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Gene Editing: Principles, Tools and Applications

*B. Mounika, Darmagaru Shivani, Kavitha Reddy A, Chandrasekhara Sarma M, Ramchander S and Dakshina Raj R
Department of Genetics and Plant Breeding, School of Agriculture, Kaveri University, Gowraram (V), Wargal (M), Siddipet Dist., Telangana-502279, India
*Corresponding Author's email: bmounica2002@gmail.com

Gene editing has emerged as a ground-breaking technology that allows scientists to precisely alter DNA with remarkable accuracy. Tools such as CRISPR-Cas9, TALENs, and Zinc Finger Nucleases have transformed biological research by making gene modification more efficient and accessible. From developing disease-resistant crops to advancing medical therapies, gene editing is reshaping multiple fields. In agriculture, it offers environmentally sustainable strategies to improve crop resilience, productivity, and quality without the introduction of foreign genes. This article explores the core principles, major tools, diverse applications, regulatory considerations, and future directions of gene editing, particularly for postgraduate students and researchers in plant sciences.

Introduction

A specific type of molecular techniques called "Gene editing" allows for the exact modification of live organisms' sequences of DNA. Unlike traditional genetic engineering, which often introduces alien genes, gene editing techniques may produce minor, precise, and predictable changes inside the native genome, such as base substitutions, insertions, deletions, or gene knockouts. CRISPR-Cas technology has transformed biological research due to its remarkable efficiency, versatility, and ease of usage. In order to produce high-yielding, nutritionally superior, climate-resilient crops that are required to feed the world's growing population while maintaining environmental sustainability, gene editing is becoming more and more common in plant sciences. Gene editing supports sustainable agriculture by reducing the use of pesticides, decreasing crop losses, and promoting efficient resource use.

Principles of Gene Editing

Gene editing relies on the ability to create a **site-specific double-strand break (DSB)** or a targeted nick in DNA. Once DNA is cut, the cell repairs the break through either:

1. Non-Homologous End Joining (NHEJ)

- Error-prone repair mechanism
- Often results in insertions or deletions (indels)
- Used for gene knockouts

2. Homology-Directed Repair (HDR)

- Uses a DNA template to repair the break
- Enables precise gene correction or insertion
- Active mainly in dividing cells

The kind of repair pathway triggered, the stage of the cell cycle, the mode of operation, and the editing component design all influence the efficiency and outcome of gene editing.

Major Gene Editing Tools

1. Zinc Finger Nucleases (ZFNs): Zinc Finger Nucleases (ZFNs) were the first genome editing tools used in plants (around 2005). They are engineered nucleases formed by fusing a

DNA-binding zinc finger domain with the FokI restriction enzyme cleavage domain. Each zinc finger recognizes a specific 3-base pair DNA sequence, and multiple zinc fingers (3–6) are combined to target a unique 9–18 bp sequence. The FokI nuclease must dimerize to create a double-stranded break (DSB), so two ZFNs bind to opposite DNA strands with a short spacer between them. ZFNs have been successfully used in crops like maize, tobacco, and soybean. However, they are complex, technically challenging, and expensive to design and assemble.

2. Transcription Activator-Like Effector Nucleases (TALENs)

TALENs are second-generation genome editing tools first applied in plant genome editing. Like ZFNs, TALENs are engineered nucleases created by fusing the FokI endonuclease with a DNA-binding domain derived from transcription activator-like effectors (TALEs). TALE proteins originate from *Xanthomonas* bacteria, which naturally bind to plant DNA during infection. Their DNA-binding domain consists of multiple repeating 33–35 amino acid sequences. Each repeat contains specific repeat variable di-residues (RVDs) that determine binding to a particular nucleotide (A, T, G, or C).

Each repeat recognizes a single nucleotide, making TALENs easier to design and more flexible than ZFNs. Like ZFNs, TALENs require FokI dimerization to create double-stranded DNA breaks.

3. CRISPR-Cas System

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) family has transformed gene editing due to its simplicity and high efficiency.

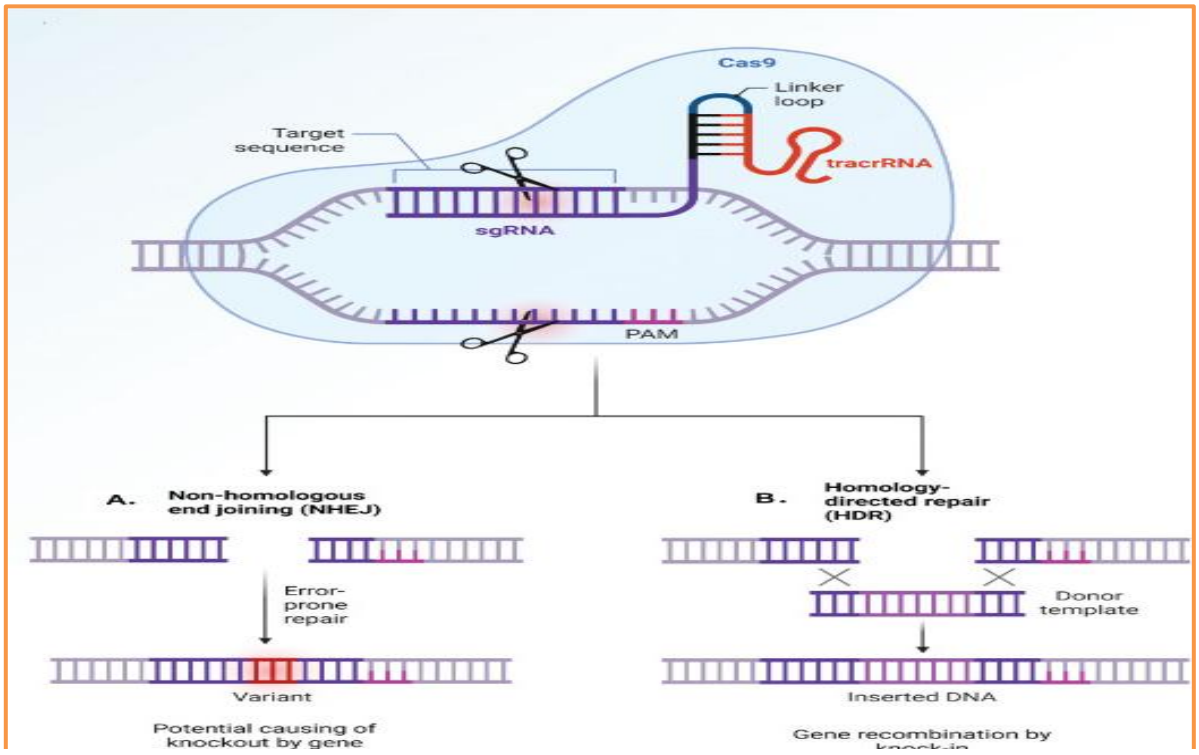
CRISPR Variants

CRISPR variants are modified or alternative forms of CRISPR-based gene editing systems developed to improve precision, flexibility, targeting range, and functionality. These variants expand the capabilities of the original CRISPR–Cas9 system and are widely used in medicine, agriculture, and molecular research.

The most important CRISPR variants include:

1. **CRISPR-Cas9 (SpCas9)**

- Derived from *Streptococcus pyogenes*
- The original and most widely used CRISPR system
- Creates **double-stranded DNA breaks**
- Requires PAM sequence: NGG



2. **Cas12a (Cpf1)**
- Alternative to Cas9
- Produces **sticky-end cuts** instead of blunt ends
- Recognizes T-rich PAM (TTTV)
- Smaller guide RNA requirement
- Useful in plant genome editing

3. **Cas13**
- Targets **RNA instead of DNA**
- Used for RNA editing and viral diagnostics
- Does not permanently alter the genome

4. **Base Editing**

Base editing is an advanced modification of the CRISPR/Cas system that enables precise single-base changes without creating double-stranded DNA breaks. Instead of using standard Cas9, it employs Cas9 nickase, which cuts only one DNA strand.

Base editors combine Cas9 nickase with enzymes such as cytidine deaminase or adenosine deaminase to directly convert one base into another:
- Cytosine (C) → Thymine (T)
- Adenine (A) → Guanine (G)

Cytidine deaminase converts cytosine into uracil within a small editing window, leading to a permanent C–G to T–A base pair change after DNA repair. A uracil glycosylase inhibitor protects the edited base during this process. Thus, base editing allows highly precise point mutations without double-strand breaks or donor DNA templates.

5. **Prime Editing**
- More versatile and precise
- Can insert, delete, or replace DNA sequences
- Does not require donor DNA template
- Lower unintended mutations compared to standard CRISPR

6. **CRISPR interference (CRISPRi)**
- Uses inactive Cas9 (dCas9)
- Suppresses gene expression
- Does not cut DNA

7. **CRISPR activation (CRISPRa)**
- Uses modified dCas9
- Activates gene expression
- Useful in functional genomics

8. High-Fidelity Cas Variants

Engineered versions to reduce off-target effects:
- eSpCas9
- SpCas9-HF1
- HypaCas9

Applications of Genome Editing

1. **Increasing Crop Production:** Enhances stress tolerance (drought, salinity, heat) to improve yield and food security.
2. **Developing Disease-Resistant Crops:** Modifies plant genes to improve resistance against pests and diseases (e.g., powdery mildew-resistant wheat).
3. **Gene Therapy:** Corrects genetic mutations to treat hereditary diseases such as sickle cell anaemia.
4. **Cancer Treatment:** Edits immune cells to better target and destroy cancer cells (e.g., CAR-T therapy).
5. **Conservation of Endangered Species:** Improves disease resistance in threatened species to support survival and biodiversity.
6. **Ecosystem Restoration:** Enhances resilience of organisms (e.g., coral bleaching resistance) to restore damaged ecosystems.

Conclusion

Gene editing is a transformative advancement in modern biological sciences, enabling precise and efficient modification of genetic material across diverse organisms. From early tools like Zinc Finger Nucleases and TALENs to advanced systems such as CRISPR-Cas9, Base Editing, and Prime Editing, the technology has evolved toward greater accuracy with minimal unintended changes. It has significantly impacted agriculture, medicine, functional genomics, and environmental conservation by supporting the development of disease-resistant crops, climate-resilient varieties, gene therapies, and novel cancer treatments. However, ethical concerns, regulatory challenges, off-target effects, and public acceptance remain important considerations for its responsible use.

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