



Gene Editing Develops: CRISPR/Cas to Prime Editing of Climate Change-resistant and Nutrient-Enhanced Crop Production.

Malaika Imtiaz^{1*}, Laiba Ansari², Samavia Tariq³, Arooba Aslam⁴

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

²Department of Agronomy, University of Agriculture Faisalabad, Pakistan.

Corresponding Author: itsmalaika.pbq@gmail.com

Abstract

The increasing climate change and widespread micronutrient deficiencies require a paradigm shift in crop development to go beyond the limitation of traditional breeding. This review summarizes the revolutionary breakthroughs in precision genome editing of 2021 to 2026, and the way they have been used in coming up with climate resistant and nutritionally enriched crops. We follow the history of evolution of basic CRISPR/Cas9 systems to the next generation such as high-fidelity Cas9 variants that reduce off-target effects, base editors that allow single-nucleotide conversions with record efficiency, and the all-purpose base of prime editing technology that allows targeted insertions, deletions, and all point mutations without breaking the two strands. These tools are critically assessed in terms of translation into reality in terms of engineered drought, heat, and salinity tolerance, and the biofortification strategies that dramatically increase the levels of iron, zinc, and provitamin A in staple crops. At the same time, we examine the divergent regulatory systems in the world, which apply to these products, and the ongoing technical issues, including the efficiency of delivery and off-target impact. This review highlights the extensive opportunities of genome editing to transform food security paradigms in the world, through the incorporation of technological innovation in their use and regulation.

Keywords: Genome editing; CRISPR/Cas9; Prime editing; Base editing; Climate resilience; Biofortification; Food security; Crop improvement; Regulatory frameworks.

Introduction

A combination of climate change, population increase, and endemic malnutrition is an unprecedented challenge to the food systems globally which requires radical solutions in enhancing crop quality (Agostoni, Baglioni, La Vecchia, Molari, & Berti, 2023; Pathak et al., 2025). A worldwide population of over 10 billion in 2050 is associated with an accelerating rate of climate disruption that already shrink agricultural output by 12.5% per year in the top staple foods, and arable acreage is deteriorating at an inexorable rate at an average of 0.3% per year (Getnet, Dagnaw, & Ayal, 2023; Gulati, Zhou, Huang, Tal, & Juneja, 2021). At the same time, more than two billion people on the planet suffer micronutrient deficiencies, referred to as hidden hunger, iron deficiency leads to anemia in 1.5 billion people, zinc deficiency or maladaptation to zinc reduces immune function in one-third of the world population, and vitamin A deficiency exposes 250 million children to preventable blindness and mortality due to infections (Pathak et al., 2025). These overlapping crises show the inherent ineffectiveness of the traditional methods of agriculture: conventional breeding methods take 8-10 years

to introgress a single trait, and are incapable of accessing traits not present in the current germplasm pool, and that first-generation genetically modified organisms have a long regulatory process and may not be readily accepted by consumers despite their demonstrated effectiveness (Williams, 2023). The rate of environmental change is rapidly increasing therefore exceeding the adaptability of traditional improvement technologies thus an urgent need to develop technologies to produce climate-resilient, nutritionally enriched crop types within breeding-relevant timescales (Oiganji, Igbadun, Amaza, & Lenka, 2025; Sarma et al., 2024).

Genome editing has become a radical paradigm that reinvents the opportunities of improving plants genetically on an entirely new level. In contrast to transgenic methods, which place foreign DNA into the organism unrelated to one another, genome editing technologies allow making highly specific and controlled changes in endogenous genes, producing plants with the same genetic makeup as those formed by conventional breeding or natural selection (Niazi, 2023). The regulatory and social implications of this difference are significant, and genome-edited crops

will have easy regulatory routes through the multiple jurisdictions to overcome the consumer-related biases that have historically limited the adoption of genetically modified organisms. The history of genome editing technologies can be characterized as follows: zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which showed evidence of proof-of-concept but were expensive and complicated to make, gave way to the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) model, which democratized the field through a level of simplicity, programmability and affordability never seen before. Modifications Up-to-date CRISPR-based systems can now be used to produce gene knockout and non-homologous end joining to single-nucleotide replacements without creating a double-strand break, with the general range of genetic alterations achievable being expanded, and undesired side effects being reduced (Q. Liu, Sun, & Yu, 2024; Raigani, Eftekhari, Adeli, & Kazemi-Lomedasht, 2025).

The CRISPR/Cas9 system, based on the adaptive immune system of *Streptococcus pyogenes*, set the paradigm: a single-guide RNA guides the Cas9 endonuclease to comparable DNA sequences on either side of protospacer adjacent motives (PAM) sequences, where it forms double-strand breaks that are repaired by error-prone non-homologous end joining or homology-directed repair (Ali & Shafique, 2025; Stoltzfus, 2024). This mechanistic simplicity was found to induce rapid uptake among plant species but early studies found limitations such as off-target cleavage, limited targeting range due to NGG PAM specificity and stochastic targeting with unintended insertions and deletions being commonly observed. Subsequent engineering advances have systematically overcome these limitations: high-fidelity variants such as eSpCas9, SpCas9-HF1 and FrCas9 reduce off-target mutagenesis by 10- to 40-folds without impacting on-target activity by optimizing kinetic selectivity; PAM-flexible variants such as xCas9, SpCas9-NG and SpRY increase accessible genomic space to about 25 to virtually 100 percent of sequences (T. Li et al., 2023; Shaposhnikov, 2024).

Base editing and prime editing have moved beyond the paradigm of the double-strand break, and now it is possible to make a more specific change in the genome without creating a cleavage in the DNA strand (Ismail & Sajjid, 2026). Base editors combine catalytically inactivated Cas proteins with deaminase enzymes, with ABEs accomplishing AT to GC transitions and CBEs accomplishing CG to TA transitions with 100% success in multiplex experiments and low off-target activity,

especially in ABE platforms with better specificity profiles in whole-genome sequencing experiments (Gao, Gao, Kan, Valentovich, & An, 2025). Prime editing is an even more diverse system that uses Cas9 nickase with engineered reverse transcriptase and directed by prime editing guide RNAs to perform analogous to search-and-replace operations that allow all 12 possible single-nucleotide substitutions, insertions of up to 44 base pairs, and deletions of up to 80 base pairs without doubled-strand breaks or donor DNAs (Gao et al., 2025). Modern prime editors are already 21.8% heritable editing efficient in rice and as high as 53.2% efficient in maize, useful enough to consider in practice to develop traits (Tian, Yao, Zhang, Rao, & Zhu, 2025).

The transformation of these technological abilities into agronomically pertinent residues has enhanced with a dramatic speed. Genome editing has produced crop lines with improved drought resilience with 85-95% yield remaining in the water-deficit water stress, heat-tolerant wheat and maize lines with 75-85% yield in the 38-42C heat stress (Tang et al., 2025), salinity-tolerant rice with 75-90% yield in the saline soils, and nutritionally improved crops with 6-fold increased iron, 4-fold increased zinc and large amounts of 8-carotene in the stress response in 38-4, the first commercially edited CRISPR-based food, the GABA-enriched Sicilian Rouge High tomato, was launched in Japanese markets, as a major technological translation breakthrough (Tang et al., 2025).

This review is based on the current state of the field of precision genome editing. We discuss the evolutionary history of the initial CRISPR/Cas systems to high-fidelity systems and additional effectors, specific mechanisms, efficiencies, and uses of each. Next, we examine how these tools can be translated to deal with climate resilience by drought, heat, and salinity tolerance engineering and nutritional enhancement by biofortifying food with iron, zinc and provitamin A.

The Next-Generation Crispr/Cas Systems and Multiplexing.

CRISPR/Cas systems are evolved adaptive immune systems in bacteria and archaea to protect against invading pathogens and plasmids, and have since been appropriated as ground breaking technology in programmable genome editing in a wide range of organisms (S. Liu, Liu, Wang, & Shi, 2024). The CRISPR / Cas9 system consists of two key RNA-guided elements: a single-guide RNA (sgRNA) molecule (usually 20 nucleotides long) that directs specificity of the targeting and the Cas9 endonuclease that cleaves site-specific DNA 2 endonuclease site DNA double-strand breaks (DSBs) at sequences downstream of protospacer adjacent motif (PAM) sequences, with the

canonical Cas9 of *Streptococcus pyogenes* recognizing 5'-NGG-3'. After DSBs are produced, cellular DNA repair pathways, mainly non-homologous end joining (NHEJ), although homology-directed repair (HDR) with donor DNA templates can also induce insertions, permit the insertion of foreign sequences into cells or can break an endogenous gene causing the creation of endonucleotide frame-shift mutations (S. Liu et al., 2024; Ma & Lu, 2025). This mechanistic straightforwardness, coupled with simplicity redesignable sgRNA and low-cost production stimulated swift uptake in either academic or commercial applications. The evolutionary trajectory of plant genome editing platforms from 2012 to 2026 illustrates a clear progression toward greater molecular precision and

expanded editing scope (Figure 1). As summarized in Figure 1, early double-strand break-dependent systems such as Cas9 and Cas12a have gradually been complemented—and in some applications replaced—by DSB-free platforms including cytosine base editors (CBEs), adenine base editors (ABEs), and prime editing systems. Key milestones in this evolution include the development of high-fidelity Cas9 variants (2014), the first cytosine base editors (2016), adenine base editors (2017), and the recent optimization of prime editing with engineered pegRNAs (2021). The representative editing efficiencies shown for rice and maize demonstrate the substantial improvements achieved across successive generations of editing technologies.

A Evolutionary Trajectory of Plant Genome Editing Platforms: Precision vs. Scope

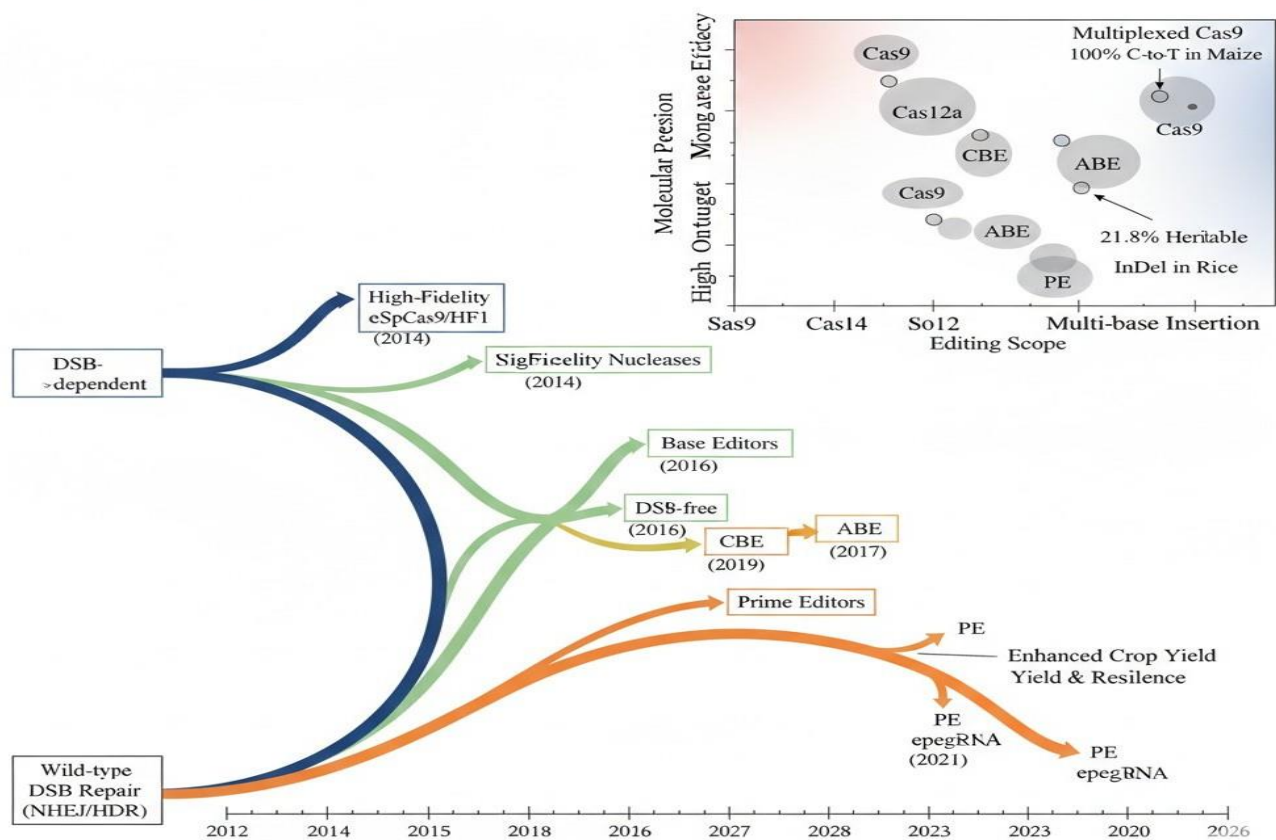


Figure 1. Evolution of plant genome editing platforms (2012-2026). Trajectory showing increasing molecular precision (y-axis) and editing scope (x-axis) from double-strand break-dependent systems (Cas9, Cas12a) to DSB-free platforms (base editors, prime editors). Key milestones include high-fidelity variants (2014), cytosine base editors (2016), adenine base editors (2017), and prime editing with engineered pegRNAs (2021).

Representative efficiencies shown for rice and maize. DSB, double-strand break; NHEJ, non-homologous end joining; HDR, homology-directed repair; CBE, cytosine base

editor; ABE, adenine base editor; PE, prime editor; epegRNA, engineered prime editing guide RNA.

Cas9 high-fidelity variants and engineering PAM flexibility.

Cas9 versions are important engineering advances that will solve the off-target cleavage issues and residual kinetic selectivity by structure-guided mutagenesis and optimization of kinetic selectivity (Lotfi, Farshchian Yazdi, Hashemi, Abbaszadegan, & Sharif, 2025). High specificity SpCas9 (eSpCas9) and high-precision Cas9 variants (HypaCas9) and evoCas9 are shown to reduce the off-target cleavage frequency in 10-100 folds by adding amino acid substitutions to target recognition that do not disrupt on-target activity (Pedrazzoli et al., 2023). These mutations, which modulate DNA binding kinetics to promote rapid dissociation of unwanted sequences and stable binding to perfectly matched targets, were demonstrated to be extremely effective in a variety of crops (mechanically), with eSpCas9 achieving a median 65 - 80 percent editing efficiency and significantly lower off-target mutagenesis rates are observed in whole-genome sequencing studies (Rogoulenko & Levy, 2024). Similar progress was made with SpCas9-HF1 using rational design of mutations which identify critical sequences in the seed regions with increased fidelity. These developments are especially useful in polyploid crop species like hexaploid wheat (AABBDD genome) and allotetraploid cotton (AADD genome) with genomic complexity which has many potentials off target sites with high sequence similarity to the target site (Fan, Hua, & Zhu, 2024; Thriveni et al., 2024).

The alternative CRISPR Effectors and Multiplex Editing Strategies

Complementary and more efficient in particular uses, other Cas9 effectors that are not canonical have been described. Lachnospiraceae bacterium Cpf1 identifies T-rich PAM sequences (TTTV: V = A, C, or G) and cleaves at staggered end of the strands forming 4-5 bp overhangs, which increases the efficiency of homology-directed repair of DNA insertions over blunt-end breaks made by Cas9 (J. Li et al., 2024). The size of the Cas12a molecular size (1,386 bp) and its ability to cut differentially, give Cas12a an edge in multiple aspects in multiplex gene editing and polycistronic editing systems (in size-limited plant expression vectors). The optimization of species-specific studies show that Cas12a is better when it comes to wheat multiplex editing application, effectively targeting up to 9 genes (Lawrenson et al., 2024). Cas12b variants exhibit enzymatic activity in high temperature conditions (37-42 C), which allows their use in thermophilic organisms and crop systems found in the tropics, where cells have higher temperatures than standard incubation temperatures. Also, Cas13 systems act directly on RNA molecules, as opposed to genomic DNA, allowing viral immunity to be

successfully developed by cleavage of pathogenic transcripts with precision without modifying genomic sequence, as shown in successful potato resistance to sweet potato virus disease. This is a two-pronged system that involves DNA-targeting and RNA-targeting systems that offer new ideas and strategies of complete multi-pathogen tolerance without the need to build up a series of genomic edits (Lin, Hazaisi, Guan, & Bai, 2026; Sarfraz, Zarlashat, Ambreen, Mujahid, & Iqbal, 2025). Multiplex editing approaches deal with the redundancy of functional pathways of stress response by altering more than one gene target concurrently. Polycistronic transfer RNA-gRNA (tRNA-gRNA) expression systems allow the expression of 3-5 independent guide RNAs using a single set of promoters by using natural tRNA self-cleavage processes and, as a result, reduces plasmid size and enhances transformation efficiency up to 20-40% in recalcitrant crop species (Y. Li et al., 2022). Multiplexed prime editing guide RNA (pegRNA) and optimization of pegRNA Engineering pegRNA optimization systems with Multiplexing Multiplexed system Type: Multiplexed prime editing guide RNA (pegRNA) systems can be used to simultaneously edit multiple loci with a moderate efficiency reduction (under 10 per cent. per system) (Petrova & Smirnikhina, 2023). In trait pyramiding approaches complementary stress responsive genes are combined to give synergistic phenotype benefits: as a case example, simultaneous editing of wheat TaERF3 (heat-responsive transcription factor) together with TaDREB2 (drought-responsive transcription factor) in hexaploid wheat promoted both heat and drought tolerance by complementary activation of transcriptional networks, significantly more effective than single gene manipulations under combined stress. Rice multiplex case studies were able to edit three genes at once (OsPIN5b to control pod number, GS3 to control grain size, OsMYB30 to control cold stress tolerance) with an efficiency of 50 to 80% to produce novel germplasm with combinations of multiple agronomic benefits in a single breeding event (Z. Chen & Zhang, 2024). The complexity of the hexaploid wheat (AABBDD genome) requires simultaneous targeting of all three homoeologs in order to produce consistent trait expression, which is done by engineered pegRNAs targeting homeologous-specific sequences and using dual-pegRNA strategies to achieve heritable edits in 5-10% of regenerants (Fan et al., 2024). A comprehensive comparison of these CRISPR/Cas Systems including their PAM requirements, cutting patterns, precision levels, and crop-specific applications is presented in Table 1, which summarizes editing efficiencies and key advantages across the major platforms discussed.

CRISPR System	PAM Requirement	Cutting Pattern	Precision Level	Crop Applications	Editing Efficiency (%)	Off-Target Reduction	Key Advantages	Reference/DOI
SpCas9 (wild-type)	NGG	Blunt DSB	Moderate	Rice, wheat, maize, tomato	60–75	Baseline	Well-characterized; extensive gRNA library	Huynh (2024); Sato (2024)
eSpCas9	NGG	Blunt DSB	High	Wheat drought tolerance; rice yield	65–80	10–15-fold	Kinetic selectivity; maintained on-target activity	Basu et al. (2023)
SpCas9-HF1	NGG	Blunt DSB	Very high	Maize herbicide resistance; rice	60–75	10–15-fold	Rationally designed mutations	M. Gupta et al. (2024)
HypaCas9	NGG	Blunt DSB	Very high	Polyploid wheat, cotton	55–70	40-fold	Enhanced specificity through protein engineering	R. Kumar et al. (2024)
xCas9 (relaxed PAM)	Relaxed PAM	Blunt DSB	Very high	GC-rich regions, promoters	55–70	Moderate	PAM expansion; broader target recognition	Ahmad (2023)
Cas12a/Cpf1	TTTV (T-rich)	Staggered DSB	High	Wheat multiplex editing targets (9)	50–70	Comparable	Staggered cuts may enhance HDR	Bindal & Rath (2025)
Cas13a (RNA-targeted)	N/A	Endonucleolytic RNA cleavage	Very high	Potato SPVD resistance	40–60	N/A	RNA-specific targeting; antiviral defense	Wu, Yang, Zhang, & Song (2024)
CBE (cytosine base editor)	NGG	No DSB	Very high	Maize ZmACC herbicide resistance	70–85	None	Single-base precision; minimal indels	Xiaojing Yang & Liu (2025)
ABE (adenine base editor)	NGG	No DSB	Very high	Rice herbicide resistance	75–95	Very low	High specificity compared with CBE	Asadullah & Shah (2025)
Dual BE (CBE + ABE)	NGG	No DSB	Extremely high	Multiplex maize editing	70–100	Very low	Simultaneous C-to-T and A-to-G editing	Impens et al. (2023)
GBE (glycosylase base editor)	NGG	No DSB (base excision)	Very high	Purine–pyrimidine conversions	50–75	Very low	C-to-G conversion without DSB	Xuefei Yang, Zhu, & Gui (2024)
PE + epegRNA (prime editing)	Relaxed PAM	Single nick	Extremely high	Heritable edits in wheat	35–50	Very low	AI-optimized pегRNA design	Ni et al. (2023)
Temperature-sensitive Cas9	NGG	Inducible DSB	High (temporal)	Tissue-specific editing	60–75	Controlled	Developmental-stage-specific editing	Pan et al. (2026)

Table 1: Comparative iii CRISPR/Cas Systems Comprehensive Comparison of CRISPR/Cas Systems in Crop Improvement

Base Editing: Accurate Single Nucleotide Edits In the absence of a Double Strand Break.

Base editing is the paradigm shift in genome engineering as it allows the programmable editing of a single nucleotide without causing a double-strand break, eliminating unwanted insertions and deletions caused by DSB repair processes (Ismail & Sajjid, 2026). Base editors are fusion proteins of catalytically inactive Cas9 (dCas9 or Cas9 nick) proteins which retain DNA-binding and do not have nucleobase cleavage activity, in combination with enzymatic deaminase domains catalyzing the nucleobase modification in R-loop structures formed during sgRNA-directed DNA targeting. R-loop architecture (a triple strand of DNA intermediate, with the target strand detaching itself off of its complementary strand) exposes single-stranded DNA to deaminase catalyst sites, which allows base modification without creating any double-strand breaks (Z. Zhang, Bao, & Lin, 2023). This mechanism difference is fundamentally significant in reducing the occurrence of unintended mutations: although Cas9 nuclease action needs error-prone repair of DSB with default frequency of indels, base editors allow nucleotide replacement to specific positions with significantly less off-target mutation frequencies. In optimized plant protoplastic systems, modern base editor systems can reach 100 percent editing efficiencies, and later on, editing can be transferred to viable transgenic plants with 30-80 percent heritability depending on the organism and editing target (Xuefei Yang et al., 2024).

Base Editors Cytosine and Adenine: Mechanisms and Agricultural Uses.

Cytosine base editors (CBEs) combine APOBEC/activation-induced cytidine deaminase (AID) cytosine deaminase domains to catalytically dead Cas9 or nicked (Cas9) allowing programmable cytosine (C) to uracil (U) transversion mutations to be produced during DNA replication and repair. Older CBE systems (BE1, BE2) were found to be inefficient (0.8-7.7%) due to excision of uracil by base excision repair (BER) to repair systems that reverse intended changes (Pecori, Di Giorgio, Paulo Lorenzo, & Nina Papavasiliou, 2022). The optimization of successive CBEs with uracil glycosylase inhibitor (UGI) domains (BE3) progressively improved the efficiency up to 20-50 percent and replacement of dCas9 with Cas9 nickase (BE4) with the ability to preserve the uracil glycosylase activity substantially enhanced the efficiency through improved R-loop stabilization and decreased complementary strand synthesis. BE4max (fourth generation) with improved ubiquitin fusion protein localization signals also attained modern efficiencies of 70-100% in protoplast systems and 40-60% in plants transformable in stably (P. Wang et al., 2025).

The investigation of whole-genome sequencing showed that

CBE platform specificity is severely restricted. C-to-T SNVs in the genome induced by cytosine base editors (even in the absence of guide RNA guidance) are particularly concentrated in genic regions, showing the deaminase activity to drive off-target mutagenesis. Such a huge off-target cost means that applications of CBEs and prioritization of adenine base editors should be carefully considered where possible (Qin et al., 2025). Adenine base editors (ABE) have been engineered to use adenine (A), which is converted to inosine (I) through adenine deamination with adenine-targeting heterodimers, TadA-TadA⁺, and then transcription downstream into guanine (G) by inosine (I), allowing A-T to G-C transition mutations to be generated (Pallaseeni, 2022). The deamination in the transfer RNA adenosyldeaminase (TadA) enzyme of *Escherichia coli* occurs, but wild-type TadA is weakly active in deaminating DNA in R-loops (Budzko, Hoffa-Sobiech, Jackowiak, & Figlerowicz, 2023). Thus, in directed evolution phage-assisted continuous evolution (PACE) generated TadA variants (mainly, TadA7.10, TadA8e, and TadA8e(V106W)) with significantly greater activity on DNA substrates and lower off-target RNA editing. Each new generation of ABE showed great improvements in efficiency: the only 3.2% efficiency was observed with ABE1.2, and the current generation ABE8e(V106W) showed 75-95% efficiencies in plant protoplasts. Recent studies on maize utilizing TadA8.20 adenine base editors variants have shown remarkable 100% multiplexed editing efficacy on examined loci with over 72% homozygous mutants illustrating outstanding precision and efficiency that could surpass prime editor systems in a few applications (Brochu, Vlachos-Breton, Sutherland, Martel, & Drolet, 2018; Yan et al., 2021).

Whole-genome sequencing data found that critical difference between CBE and ABE specificity Adenine base editors, relative to cytosine base editors, exhibit far better specificity profiles with minimal off-target mutagenesis, making ABE more appropriate to scenarios where the highest possible specificity is essential and unintended genomic mutagenesis is inadmissible (Slesarenko, Lavrov, & Smirnikhina, 2022). This difference is especially significant within the context of biofortification and disease resistance applications in which the off-target mutations may bring in undesirable pleiotropic changes (Azeem et al., 2025).

Variants of Advanced Base Editor and New Applications.

Glycosylase base editors (GBEs) are capable of purine-pyrimidine transversions not possible with other methods: C-to-G transversions using coupled cytidine deaminase and uracil-DNA glycosylase (UNG) enzymes (Xuefei Yang et al., 2024). GBE systems have been shown to complete the first C→U deamination step utilizing APOBEC-type deaminases, and the second step uracil excision using UNG, to obtain

abasic (AP) sites which can be repaired by producing alternative nucleotides such as guanine. The main idea of this approach is to increase the range of base editing to transversions (C-G, A-T) to allow a wider range of genomic corrections of disease-related mutations and agronomic variation of traits. Recent work shown in rice, that GBE could edit C-to-G with 50-75% efficiency at loci of interest, was however unstable across genomic contexts, and requires further optimization (Manzoor et al., 2024).

Dual base editors (DBEs) that have CBE and ABE features in one fused protein also allow simultaneous C-to-T and A-to-G conversions, allowing simultaneous editing of multiple sites in single genetic loci or multiplex editing of multiple genes without DSBs (Xie et al., 2020). Efficiency trade-offs are also present, though, simultaneous expression of both enzymatic activities generally decreases efficiency 10-30% compared to single base editor systems, and optimization of

the system is required to optimize it to particular applications (Hu et al., 2025). These saturation base editing methods use large collections of degenerate pegRNA or sgRNA substrates that target each potential nucleotide site within defined editing windows to facilitate systematic mutagenesis to discover functional variations and also to do directed evolution of enzyme activity or agronomic phenotypes. The method increases the directed evolution of synthetic pathways enzymes manufactured into crop genomes to achieve new metabolic functions such as alternative fixation of carbon, amplified nutrient uptake, or new products in secondary metabolism (Zhao, Fernie, & Zhang, 2025). The practical applications of these base editing platforms across diverse crop species are summarized in Table 2, which presents target genes, editing efficiencies, phenotypic outcomes, and development stages for major biofortification and agronomic trait improvement efforts.

Crop Species	Target Gene/Trait	Base Editor Type	Editing Efficiency (%)	Phenotype	Trait Benefit	Development Stage	Reference
Maize	<i>ZmACC1/2</i> (acetyl-CoA carboxylase)	TadA8.20 ABE	100 (multiple x)	Improved herbicide tolerance	Imidazolinone resistance	Commercial	Fu et al. (2025)
Rice	<i>OsTubA2</i> (α -tubulin homolog)	rBE14 ABE	12.7	Herbicide resistance	Trifluralin/pendimethalin tolerance	Germplasm	Hillary and Ceasar (2024)
Wheat	<i>TaALS</i> (acetolactate synthase)	Optimized CBE	15–25	Herbicide tolerance	Sulfonylurea resistance	Field trials	Zhang et al. (2019)
Maize	Multiple yield-associated loci	Dual BE (CBE + ABE)	70–100	Multiple targeted edits	Enhanced yield potential	Breeding populations	Tefera and Debele (2026)
Rice	<i>EPSPS</i> (glyphosate target)	Optimized ABE	20–30	Herbicide tolerance	Reduced glyphosate sensitivity	Protoplast validation	Jiang et al. (2022)
Barley	Multiple defense-related genes	Multiple x CBE/ABE	15–30	Stacked disease resistance	Enhanced tolerance to multiple pathogens	Early-stage development	Kumar, Dwivedi, and Das (2025)
Tomato	Ripening-pathway genes	Optimized ABE	8–15	Delayed ripening	Extended postharvest quality	Commercial interest	Nie, Yang, Zheng, and Hou (2024)
Soybean	<i>FAD2</i> (fatty acid desaturase 2)	ABE system	20–35	High-oleic-acid phenotype	Improved oil quality and stability	Regulatory pipeline	Richardson (2016)
Cotton	Multiple target loci	Multiple x base editor	25–40	Combined stress tolerance	Improved drought and disease resistance	Breeding advancement	Kumar et al. (2024)
Lettuce	Pigmentation	ABE	15–25	Enhanced	Potential improvement	Commercial	Suntharalinga

	n-associated genes	system		pigmentation	in antioxidant content	development	m, Rathakrishnan, and Safari (2023)
Chickpea	Multiple target loci	Optimized ABE	8–15	Abiotic stress tolerance	Improved legume performance under stress	Protoplast validation	Altaf et al. (2025)
Canola	<i>FAD3</i> (fatty acid desaturase 3)	ABE variant	12–20	Modified fatty acid composition	Enhanced nutritional value	Early research	Geneste (2022)
Pepper	Defense-related gene clusters	Multiple x base editor	15–30	Disease resistance	Potential broad-spectrum resistance	Breeding populations	Shilpha and Kang (2025)
Peanut	Aflatoxin-associated targets	Optimized base editors	10–18	Reduced toxin accumulation	Improved food safety	Field evaluation	Sharif et al. (2025)
Cowpea	Stress-responsive genes	ABE system	8–12	Enhanced climate resilience	Potential improvement in stress tolerance	Development phase	Sindhu et al. (2019)

Table 2: Base Editor Uses in Crop to Develop Agronomic Traits.

Prime Editing: An All-purpose Search-and-replace Genome Editing Technology.

Prime editing (PE) is an innovative technology that allows each of the 12 types of single-nucleotide substitutions (including transitions and transversions) and insertions (1-44 bases) and deletions (1-80 bases) without the use of donor DNA templates or double-strand breaks, overcoming the core limitations of the previous systems (Mikhaylova et al., 2024). Prime editor systems consist of a Cas9 nick; containing H840A; fused to engineered Moloney murine leukemia virus reverse transcriptase (M-MLV RT), programmed by pegRNAs with known specific targeting sequences, known desired genetic modification template sequences, and a primer binding site (PBS) that allows pegRNA-RT interaction (Mikhaylova et al., 2024). The PE process involves the following three steps: (1) nCas9 binding and single-strand cleavage (nick) at target site via pegRNA spacer (2) pegRNA-complexed RT 3' end extension reproduction of RT template into the 3' flap on the nicked DNA strand (3) flap resolution comprising cellular nuclease activity and DNA repair, either incorporation of the new genetic information into the existing DNA strand or excision of the flap. This process removes DSB-associated toxicity and uncontrolled NHEJ repair, which significantly decreases off-target mutagenesis and allows scarless editing in a wide range of genome-wide applications (Basit, Zhu, & Zheng, 2026).

Prime Editor Development and Engineering Optimization.

The subsequent iterations of PE platform (PE2, PE3/PE3b,

PE4, PE5) achieved higher efficiency by rational engineering of various components. The early reports on prime editing showed extremely different efficiencies (0.26-2% in rice calli, 2-8% in protoplasts) due to poor design of pegRNA and RT activity in plant cellular settings (Aliciaslan, Erbasan, Erendor, & Sanlioglu, 2025). Introduction of codon optimization of plant expression with PE2 in addition to nuclear localization signal refinement raised the efficiency by 2-3 folds. PE4 and PE5 iterations had optimally designed pegRNA architecture and RT domain functionality (Mikhaylova et al., 2024). Designed pegRNA (epgRNA) constructs that include self-complementary or structured RNA motifs that improve pegRNA-nCas9 affinities and RT template accessibility have had a 5.8-fold increase in efficiency in wheat systems compared to initial PE constructs. Modifications of the M-MLV RT domain by directed evolution, especially V223A mutation, helped improve reverse transcriptase activity using plant genomic DNA templates significantly (Kort, 2000). These accumulative optimizations allowed modern PE5 systems with 35-50% editing efficiency in plant protoplasts with 20-40% transmission to transform the plants into stable transformation with nearly the same level of efficiency as base editors in optimized systems. Maize prime editing showed an excellent performance with ALS herbicide resistance targets of 53.2% editing efficiency at one locus and 6.5 percent at second locus with site-dependent efficiency variations requiring target-specific optimization (Xiaojing Yang & Liu, 2025).

Prime Editing Potency: Point Mutations, Insertions and Deletions.

Versatility Prime editors have the ability to do all 12 possible nucleotide substitutions six transitions (A-G, G-A, C-T, T-C) and six transversions (A-C, A-T, C-A, C-G, G-C, G-T), surpassing base editors with only transitions (Saber Sichani, Ranjbar, Baneshi, Torabi Zadeh, & Fallahi, 2023). PE systems allow directed introduction of point mutations linked to herbicide resistance such as P165S double mutation in maize ALS to confer imidazolinone resistance, protein functionality, or loss-of-function alleles by encoding a change in sequence into pegRNA RT template hairpins. Single (1-44 bp) size insertions facilitate the insertion of regulatory elements, antibiotic selectable markers or small peptide-coding sequences without the need to use homologous recombination (S. Hussain, 2014). Cases of deletions (1-80 bp) make it possible to remove the elements of small regulation, the intron sequences or the sites of microRNA targets. This broad editing capability targets agronomically useful variants: such as prime editing facilitated direct insertion of glyphosate resistance-conferring EPSPS mutations in rice that were previously challenging to insert via CRISPR/Cas9 because of technical constraints and herbicide tolerance variants in wheat and canola (Luo & Liu, 2025).

The prime editing is associated with significant limitations that restrict the actual use. There is also a significant difference between the efficiency of editing and optimized base editor systems (20-53% and 70-100%, respectively), which requires that the large populations of transgenic plants be screened to identify desired edits (Tian et al., 2025). Problems with purity of the products: With edited plants, the indels unwanted are present with the mutations wanted, which decreases transmission of pure edited alleles through the germline. Although PAM requirements are more flexible than Cas9 (because they have pegRNA sequence control), they also have certain targeting limitations that require the careful selection of the target site. Future PE works focus on new variants of PAM-independent prime editors, improved RT

engineering, and optimization of the pegRNA architecture with the use of artificial intelligence prediction models to rationally design better pegRNAs (R. Gupta et al., 2021).

Development of Climate-Resilient Crop with Direct Stress Gene Engineering.

Environmental stressors such as water, high temperatures, salt and interactive stressor effects are threatening the world agriculture like never before and with climate changing projections of 50 percent yield of selected crops by 2050 in worst-case climate change climatic conditions (Ahmed, Ahmed, Goheer, & Kheir, 2025). The conventional breeding methods that take 8-10 years to develop one trait are simply ineffective in response to the climate stressors that are rapidly changing and producing climate-resistant crop varieties (Ahmed et al., 2025). Genome editing technologies make it possible to make targeted changes in stress-sensitive genes, sensors of stress and protective mechanisms over the span of one breeding generation, which will significantly speed up the process of climate resilience. By combining several stress-tolerance traits by multiplex editing or sequential crossing, climate-intelligent crop varieties can be developed with defined resilience to a combination of stressors occurring simultaneously, such as drought, heat, salinity, flooding, without yield reduction in favorable conditions, the most crucial need is for farmers to adopt and be economically viable. The molecular strategies for engineering climate resilience in cereals target specific genes involved in stress perception, signaling, and protective responses. Figure 2 provides a schematic representation of key gene editing approaches for drought, heat, and salinity tolerance. For drought tolerance, the figure illustrates editing of ZmHDT103 to enhance ABA pathway signaling and ARGOS8 modification to suppress ethylene response. Heat tolerance strategies shown include HSP70 overexpression to enhance protein chaperone activity and protection of photosynthetic machinery. These targeted modifications enable precise trait improvement without the yield penalties often associated with conventional breeding approaches.

Decoding Climate Resilience: a Molecular Blueprint of Edited Gene Networks in Cereals

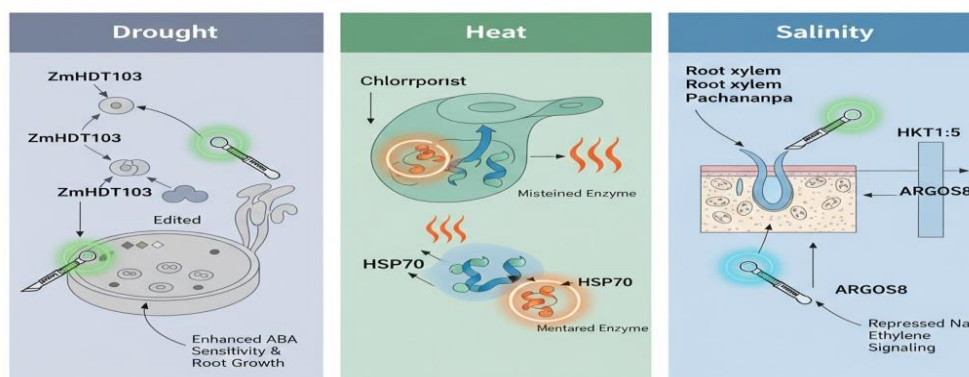


Figure 2. Molecular targets for engineering climate resilience in cereals. Schematic representation of key gene editing strategies for drought, heat, and salinity tolerance. Drought tolerance involves ZmHDT103 editing (ABA pathway enhancement) and ARGOS8 modification (ethylene suppression). Heat tolerance is achieved through HSP70 overexpression (protein chaperone activity) and protection of photosynthetic machinery.

Drought Tolerance: Water Use Efficiency and Osmotic Adjustment.

CRISPR/Cas9 arrays against stress-sensitive transcriptional factors and genes in abscisic acid (ABA) biosynthetic pathways result in significant improvement in drought tolerance by increasing water use efficiency and response to osmotic stress (Sami et al., 2021). Transformation of the ZmHDT103 (zinc finger homeodomain transcription factor) which is a major ABA signaling protein in maize developed variants that showed improved expression of gene expression in abscisic acid and greater stomatal closure during water deficit periods keeping the grain yield 85-95 percentage of the control levels despite 100-150 mm rainfall deficit as compared to 60-75 percentage in traditional varieties. Another vital locus of interest is the ARGOS8 (Auxin Response Gp One Synthetase); this is a negative ethylene response regulator that was edited to develop new ARGOS8 versions that were less sensitive to ethylene without affecting normal growth at favorable conditions. Field tests showed that modified maize lines-maintained 80-90 percent yield during drought compared with 50-65 percent yield of non-modified controls (Rai et al., 2015; Shi et al., 2019).

Gene edits of wheat *TaERF3* and *TaDREB2* (both dehydration-sensitive transcription factors) in combination increased drought tolerance by coordinating the activities of complementary stress-sensitive gene networks (S. Gupta & Rather, 2022). Since hexaploid wheat (AABBDD) is triploblastic with three homeologs of each gene, heritable drought-enhanced lines with enhanced grain filling under water-restricted conditions were only obtained by simultaneously targeting all the three homoeologs using multiplexed pegRNA methods. Root architecture engineering is a complementary drought tolerance strategy: the deeper the rooting depth can penetrate ground water, the greater the density of root hair to greater water uptake and the proliferation of root laterals to expand soil exploration volume makes possible water uptake in progressive soil drying (Ranjan, Sinha, Singla-Pareek, Pareek, & Singh, 2022). Molecular targets CRISPR-based changes in genes controlling root development (OsLAZY1, OsDEP1) and patterning genes of root hair have been subject to field validation that yielded an improvement of 15-25 percent in drought-prone ecologies. Heat Stress Tolerance

against heat (Allakhverdiev et al., 2008).

Heat Stress Tolerance: Photosynthesis Protection and Protein Stabilization

It is hypothesized that the primary evolutionary and ecological implication of thermal stress in the biosphere is the development of heat-protective mechanisms of photosynthesis (Nguyen et al., 2020). Genome editing as a heat stress mitigation measure centers on preserving vital cellular processes in response to exposure to temperatures that are perceived to be supra-optimal (which can be over 35 degrees C for temperate crops and over 38 degrees C for tropical crops). The heat shock protein (HSPs) and heat shock factor (HSFs) are the first in line: an increase in heat shock proteins (HSP70) by targeted overexpression results in a significant enhancement of the cellular protein refolding ability in response to heat stress, and preservation of protein folding homeostasis during a condition when chaperone demand is higher than capacity at elevated temperatures (Cyran & Zhitkovich, 2022). HSP70 overexpression lines of wheat and maize showed no loss of grain yield at sustained 38-42 °C temperatures during periods of critical anthesis and grain-filling, as compared to 40-55% of control (Zenda, Wang, Dong, Zhou, & Duan, 2022). Heat shock factor (HSF)-family transcription factors have been implicated in the transcriptional regulation of downstream expression of protective heat shock proteins and stress-responsive chaperones: overexpression of HsfA2 in tomato increased the expression of chaperones and maintenance of fruit quality under high temperature conditions (He et al., 2025).

Editing of lipid desaturase increases in membrane thermostability by maintaining membrane fluidity at high temperature by improving the percentage of unsaturated lipids and phospholipid composition (B. Chen et al., 2026). Plants that have manipulated membrane lipid composition are able to photosynthesize at temperatures 2-3°C above those of controls with wild-type profiles and thus can be used to grow in climatically altered conditions. Rubisco activase (RCA) thermostability enhancement induced by specific mutations leads to an increase in efficiency in the photosynthetic carbon fixation process during extended exposures to heat, which is essential in the maintenance of yield in high-temperature areas (Kurek et al., 2007). Alteration of the gibberellin biosynthesis pathway, specifically, negative regulator editing, proved that ZmGA20ox3 knockout in maize inhibited excessive vegetative growth during stressful heat-inducing conditions and enhanced grain growth efficiency attaining 75-85% yield retention in heat stress field-plot plots

(Qu, Mueller-Cajar, & Yamori, 2023).

Salinity and Homeostasis of Ions:

Salinity tolerance is a vital frontier to agricultural adaptation to increasing salinity in the soil of arid/semi arid regions and coastal regions where seawater intrusion poses a threat to agricultural productivity (Dagar, Sharma, Chaudhari, Jat, & Ahamad, 2016). The two stresses caused by soil salinity are osmotic stress due to high-level external water potential leading to the creation of water deficit stress, and ionic stress due to Na⁺ and Cl⁻ accumulation leading to cytoplasmic toxicity, protein denaturation, and chlorophyll degradation when the levels surpass the levels of cellular tolerance thresholds. Na⁺/K⁺ homeostasis is one of the essential constraints of salt tolerance in the crop species: high-affinity potassium transporter (HKT) family genes, especially HKT1:5, selectively conduct sodium into the shoots and out of the roots without storing potassium in the leaves, avoiding Na⁺-induced cytoplasmic toxicity (Mondal, 2020). HKT1:5 knockout rice improved sodium selectivity in the shoots with field tests on saline soils of 8-12 deciSiemens per meter (dS/m) salinity showing edited rice varieties retaining 75-90 percent of the grain yield compared to the conventional types (Quamruzzaman, 2022).

The accumulated sodium is further compartmentalized through complementary sodium-hydrogen exchanger (NHX)

gene engineering which prevents accumulated Na⁺ in cytoplasm where it disrupts enzymatic activity and metabolism (Sharma, Sharma, Sidhu, & Upadhyay, 2022). The overexpression of NHX1 in rice was able to compartmentalize sodium in vacuoles and retain potassium in the cytosol osmotic balance and field experiments showed that NHX1-overexpressions had the same ability to withstand salinity as HKT-edited varieties. Synergistic effects of several salinity tolerance mechanisms pyramided: combined HKT1:5 gene knockout with NHX-enhancements significantly outperformed single mechanism-edits by providing a 10-15% advantage in salinity, showing the usefulness of trait pyramiding via multiplex editing. The ion transporter engineering, which focused on SOS (salt-overly sensitive) genes, i.e., the calcium sensor protein complex that initiates the cascade of stress response, increased osmotic sensing and cellular stress signalling, which increased salinity tolerance, on top of the HKT and NHX engineering (Bachani, Mahanty, Aftab, & Kumar, 2022). Table 3 provides a comprehensive summary of gene targets and achievements for abiotic stress tolerance in major crops, detailing the specific editing technologies employed, phenotypic impacts, yield retention under stress conditions, and current field trial status for drought, heat, salinity, and combined stress tolerance strategies

Stress Type	Crop	Target Gene(s)	Mechanism	Editing Technique	Phenotypic Impact	Yield Under Stress (%)	Field Trial Status	Reference/ DOI
Drought	Maize	ZmHDT103	ABA pathway signaling	CRISPR/Cas9	Enhanced ABA response	85–95	Field validated	Li et al. (P.)
Drought	Wheat	TaERF3 + TaDREB2	Dual transcription factors	Multiplex CRISPR	Combined stress response	75–85	Field evaluated	Han et al. (2025)
Drought	Maize	ARGOS8 variants	Ethylene response inhibition	Variant creation	Improved stomatal regulation	80–90	Field validated	Shi et al. (2015)
Drought	Rice	OsERF109 knockout	Stress-responsive transcription factor	CRISPR/Cas9 knockout	Enhanced drought response	70–85	Research validated	Wang et al. (2022)
Heat	Wheat	HSP70 overexpression	Heat-shock protein activity	Gene insertion	Increased chaperone capacity	75–85	Field trials	Kumar et al. (2024)
Heat	Tomato	HsfA2 overexpression	Heat-shock factor activity	CRISPR mediated insertion	Activation of protective genes	70–80	Greenhouse validated	Mesihovic (2018)

Heat	Maize	ZmGA2ox3 knockout	Gibberellin metabolism	CRISPR/Cas9 knockout	Improved grain performance under heat	75–85	Field evaluated	Liu et al.(2024)
Salinity	Rice	HKT1;5 knockout	Sodium transport and exclusion	CRISPR/Cas9 knockout	Altered sodium exclusion	75–90	Extensive field trials	Javaid et al. (2025)
Salinity	Tomato	NHX overexpression	Vacuolar Na ⁺ compartmentalization	CRISPR-mediated insertion	Improved cellular ion balance	70–80	Greenhouse validated	Kumari et al. (2017)
Combined drought and salinity	Wheat	HKT1;5 + NHX	Dual ion transport	Multiplex editing	Combined stress tolerance	70–85	Field trials	Shabala et al. (2025)
Cold	Barley	CBF1/CBF3 overexpression	Cold-responsive transcription factors	Gene insertion	Improved cold acclimation	65–75	Research validation	Båga et al. (2022)
Submergence	Rice	SUB1A enhancement	Submergence tolerance pathway	Base editing	Improved flood tolerance	60–75	Breeding pipelines	Anumalla et al. (2025)
Combined heat and drought	Maize	Multiple genes	Trait pyramiding	Multiplex prime editing	Enhanced resilience to multiple stresses	70–85	Breeding advancement	Malenica et al. (2021)
Salinity	Cotton	SOS pathway genes	Osmotic sensing and ion homeostasis	Multiplex CRISPR	Improved ionic and osmotic balance	65–75	Research phase	Akhtar et al. (2026)
Cold	Cassava	CBF pathway genes	Cold-responsive gene regulation	Heterologous expression	Potential expansion into cooler environments	50–65	Early development	Jiang (M., 2025)
Combined drought and heat	Sorghum	ABA biosynthesis genes	Stress-hormone signaling	Base editing	Potential improvement in stress resilience	75–85	Breeding pipelines	Wang et al. (2025)

Table 3. Gene Targets and Achievements for Abiotic Stress Tolerance in Major Crops

The Biofortification of food and Enhanced nutrition to solve Micronutrient deficiencies.

Micronutrient malnutrition, also termed as hidden hunger by the international health bodies, is a condition that affects over 2 billion people in the world, with children and pregnant women being the most affected in the developing regions where dietary diversity is limited and people are dependent on the staple crops which inherently lack the essential minerals, vitamins and amino acids (A. Kumar et al., 2023). Zinc deficiency is the fifth most common risk factor in terms of disease burden in the world and has been known to cause impaired immune capacity, stunted growth and increase the mortality rate of infectious diseases among the affected people. Iron deficiency is a cause of anemia in more than 1.5 billion people and it leads to poor cognitive developments, work productivity and maternal mortalities (A. Hussain et al., 2022). Childhood blindness, which is preventable, is caused by vitamin A deficiency in more than 250 million people. Traditional breeding methods do not have a significant effect on increasing the levels of micronutrients in staple crops because of natural genetic variation limits: rice grains have nearly no 8-carotene in them because rice endosperm did not evolve an ability to synthesize 8-carotene, and white rice in conventional stores has less than 5 ppm of iron in it, which is a very low level of concentration to solve deficiency by diet. Direct pathway engineering, metabolic flux redirection, and optimization of nutrient transporters can be done through genome editing to obtain a significant improvement in micronutrient levels without the limitations of natural genetic variation, which is a sustainable and cost-efficient alternative to supplementation or food fortification with constant external inputs (Zhu et al., 2020).

Biofortification of Iron and Zinc with Nutrient Transporter and Chelator Engineering.

The biofortification of iron and zinc focuses on various steps in the pathway that allow the accumulation of micronutrients in a synergistic approach to avoid agronomic costs. The major iron and zinc chelator (nicotianamine) that is encoded by Nicotianamine synthase (NAS) genes aids long-distance nutrient transport to edible tissues by roots in rice: the optimization of OsNAS gene in rice created nicotianamine, and the nicotianamine levels improved up 2-fold by increasing the amounts of zinc in polished rice (25 ppm zinc versus 20 ppm conventional) and 1.5-fold by increasing the chelation and transport capacity (Seregin & Kozhevnikova, 2023). Simultaneous enhancement in iron levels (3-fold), zinc levels (4-fold), and 6.4X 0-carotene was successfully obtained through combined expression of several biofortification genes in transgenic rice-OsNAS1, bean ferritin (PvFERRITIN), bacterial carotene desaturase (CRTI), and maize phytoene synthase (ZmPSY) which can be transduced into rice,

yielding polished rice with 6-fold higher levels of iron (S. Kumar, Palve, Joshi, & Srivastava, 2019).

Metal transporter engineering is aimed at IRT (iron-regulated transporter), ZIP (zinc-regulated transporter and iron-regulated transporter protein) and YSL (yellow stripe-like) family genes that facilitate the transport of iron and zinc between cellular membranes (Song et al., 2024). OIRT1 and OsIRT2 were able to enhance the capacity of iron uptake by 1.5-2-fold and OsZIP (zinc transporter) was able to enhance zinc accumulation in grain 1.8-fold. Phytate degradation as a means of increasing bioavailability is vital supplementary approach: phytic acid (inositol hexaphosphate) chelates iron and zinc in grain bran, which makes them inaccessible to intestinal absorption even in large amounts (A. Kumari & Roy, 2023). CRISPR-mediated knockout of inositol phosphate kinase (IPK) or multiple inositol polyphosphate phosphatase (MIPP) gene reduced phytate by 50-80 percent and bioavailability of iron and zinc 2-3-fold compared to high-phyta lines (Pandey, Aggarwal, Meena, & Kumar, 2021).

Provitamin A Fortification and Golden Rice 3.

Golden Rice is a major success story of biofortification that was used to solve vitamin A deficiency affecting 250 million susceptible people, especially in Southeast Asia and Sub-Saharan Africa. Traditional white rice endosperm bears essentially no 0-carotene since rice never developed carotenoid production in grain tissue even though it had all the enzyme machinery in the leaves (Alex, Karuppanchetty, & Bhushesh Kumar, 2014). Later developments which employed other gene regulation optimization yielded the Golden Rice 2 varieties with 7.9 0g/g of 0-carotene which is almost 5-fold improvement. The evidence of human feeding trials shows that β -carotene in Golden Rice can be converted to vitamin A whose bioavailability is equal to that of purified supplements and far better than that of plant foods such as spinach, i.e. one cup a day can supply about half of recommended dietary allowance of children (Amna et al., 2020).

In addition to rice, vitamin A deficiency in populations taking various staple grains and roots is solved by the enrichment of β -carotene in a variety of staple foods (Ashokkumar, Govindaraj, Karthikeyan, Shobhana, & Warkentin, 2020). Golden Maize modified to produce provitamin A by the introduction of specific genes was 5-10-fold higher in carotenoids than control maize and field tests in Africa showed that it was acceptable and promising as part of integrated disease risk management (Mudadirwa, Ndhlela, Olakojo, & Gonhi, 2023). Many crops eaten by populations with low dietary diversity such as cassava, sweet potato, banana and others are a significant source of carotenoid fortification. The addition of other carotenoids other than carotenes, especially the

eye-protective and macular disease preventive lutein and zeaxanthin is another new area of horticultural crop bio-fortification (Bungau et al., 2019).

Future perspectives and Challenges of Precision Genome Editing in Crop Improvement.

Limits of the Technical Optimization and Delivery System.

Although there have been astonishing improvements in the efficiency of editing, including 70-100 percent efficiency in base editors using protoplast systems and 53.2 percent with prime editors in maize, enormous technical challenges are present in the range of crop species and genotypes (Tian et al., 2025). The factors of the delivery system are leading, because the existing techniques are characterized by high levels of genotype dependence, with the efficiency of transformation in different cultivars of the same species ranging in 20-40%. Remaining crops such as legumes, trees and most orphan crops still exhibit editing efficiencies under 15 and this limitation restricts the accessibility of the technology most needed in nutritionally deficient areas (Venezia & Creasey Krainer, 2021). This issue with purity of products adds a further level of difficulty to development directions unwanted indels are observed in 10-30% of prime-edited plants, so vast screening populations are required to separate homozygous edited lines (Velimirovic, 2024). The discrepancy in the efficacy of optimized model systems (75-95% in the case of ABEs) and consistent transformation results (30-60% heritability) accounts for basic gaps in comprehending the biology of repairing plant DNA and the dynamics of accessing chromatin that current studies have failed to address (Vieira, Nogueira, & Fritsche-Neto, 2025).

Regulatory Barriers to Market Access and Harmonization.

Conflicting international regulatory systems establish fragmented developmental aspects that overweight the small-to-medium enterprise development and enhancement of orphan drugs (McConnell, 2022). Genome-edited products are subject to vastly different classification results across jurisdictions: the GABA-enriched Sicilian Rouge High tomato released in Japanese markets was assigned to the lighter notification processes, whereas similar products in the European Union will have to meet GMO directives and pay 8-10 years of regulatory costs and 10 to 15 million (Ezura, 2022). Reciprocity in this regulation introduces barriers to market entry that divide the world commodity chains and discourages the development of traits that are specific to region-specific problems. The interpretations of the Cartagena Protocol on Biosafety differ greatly with certain countries adhering to process (presence of editing machinery) and other to product (final genetic composition), which is a direct

contradiction to the scientific agreement that SDN-1 edits (no foreign DNA integration) are identical to conventionally-derived mutations (Yasir, 2023). The intellectual property environments also make technology implementation more challenging: fundamental CRISPR-Cas9-related patents are still disputed in multiple jurisdictions, and it is unclear how the organization licences its technology to those in the public sector, who develop staple crop varieties in the service of smallholder farmers (van der Sluis, Husovec, & Waterson).

Traits of Stacking Climate Resilience and Field Performance.

Transduction of molecular edits into consistent field performance in varying environmental conditions is a severe validation problem which needs to be systematically studied (Mete et al., 2025). Although studies have been done under controlled environments which show 85-95% yield retention under single stressors, the field environment presents the combination of drought, heat and salinity stressors which show unexpected phenotypic trade-offs (Suresh et al., 2025). The success of multiplex editing (the ability to edit up to nine wheat genes using Cas12a and three rice genes using 50-80 percent efficiency) needs extensive field testing over several seasons and locations to determine stability of yield and pleiotropy. The redundancy of polyploid crops such as that of hexaploid wheat (AABBDD genome) requires homoeolog-specific targeting approaches to deliver stable traits expression and genetic diversity (Borrill, Harrington, & Uauy, 2019). Little has been done on long-term ecological interaction: edited stress tolerance characteristics have the potential to shift plant-microbiome interactions, pollination interactions, and competitive interactions with weeds, which only take multi-year agroecological evaluation to provide broad deployment recommendations (Liang, Yu, Ju, Wang, & Yin, 2025; Mikiciuk et al., 2024).

Fair Access to Technology and Prioritization of Orphan Crop.

The fact that the research on genome editing is concentrated in major production crops (maize, rice, wheat, soybean) is a threat to increase agricultural inequality because most of the published literature is on staple cereals, whereas the orphan crops that are important in the food-insecure community are underrepresented (Adegaju et al., 2024). There are already research programs underway to use base editing to modify chickpea (8-15% efficiency), cowpea (8-12%), and cassava (50-65% cold tolerance changes), although these activities are not well-funded against commercial crop priorities (Duvick & Committee, 2001). It is necessary to strengthen technology transfer mechanisms that allow the deployment of proprietary editing systems by the public sector institutions on humanitarian purposes since the

existing models of licensing tend to limit the freedom-to-operate when it comes to non-commercial crop improvements (Kock, 2021). Cooperation between the artificial intelligence-directed pegRNA design and low-cost delivery technologies creates a possibility of democratizing editing technology, but informatics infrastructure and trained personnel are limited to geographic areas with the highest malnutrition incidence (Abdul, Adeghe, Adegoke, Adegoke, & Udedeh, 2024). Development of globally distributed centers of excellence in genome editing, in combination with open-access reagent repositories and simple regulatory navigation services is critical infrastructure to ensuring that technological sophistication can be converted into fair nutritional impact as opposed to concentrating agricultural biotechnology capability in already advantaged locations (Harbansh, 2025; Lockie et al., 2020).

Conclusion

The history behind the genome editing can be understood as a fundamental re-programming of crop enhancement, shifting between the use of stochastic modification to programmed precision. High-fidelity variants of CRISPR, base editors with near-quantitative conversion rates, and prime editing systems that can perform the full range of nucleotide substitutions have all helped extend the editable genome and shrink the range of unintended outcomes. These technical innovations have made possible quantifiable increases in the field performance of crops that have been engineered to be resistant to drought, heat and salinity, as well as nutritional improvements on a scale significant enough to have a meaningful impact on iron, zinc and vitamin A deficiency at the population scale.

However, the conversion of the editing capability into the agricultural effect is still conditioned by the aspects outside of the molecular innovation. The ability to perform delivery in a wide range of genotypes, effective editing of recalcitrant species, and the purity of products remain unsorted technical limitations to many combinations of crops. The heterogeneity in regulations between the key agricultural markets will disaggregate development pathways and the intellectual property environments and technology transfer models will dictate whether or not these tools will find their way to the orphan crops and the smallholder farmers where nutritional inadequacies are most severe. The combination of the developed editing platforms and the growing speed of climate disruption and endemic malnutrition creates an urgent need as well as a chance. To understand the substantive value of the role of genome editing in the global food security system, it is important to maintain robust science, regulatory integrity and willful institutions that will guarantee the technological use of the technology to the entire range of agricultural producers and consumers. The next decade will

be the one that determines whether the technological sophistication observed in the controlled environments can be adapted to offer fair, long-term solutions to the most acute problems of the world agriculture.

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