Pandey, S., Karp, P. H., McCray, P. B., & Liu, D. R. (2024). Systematic optimization of prime editing for the efficient functional correction of CFTR F508del in human airway epithelial cells. *Nature Biomedical Engineering*, *9*(1), 7–21. https://doi.org/10.1038/s41551-024-01233-3

Tian, S., Yao, L., Zhang, Y., Rao, X., & Zhu, H. (2025). Prime editing for crop improvement: A systematic review of optimization strategies and advanced applications. *Genes*, *16*(8), 965. https://doi.org/10.3390/genes16080965

Vu, T. V., Nguyen, N. T., Kim, J., Das, S., Lee, J., & Kim, J.-Y. (2022). The obstacles and potential solution clues of prime editing applications in tomato. *BioDesign Research*, *2022*, 0001. https://doi.org/10.34133/bdr.0001

Vu, T. V., Nguyen, N. T., Kim, J., Song, Y. J., Nguyen, T. H., & Kim, J.-Y. (2024). Optimized dicot prime editing enables heritable desired edits in tomato and Arabidopsis. *Nature Plants*, *10*(10), 1502–1513. https://doi.org/10.1038/s41477-024-01786-w

Xu, R., Li, J., Liu, X., Shan, T., Qin, R., & Wei, P. (2020). Development of plant prime-editing systems for precise genome editing. *Plant Communications*, *1*(3), 100043. https://doi.org/10.1016/j.xplc.2020.100043

Yu, G., Kim, H. K., Park, J., Kwak, H., Cheong, Y., Kim, D., Kim, J., Kim, J., & Kim, H. H. (2023). Prediction of efficiencies for diverse prime editing systems in multiple cell types. *Cell*, *186*(10), 2256–2272.e23. https://doi.org/10.1016/j.cell.2023.03.034

Zhang, H., Si, X., Ji, X., Fan, R., Liu, J., Chen, K., Wang, D., & Gao, C. (2018). Genome editing of upstream open reading frames enables translational control in plants. *Nature Biotechnology*, *36*(9), 894–898. https://doi.org/10.1038/nbt.4202

Zhang, G., Liu, Y., Huang, S., Qu, S., Cheng, D., Yao, Y., Ji, Q., Wang, X., Huang, X., & Liu, J. (2022). Enhancement of prime editing via xrRNA motif-joined pegRNA. *Nature Communications*, *13*, 1856. https://doi.org/10.1038/s41467-022-29507-x

Zhang, W., Petri, K., Ma, J., Lee, H., Tsai, C.-L., Joung, J. K., & Yeh, J.-R. J. (2024). Enhancing CRISPR prime editing by reducing misfolded pegRNA interactions. *eLife*, *12*, RP90948. https://doi.org/10.7554/eLife.90948

Zhao, Z., Shang, P., Mohanraju, P., & Geijsen, N. (2023). Prime editing: Advances and therapeutic applications. *Trends in Biotechnology*, *41*(8), 1000–1012. https://doi.org/10.1016/j.tibtech.2023.03.004

Zhong, Z., Fan, T., He, Y., Liu, S., Zheng, X., Xu, Y., Ren, J., Yuan, H., Xu, Z., & Zhang, Y. (2024). An improved plant prime editor for efficient generation of multiple-nucleotide variations and structural variations in rice. *Plant Communications*, *5*(9), 100976. https://doi.org/10.1016/j.xplc.2024.100976

Zou, J., Meng, X., Liu, Q., Shang, M., Wang, K., Li, J., Yu, H., & Wang, C. (2022). Improving the efficiency of prime editing with epegRNAs and high-temperature treatment in rice. *Science China Life Sciences*, *65*(11), 2328–2331. https://doi.org/10.1007/s11427-022-2147-2

Zou, J., Li, Y., Wang, K., Wang, C., & Zhuo, R. (2024). Prime editing enables precise genome modification of a *Populus* hybrid. *aBIOTECH*, *5*(4), 497–501. https://doi.org/10.1007/s42994-024-00177-1