



Genome editing: Techniques, regulatory frameworks and recent advancements

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Abstract

Genome editing is an emerging technology that is transforming modern crop improvement by enabling precise and targeted modification of genes associated with crucial agronomic traits. Conventional breeding is often tedious and constrained by the complex nature of quantitative traits, long breeding cycles and influence of environment on phenotypic expression. Genome editing technologies, particularly CRISPR/Cas systems, provide a precise, efficient and versatile approach for modifying genes associated with abiotic stress tolerance, yield, nutritional quality, disease resistance and other agronomically important traits. Different genome editing approaches, including base editing and prime editing, further expand the potential for introducing specific genetic changes with improved precision. The integration of genome editing with speed breeding, high-throughput phenotyping, next-generation sequencing can further speed up crop improvement by enabling rapid generation advancement, precise characterization of edited genotypes and selection of desirable lines. Genome editing, when combined with modern breeding and genomic approaches, offers immense potential for developing improved crop varieties with enhanced productivity, quality and resilience to changing environmental conditions.

Keywords: Genome Editing, Crispr/Cas Systems, base editing, prime editing, crop improvement, abiotic stress tolerance

Introduction

India's tribunal system has repeatedly generated the same constitutional anxiety: bodies created to exercise judicial or quasi-judicial power have often remained administratively dependent on the executive departments whose decisions Conventional breeding is a time-consuming process. However, the emergence of genetic engineering techniques has significantly reduced the time required for crop improvement. Genetically modified organisms (GMOs) often face regulatory challenges due to the random integration of foreign DNA, unlike genome-edited organisms. Genome editing enhances the efficiency of crop improvement by enabling precise modifications at specific genomic locations while reducing the time and resources required. Genome editing can also speed up the development of improved varieties by modifying existing elite cultivars without extensive backcrossing to recover their original genetic background. Its ability to target multiple genes simultaneously offers opportunities to improve complex traits more efficiently than conventional breeding approaches alone.

Different genome editing techniques

1. First generation tools

Meganucleases are naturally occurring enzymes found in bacteria and fungi that recognize long DNA sequences, typically ranging from 14-40 base pairs, with high specificity and induce double-strand breaks. These breaks are repaired by cellular mechanisms, resulting in gene knockouts, insertions, or other targeted modifications.

2. Second generation tools and third generation tools

Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) are engineered genome-editing tools that recognize specific DNA sequences and induce double-strand breaks through the action of the FokI nuclease domain. FokI dimerizes upon binding to the target DNA, enabling DNA cleavage. TALENs are comparatively easier to design and offer high target specificity, whereas ZFNs are relatively expensive to develop and may exhibit off-target effects.

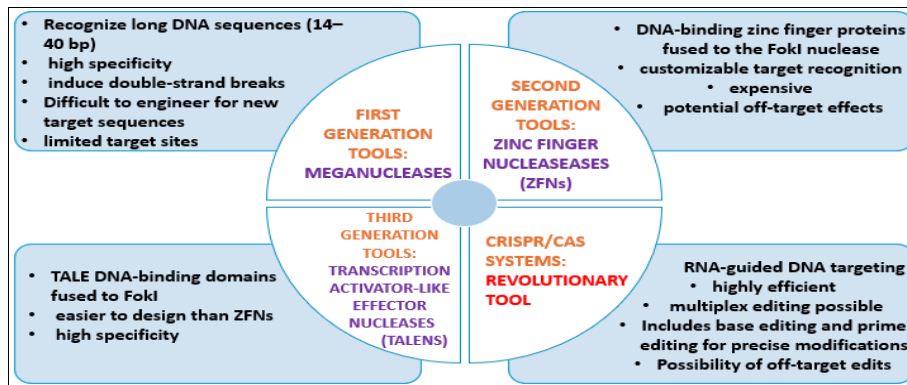
3. Crispr/cas systems

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems use single-guide RNA (sgRNA) to recognize specific target DNA sequences. The Cas9 nuclease forms a complex with sgRNA and cleaves the target DNA by generating a double-strand break. Cas9 contains two major nuclease domains: RuvC: Cleaves the DNA strand that is non-complementary to the sgRNA. HNH: Cleaves the DNA strand that is complementary to the sgRNA. CRISPR/Cas9 is a highly efficient genome-editing system that supports multiplex editing, enabling simultaneous modifications of two or more genes.

4. Cell repair mechanisms

Non-homologous end joining: Repairs double-strand breaks by directly joining the broken DNA ends, often resulting in insertions or deletions (indels) that can lead to gene knockouts.

Homologous direct repair: Uses a donor DNA template to repair double-strand breaks, enabling precise sequence modifications or the targeted insertion of genetic material.



5. Base editing and prime editing

CRISPR-based genome editing can be further classified into base editing and prime editing techniques. A major similarity between these approaches is their ability to introduce precise point mutations without generating double-strand

DNA breaks or requiring an external donor DNA template. Base editing enables four types of base-to-base conversions: C to T, T to C, A to G, and G to A. In contrast, prime editing supports all 12 possible types of base substitutions, allowing a broader range of targeted nucleotide modifications.

5.1 Types of base editors

Cytosine base editors	Adenine base editors
Converts C-G pairs to T-A base pairs	Converts A-T to C-G base pairs
Uses cytidine deaminase that fuses to either Cas9 nickase or a dead Cas9	Uses adenine deaminase that fuses to various forms of Cas9

Cas9 contains two nuclease domains, RuvC and HNH, which are responsible for cleaving the two strands of DNA. When one of these domains is inactivated, Cas9 functions as a nickase (nCas9),

producing a single-strand break. When both domains are inactivated, it becomes dead Cas9 (dCas9), which retains its DNA-targeting ability but loses its DNA-cleavage activity.

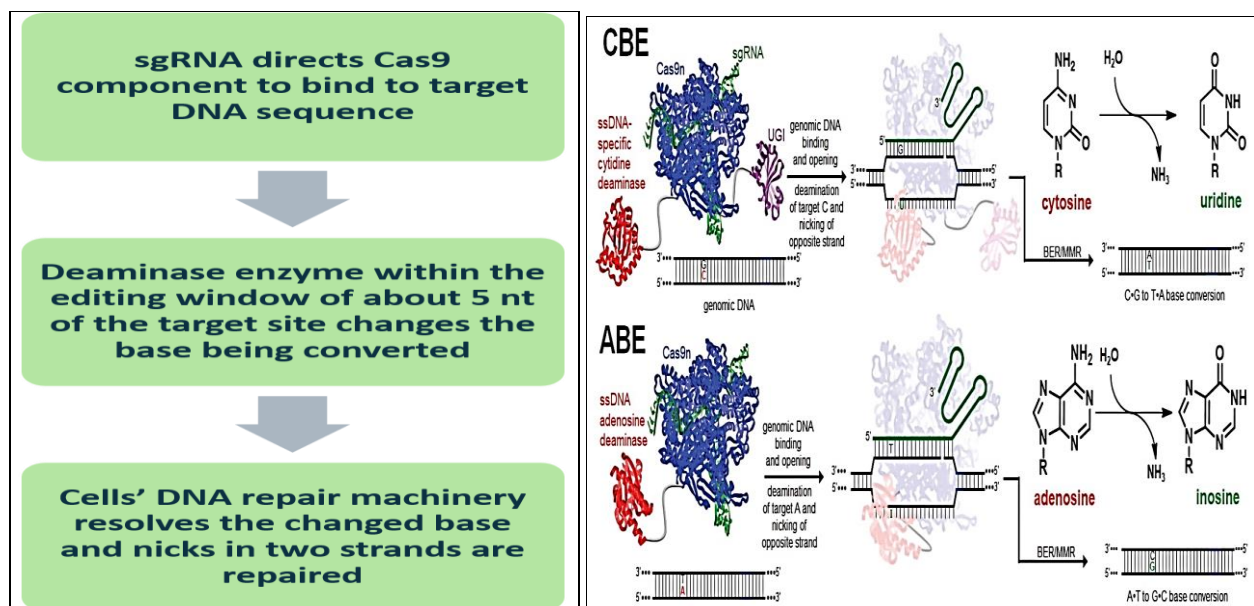


Fig 1: Base editing mechanism

5.2 Procedure Of Prime Editing:

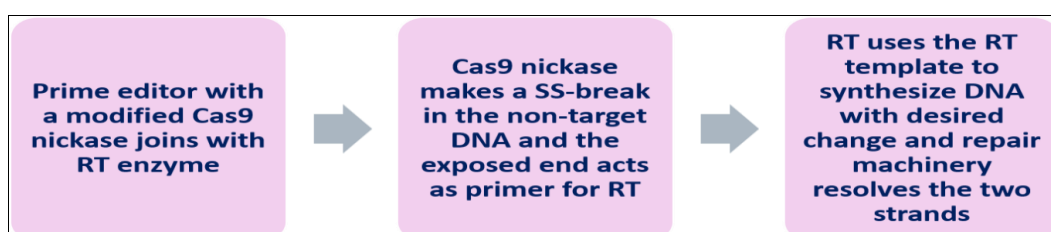


Fig: Prime editing mechanism

The cell repair mechanism differs as BER/MMR are used which reduce the occurrence of indels. BER (Base Excision Repair) repairs damaged or chemically modified individual DNA bases by removing the abnormal base and replacing it while MMR (Mismatch Repair) corrects incorrectly paired or mismatched bases that arise mainly during DNA replication or genome editing.

Regulatory mechanisms

Product-based regulation focuses on the characteristics and risks of the final crop, while trait-based regulation focuses on the specific novel or modified trait. Process-based regulation considers the technique used to develop the crop. Canada, US, Brazil follow product-based approach while Argentina follow trait based and in European Union genome edited crops must undergo comprehensive risk assessments and authorization. India issued the Guidelines for Safety Assessment of Genome Edited Plants, 2022 under the Department of Biotechnology (DBT). The 2022 guidelines specifically define these three categories:

Category	Type of edit	Regulatory position in India
SDN-1	Small mutation/indel without a DNA template; no foreign DNA	Simplified regulatory pathway
SDN-2	Precise small change using a DNA template	Simplified regulatory pathway
SDN-3	Insertion of a gene/large DNA sequence	Regulated more like GM plants

product-oriented regulation for SDN-1 & SDN-2 + stricter process-based biosafety oversight for SDN-3.

Recently released products based on genome editing:

1. Drr Dhan 100 (Kamala)

DRR Dhan 100 (Kamala) is an improved genome-edited rice variety developed by the ICAR–Indian Institute of Rice Research (IIRR), Hyderabad, using Samba Mahsuri (BPT 5204) as the parental variety. The variety was developed using CRISPR/Cas9 genome editing to modify the OsCKX2 (cytokinin oxidase 2) gene, which is involved in cytokinin degradation and influences grain number. The targeted modification aims to enhance grain number and yield while reducing crop duration. It underwent molecular screening, confirmation of the edited mutations and absence of foreign DNA, followed by multi-location field evaluation under the All India Coordinated Research Project on Rice (AICRPR) before its identification in 2025.

2. Pusa Dst Rice 1

Pusa DST Rice 1 is a genome-edited rice variety developed by the ICAR–Indian Agricultural Research Institute (IARI), New Delhi, using MTU 1010 as the parental variety. It was developed using CRISPR/Cas9 technology by precisely modifying the DST (Drought and Salt Tolerance) gene to improve tolerance to drought and salinity. The variety exhibits enhanced adaptability to inland saline, alkaline and coastal saline soils, making it suitable for stress-prone rice-growing environments. Field evaluations demonstrated a

yield advantage of approximately 9.66–30.4% under targeted stress conditions while retaining the desirable grain characteristics of the parent variety. It is suitable for both Kharif and Rabi cultivation. According to ICAR (2025), these genome-edited rice varieties have a combined recommended cultivation area of approximately 5 million hectares (50 lakh hectares) across suitable rice-growing regions of India contributing to an additional 4.5 million tonnes of paddy production. (Acreage represents the combined potential area suitable for the two varieties and does not indicate the area currently under cultivation)

Case studies

1. Case Study 1: CRISPR-Cas9 Editing of Starch Branching Enzyme in Potato

Batta *et al.* (2025) ^[1] used CRISPR-Cas9 to target SBE2.1 and SBE2.2 genes in potato cv. Kufri Chipsona-I to improve nutritional quality. Four target sites were designed and introduced through *Agrobacterium*-mediated transformation. Six mutant lines (K301-K306) were identified through PCR and Sanger sequencing, with K304 showing the highest editing efficiency. These lines showed altered crystalline index when starch was evaluated using X-ray crystallography. Genome editing reduced starch branching and increased amylose and resistant starch. K304 recorded 95.91% amylose and 8.69 g/100 g resistant starch, demonstrating the potential of genome editing for improved nutritional quality.

2. Case Study 2: Establishing a CRISPR/Cas9 genome editing framework in pigeonpea (*Cajanus cajan* L.) by targeting phytoene desaturase (PDS) gene disruption

Senthil *et al.* (2025) established a CRISPR/Cas9 genome-editing system for pigeonpea by targeting the phytoene desaturase (PDS) gene, which is involved in carotenoid biosynthesis ^[8]. A pigeonpea-specific CRISPR vector was developed using the CcU6 7.1 promoter to drive sgRNA and Cas9 under a potato ubiquitin promoter. The system was delivered through *Agrobacterium*-mediated in planta apical-meristem transformation and *in vitro* embryonic-axis transformation. Disruption of *PDS* caused albino/bleached phenotypes, providing a convenient visual marker for successful editing. Editing efficiencies of 8.80% and 9.16% were obtained in in planta and *in vitro* approaches, respectively, and mutations were confirmed by sequence analysis.

3. Case Study 3: Knockout of a pectate lyase in tomato increases fruit firmness and reduces foliar susceptibility to pathogens

Fumelli *et al.* (2026) ^[2] used CRISPR/Cas9-mediated genome editing to knock out the SIPL (pectate lyase) gene in tomato (*Solanum lycopersicum*) to improve fruit firmness and disease resistance ^[2]. The mutations were confirmed through sequence analysis, and edited lines carrying small insertions or deletions were evaluated for fruit quality and pathogen susceptibility. Disruption of SIPL, which is involved in cell-wall degradation, resulted in increased fruit firmness and reduced post-harvest weight loss. The edited plants also exhibited reduced foliar susceptibility to *Botrytis*

cinerea, *Phytophthora capsici* and *Pseudomonas syringae* pv. tomato.

Advantages: Genome editing technologies offer high precision, targeted modification, rapid trait improvement, reduced breeding time, and the ability to modify multiple genes simultaneously. CRISPR/Cas systems are particularly simple, cost-effective, and versatile compared with older technologies such as ZFNs, TALENs, and meganucleases.

Limitations: Major limitations include off-target effects, PAM/target-site restrictions, variable editing efficiency, and difficulties in delivering editing components into plant cells. Some technologies also require complex and time-consuming design, while base and prime editing have limitations in the types and efficiency of genetic changes they can introduce.

Future Prospects: The future of crop improvement lies in integrating genome editing with NGS, HTP, speed breeding, de novo domestication and synthetic biology. NGS can identify and validate candidate genes, while genome editing enables their precise modification and HTP provides rapid, quantitative screening of resulting phenotypes. Speed breeding can accelerate generation advancement and development of homozygous and elite lines, while multiplex editing and machine learning can support improvement of complex traits. These integrated approaches can enhance climate resilience, nutritional and industrial traits, and accelerate the development of superior crop varieties.

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