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Harnessing genome editing for developing climate-resilient crops

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Climate change poses a serious threat to world agriculture and food security due to the increasing frequency of drought, salinity, heat, and other biotic or abiotic stresses. As a result, the development of climate-resilient crops has become an urgent priority to maintain productivity in vulnerable ecosystems. Recent technologies of genome editing using CRISPR/Cas are precise, accurate and efficient tools for accelerating crop improvement. With genome editing, stress-responsive genes, regulatory components, and metabolic pathways can be specifically altered without the hassles of linkage drag or drawn-out breeding cycles. Recent advancements have successfully edited genes linked to osmotic adjustment, antioxidant regulation, ion homeostasis, disease resistance and stress signalling pathways in cereals, legumes, and oilseeds, leading to improved crop plants. In addition to resilience against abiotic stresses, editing for characteristics such as nitrogen-use efficiency, photosynthetic performance, and yield stability in variable climates presents a promising pathway for sustainable agriculture. The integration of genome editing with genomic selection, speed breeding, and high-throughput phenotyping can further expedite the creation of resilient varieties. This article emphasizes significant breakthroughs, potential gene targets, and translational strategies for implementing genome editing in crop breeding initiatives. Leveraging these innovations can greatly enhance global food and nutritional security in the context of climate change.

Keywords: Abiotic stress, Cas9, CRISPR, Crop improvement, Genome editing

AGRICULTURAL productivity is increasingly threatened by various abiotic stresses such as drought, salinity, extreme temperatures (heat and cold), and nutrient imbalances. These environmental stressors significantly affect plant growth, development, and yield, posing a major challenge to global food security especially in the face of climate change and a growing world population. Although, conventional breeding methods have been employed for decades to develop stress-tolerant crop varieties, but the major limiting factor is the time, as to develop improved varieties takes years.

In recent years, genome editing technologies have emerged as revolutionary tools for precise and targeted genetic modifications, offering new avenues for enhancing crop resilience to abiotic stress conditions. Unlike conventional breeding or transgenic approaches, genome editing allows for the direct manipulation of specific genes or regulatory elements responsible for stress responses, without necessarily introducing foreign DNA. This precision reduces unintended effects and accelerates the development of improved crop varieties.

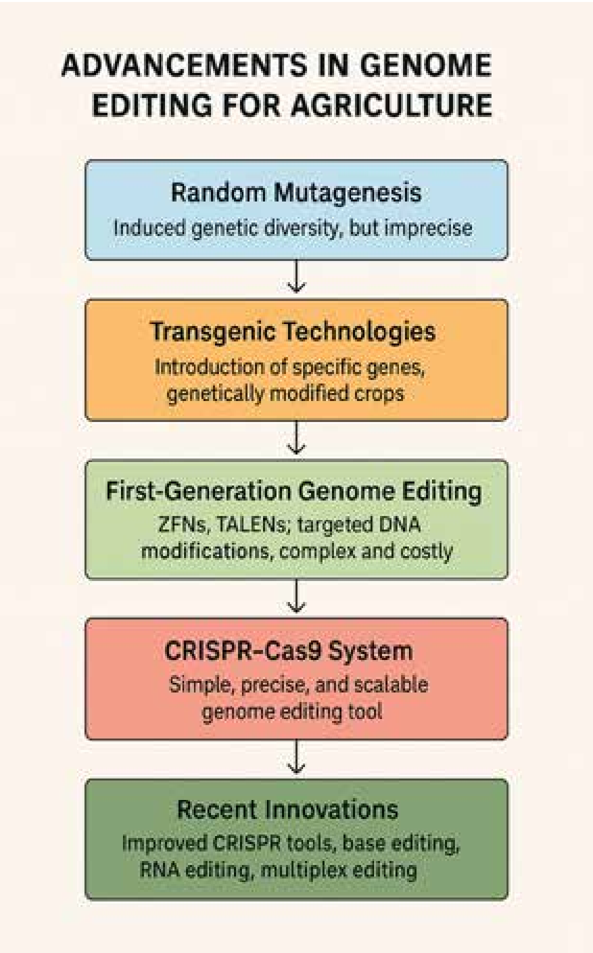
The most prominent genome editing tool, CRISPR-Cas9, along with other systems such as TALENs (Transcription Activator-Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases), has been successfully applied to a wide range of crops including rice, wheat, maize, tomato, and soybean. These technologies are being used to knock out negative regulators, enhance expression of stress-responsive genes, or even introduce beneficial alleles for improved stress tolerance. In addition to providing greater control over plant traits, genome editing offers the possibility of multiplex gene editing-modifying multiple genes simultaneously to address complex stress responses.

As a result, genome editing holds tremendous promise not only for improving abiotic stress tolerance but also for contributing to sustainable agriculture, reducing the need for chemical inputs, and supporting adaptation to changing environmental conditions. Recent studies have highlighted the potential of CRISPR/Cas system (cas9, 12, 13) in enhancing crop resilience to various biotic or abiotic stresses, including diseases, drought, salinity, heat, and cold. Furthermore, advancements in genome editing techniques, such as base editing and prime editing, offer even more precise and efficient tools for crop improvement.

Advancement in genome editing technologies

Early methods, such as random mutagenesis using radiation or chemicals, were crucial in generating diversity but lacked precision. The introduction of transgenic technologies in the 1980s enabled the insertion of specific genes, leading to genetically modified crops like Bt cotton, though public and regulatory concerns limited their adoption.

A major shift came with first-generation genome editing tools-ZFNs and TALENs which allowed targeted DNA modifications but were complex and costly. The real breakthrough was CRISPR-Cas9 in 2012, offering a simple, precise, and scalable system guided by RNA. Since then, tools like Cas12a, base editors, and prime editing have expanded capabilities to include fine-tuned gene changes without cutting DNA, or even editing RNA.



Recent innovations include epigenome editing, RNA editing, synthetic biology integration, and multiplex editing, enabling simultaneous modification of multiple genes for complex trait improvement. Together, these tools mark a shift toward precision agriculture, with the potential to sustainably boost crop performance and global food security in the face of climate challenges.

Genome-editing enzymes

Genome-editing enzymes can be broadly categorized into two main types:

- Site-specific recombinases (SSRs)
- Site-specific nucleases (SSNs)

Table 1. Comparison of site-specific recombinases and site-specific nucleases in plants

Features	Site-specific Recombinases (SSR's)	Site-specific Nucleases (SSN's)
Definition	Enzymes that catalyse recombination between specific DNA sequences (recombination without cutting)	Enzymes that introduce double-strand breaks at specific DNA sequences
Mechanism	DNA rearrangement: excision, integration, inversion	DNA cleavage followed by repair (NHEJ or HDR)
DNA Cut	No, recombination without breaks	Yes, induces double-strand breaks (DSB)
Repair Pathway	Direct recombination	Requires DNA repair (NHEJ or HDR)
Examples	Cre/loxP, FLP/FRT, R/RS	ZFNs, TALENs, CRISPR-Cas9, CRISPR-Cas12a, Cas13
Target Sites	Specific recombination sites	Custom-designed DNA sequences (can be any sequence)
Precision	Very high (recombines only defined sites)	High, but off-target effects may occur
Use in Plants	Marker gene removal, transgene stacking, reversible gene expression	Gene knockout, gene replacement, base editing, trait improvement
Delivery in Plants	Requires transformation methods (e.g. Agrobacterium or particle bombardment) to introduce recombinase genes	Delivered via plasmids, Agrobacterium, RNPs, or virus-based vectors; some edits can be DNA-free (transgene-free)
Flexibility	High if sites are pre-inserted	Variable (depends on design, delivery, and repair pathway)
Applications	Gene cassette excision, conditional expression, transgene stabilization	Trait modification, genome editing, targeted mutagenesis

Types of site-specific nucleases

There are four types of site-specific nucleases- i) Meganucleases ii) Zinc finger nucleases (ZFNs) iii) Transcription activator like effector nucleases (TALENs) iv) CRISPR-Cas

- ***Meganucleases:*** Meganucleases, a rare class of endonucleases which recognise and cleave long DNA sequences ranging from 14–-30 base pairs. Their high specificity makes them powerful tools in gene editing. Originating from microbial sources like yeast and bacteriophages, meganucleases are naturally involved in intron and intein mobility. In gene editing, they induce double-strand breaks (DSBs) at specific genomic locations, which are repaired by the cell's mechanisms (NHEJ or HDR) leading to gene disruption or precise sequence insertion. Meganucleases' exceptional target specificity minimises off-target effects, but designing new ones for each target is complex and time-consuming.
- ***Zinc finger nucleases:*** Zinc finger nucleases (ZFNs) are engineered gene-editing tools that combine a DNA-binding zinc finger protein with the FokI endonuclease's cleavage domain. Each zinc finger domain recognises a specific three-base pair DNA sequence, and multiple domains can target longer sequences. When two ZFN construct bind opposite DNA strands at the target site, the FokI domains dimerize, creating a double-strand break (DSB). This activates the cell's repair pathways, leading to gene disruption or precise sequence modification. ZFNs, among the first programmable nucleases, have been applied in various crops, viz. for improving herbicide tolerance in maize and oil quality in soybean. Their high specificity, achieved through careful zinc finger domain design, is strength. However, developing effective ZFNs for new targets is labour-intensive and requires significant protein engineering expertise.
- ***Transcription activator like effector nucleases:*** Transcription Activator-Like Effector Nucleases (TALENs) are customizable gene-editing tools composed of two main parts: A DNA-binding domain derived from transcription activator-like effectors (TALEs) and a FokI nuclease domain that cuts DNA. Each TALE repeat binds to a single nucleotide, allowing researchers to design TALENs that target nearly any DNA sequence with high precision. To function, two TALENs bind to opposite strands of DNA flanking the target site. The FokI domains then dimerize to introduce a double-strand break (DSB) at the specified location. The cell repairs this break through either non-homologous end joining (NHEJ), which can disrupt genes, or homology-directed repair (HDR), which allows precise genetic changes. TALENs are known for their accuracy and versatility, with fewer off-target effects compared to earlier tools. However, TALENS mechanism of gene editing is costly, labour intensive and time consuming.
- ***CRISPR-Cas system:*** CRISPR-Cas is a gene-editing technology that allows scientists to make precise, targeted changes to the DNA of living organisms. The term CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, which are specific sequences found in the genomes of bacteria and archaea. These sequences are part of a natural defence mechanism used by microorganisms to identify and cut viral DNA during an infection. The Cas9 protein (short for CRISPR-associated protein 9) is an endonuclease enzyme that functions as molecular scissors. It cuts double-stranded DNA at locations defined by a guide RNA (gRNA), which is engineered to match a specific sequence of interest. When introduced into a cell, the CRISPR-Cas9 complex can locate the target DNA, introduce a double-strand break, and allow for gene modification through the cell's own repair pathways-typically non-homologous end joining (NHEJ) or homology-directed repair (HDR).This programmable system enables precise editing of genes and has wide applications in research, medicine, and biotechnology. Because of its efficiency, simplicity, and versatility compared to earlier tools like zinc finger nucleases (ZFNs) and TALENs, CRISPR-Cas9 has become the method of choice for many genetic engineering tasks.The importance of this discovery was recognized in 2020 when Doudna and Charpentier were awarded the Nobel Prize in Chemistry, marking a milestone in the history of genetic engineering. There are other Cas enzymes also like, Cas12a, which is a Type V enzyme that creates staggered DNA cuts and recognizes a T-rich PAM. Cas13 is a Type VI enzyme that targets and cleaves RNA molecules.

Mechanism in plants

CRISPR-Cas9, a system similar to its animal counterpart, enables precise genome editing in plants using RNA guidance. The guide RNA matches a specific target site in the plant genome, directing the Cas9 protein, an endonuclease from *Streptococcus pyogenes*, to the intended location. Once attached, Cas9 induces a double-strand break (DSB) at the targeted site.Plant cells activate their DNA repair machinery, employing non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ introduces small insertions or deletions (indels), potentially disrupting gene function, while HDR can introduce specific DNA sequences if a repair template is provided, enabling more precise gene insertions or replacements. Delivery of CRISPR-Cas9 components into plant cells poses a unique challenge. *Agrobacterium tumefaciens* mediated transformation is commonly used in dicotyledonous plants, while biolistic delivery, or the gene gun method, is used in monocots and recalcitrant species. The choice of delivery method depends on the plant species, the targeted tissue, and the desired outcome. CRISPR-Cas9 offers a powerful and precise tool for plant genetic engineering, revolutionising plant biology and crop improvement.

Key applications in plants

Crop yield enhancement: CRISPR-Cas9 can be used to enhance crop yield by targeting genes limiting grain size and improving fruit size.

Disease resistance: CRISPR-Cas9 confers disease resistance in crops by editing susceptibility genes, leading to resistance against diseases like powdery mildew and cassava brown streak virus or any other targeted disease.

Stress tolerance and nutrition: CRISPR-Cas9 enhances abiotic stress tolerance in crops and improves nutritional content by editing stress-response regulators and metabolic pathways.

Challenges in plant CRISPR applications

- ***Delivery efficiency:*** Unlike animal cells, plant cells have rigid walls, making gene delivery more difficult.
- ***Regulatory hurdles:*** Many countries have complex laws regarding genome-edited crops; some regulate CRISPR-modified crops similarly to GMOs.
- ***Off-Target effects:*** Although rare, unintended mutations are a concern, especially in polyploid species.
- ***Multi gene copy:*** In many crops like wheat, there may be more than one gene copy for one trait which makes it difficult to edit all copies.

Future perspectives

Advancements in genome editing, like CRISPR-Cas12a, base editing, and prime editing, significantly improve precision and efficiency in plant genome engineering. Unlike CRISPR-Cas9, these newer tools enable precise gene modifications without DSBs, reducing the risk of off-target effects and unintended mutations. CRISPR-Cas12a has a unique PAM recognition and staggered cuts, making it suitable for targeting inaccessible genomic regions and multiplex editing with higher specificity. Base editing directly converts one DNA base to another, correcting single-nucleotide polymorphisms or introducing subtle variations. Prime editing, often called a "search-and-replace" tool, combines a catalytically impaired Cas9 with a reverse transcriptase, enabling precise insertions, deletions, and substitutions without donor templates or DSBs. Integrating these technologies with high-throughput phenotyping, artificial intelligence, and epigenome editing will create climate-smart and resource-efficient crops. Breeders will gain access to traits like nitrogen-use efficiency, improved photosynthesis, and abiotic stress tolerance. Innovations in delivery systems, like nanocarriers or DNA-free genome editing, could address regulatory challenges and public concerns related to genetically modified organisms (GMOs).

SUMMARY

CRISPR-Cas9 has revolutionised plant genetics by offering a precise, cost-effective, and adaptable genome editing platform. Its successful implementation across various plant species has opened new avenues for addressing global agricultural challenges. As climate change, soil degradation, limited arable land, and population growth pose significant threats, genome editing tools provide a strategic solution for enhancing crop resilience, yield, and nutritional quality. Next-generation gene editing tools offer more refined, safe, and predictable genome modifications, paving the way for sustainable and resilient crops that reduce agrochemical dependence and improve food security in a changing climate. However, responsible and equitable use of these powerful tools requires interdisciplinary research, robust biosafety frameworks, and transparent public engagement.

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