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Potential and Challenges in Gene Editing in Plants

Yogendra Singh[1], Ponnavada Abhishek[2], Rashi Mishra[3], Harshada Mankar[4] and Pratibha Goud[5]

[1]Assistant Professor (Senior Scale)-Biotechnology, Department of Genetics and Plant Breeding, JNKVV, Jabalpur (M.P)
[2]Ex-M.Sc Research Scholar, Department of Genetics and Plant Breeding, JNKVV, Jabalpur (M.P)
[3]M.Sc Research Scholar, Department of Genetics and Plant Breeding, JNKVV, Jabalpur (M.P)
[4]M.Sc Research Scholar, Biotechnology Centre, JNKVV, Jabalpur (M.P)
[5]Ph.D Research Scholar, Department of Plant Physiology , JNKVV, Jabalpur (M.P)

*Corresponding Author
Yogendra Singh*

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INTRODUCTION

Gene editing (Genome Editing) is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. Unlike early genetic engineering techniques that randomly insert genetic material into a host genome, genome editing targets the insertions to site-specific locations. CRISPR-Cas9 is the most prominent, cost-effective, and accurate method, acting as "scissors" to cut specific DNA sequences. Key applications include treating human diseases, enhancing agricultural resilience, and scientific research. Conventional breeding techniques have contributed significantly to addressing the challenges of a growing population, particularly through the Green Revolution. However, these methods have now reached a yield plateau, highlighting the need for a second Green Revolution. The depletion of wild species and wild relatives of cultivated crops has led to a reduction in the genetic diversity of the gene pool, resulting in the loss of many desirable genes. Additionally, modern cultivated plant species, due to their genetic uniformity and the presence of susceptible alleles, are increasingly vulnerable to both biotic and abiotic stresses. To overcome this, it is essential to replace these susceptible genes with resistance-conferring alleles to obtain climate resilient varieties. Transgenic technologies, which involve introducing genes responsible for desirable traits into elite cultivars, serve as an alternative approach to address crop yield losses. However, developing genetically modified (GM) crops with targeted traits is both time-consuming and costly. Moreover, this approach faces significant limitations due to low public acceptance and stringent regulatory safety requirements (Herman et al., 2019).Gene editing (GE) offers a solution to these challenges. It is a powerful and precise tool for modifying plant genomes to create novel, desirable traits (Mao et al., 2019). Genome editing enables precise alterations in chromosomes, such as single-nucleotide insertions, deletions, and duplications, which can lead to subtle changes in gene expression these, are known as site-directed nuclease 1 or 2 (SDN-1 or SDN-2) events.

Mechanism and tools of Gene Editing

Gene editing technologies such as mega nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR-Cas systems enable accurate modification of specific DNA sequences through similar three-step process.

1. An engineered nuclease is introduced, which includes a DNA-binding domain for recognizing the target site and a nuclease domain that introduces cuts.
2. The nuclease binds to the target DNA and generates double-strand breaks (DSBs) either directly at or near the intended location.
3. These DSBs are repaired by one of two cellular pathways: non-homologous end joining (NHEJ), which is prone to errors and can introduce insertions or deletions (Indels), or homologous recombination (HR), which allows for precise and accurate repair (Wada et al., 2020).

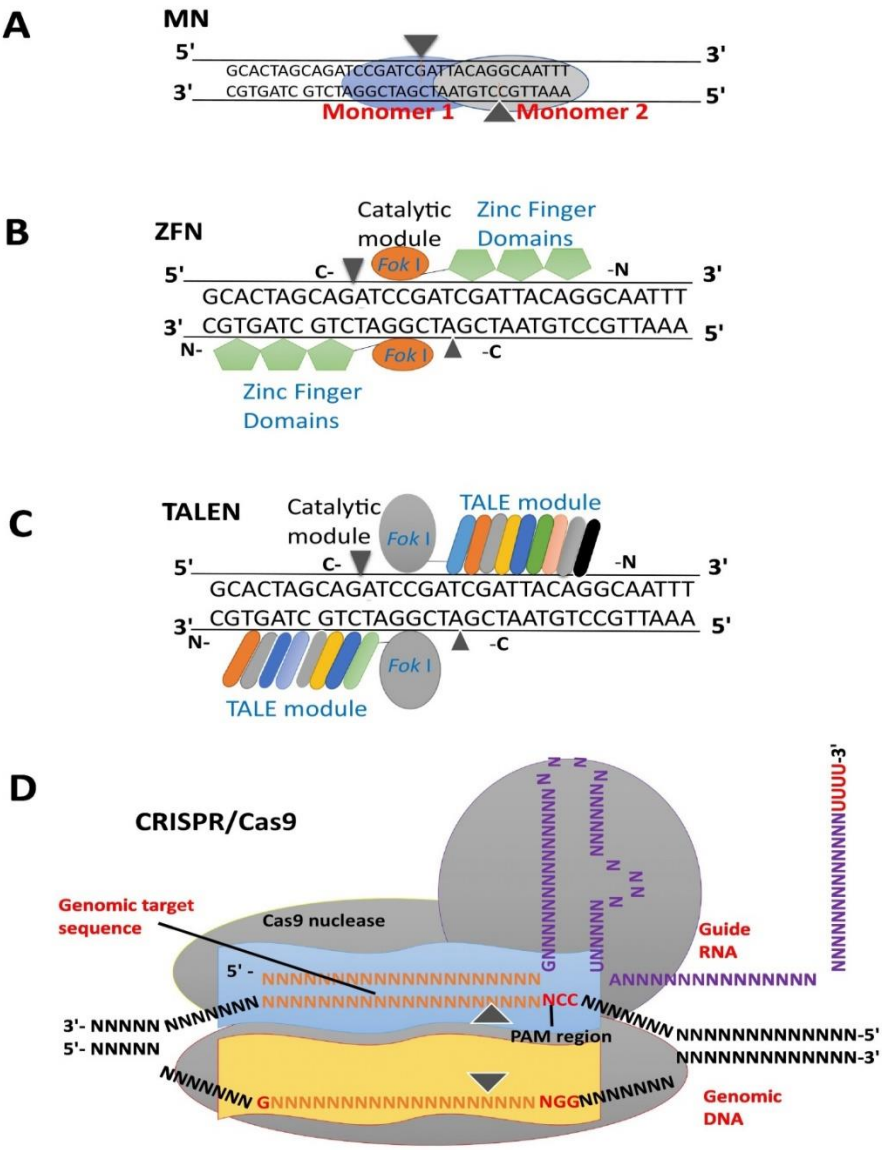

Fig. 1 The figure illustrates four major genomes editing tools (A) Meganucleases, (B) Zinc Finger Nucleases (ZFNs), (C) TALENs, and (D) CRISPR/Cas9—highlighting their structural components and mechanisms used for targeting and cutting specific DNA sequences, **source:https://images.app.goo.gl/REMkDn8cZGFvcmQm9 Mega nucleases**

These endonucleases occur naturally encoded by mobile introns which are highly suitable for genome engineering due to their ability to recognize large and specific DNA sequences.

Zinc finger nucleases

The C-terminal region of each zinc finger is responsible for recognizing specific DNA sequences, typically around three base pairs in length. The technique involves combining two engineered proteins each containing carefully selected zinc finger domains (which bind to DNA) with the catalytic domain of the Fok1 endonuclease.

Transcription activator-like effector nucleases

TALENs are considered a powerful genome editing tool due to their ease of use and high efficiency in inducing DNA cleavage. Structurally similar to zinc finger nucleases (ZFNs), TALENs consist of a non-specific Fok1 nuclease domain and a DNA-binding domain made up of transcription activator-like effectors (TALEs). Their target sites include both the TALE-binding regions and the Fok1 recognition sequence .).

CRISPR-Cas

The CRISPR-Cas system, originally derived from the prokaryotic immune response against invading DNA, is rapidly being adapted for a wide range of genome editing (GE) applications, utilizing single-guide RNAs (sgRNAs) to direct activity (Gupta et al., 2019). The CRISPR locus is composed of three key components: a series of repeat sequences, interspersed with unique spacer sequences.

Among the three major CRISPR/Cas system types (I, II, and III), type II is the most extensively studied due to its ability to create double-strand breaks (DSBs) at specific DNA targets. The Cas9 protein, part of the type II system, identifies the target DNA through base pairing between the DNA and a 20-base-pair sgRNA sequence that is designed to be complementary to the target site at the 5′ end. Recognition of the target also depends on the presence of a protospacer adjacent motif (PAM) located just downstream of the target site (Yamamoto et al., 2018).

Application of Gene Editing in Agriculture

Table 1: Gene edited crops for inducing disease resistance

Technology used	Targeted gene	Crop	Resistance against	References
TALENS	*SWEET14*	Rice	Bacterial blight	Li *et al.* 2012
CRISPR/Cas9	*SWEET11*	Rice	Bacterial blight	Zhou *et al.* 2014
TALEN and CRISPR/Cas9	*TaMLO* homoeologs and *TaMLO-A1*	Wheat	Powdery mildew	Wang *et al.* 2014
CRISPR/Cas9	OsERF922	Rice	Rice blast	Wang *et al.* 2016
CRISPR/Cas9	*SlDMR6-1*	Tomato	Bacterial speck	De Toledo *et al.* 2016
CRISPR/Cas9	*Taedr1*	Wheat	Powdery mildew	Zhang *et al.* 2017
CRISPR/Cas9	*Xa13 promoter*	Rice	Bacterial blight	Li *et al.* 2019

Prospects and Challenges of Gene Editing in Agriculture

One of the key potential applications of gene editing in agriculture is the enhancement of crop yield and productivity. By making targeted changes that boost photosynthetic efficiency, reduce losses caused by pests and diseases, and improve overall plant performance, gene editing can contribute significantly to increasing crop output and global food security. Another vital application is improving stress tolerance in crops such as resistance to drought, salinity, and other environmental stresses which help enhance crop resilience in the face of climate change. Additionally, gene editing can be employed to enhance the nutritional quality of crops. For instance, researchers have successfully used CRISPR-Cas technology to raise beta-carotene levels in rice, offering a potential solution to vitamin A deficiency in developing countries. Currently, there is no unified international regulatory framework for genome-edited crops. Ongoing debates about how these crops compare to conventional GMOs have slowed their adoption and hindered their deployment in agricultural systems. To fully realize the benefits of gene editing in agriculture, harmonized global policies and clear definitions will be essential. Furthermore, as regulatory frameworks evolve, the commercialization of gene edited crops is expected to accelerate. It is crucial for policymakers, scientists, and stakeholders to work together to establish guidelines that ensure the safety and efficacy of genome-edited crops while addressing public concerns and ethical considerations.

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