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Angira Dutta
Swami Vivekananda
University, Barrackpore, West
Bengal, India

Avishek Chatterjee
Swami Vivekananda
University, Barrackpore, West
Bengal, India

Corresponding Author:
Avishek Chatterjee
Swami Vivekananda
University, Barrackpore, West
Bengal, India

Harnessing genome editing: Innovations and applications in crop science

Angira Dutta and Avishek Chatterjee

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Abstract

Genome editing has emerged as a revolutionary approach in crop science, offering precise modifications that enhance agricultural productivity and resilience. This review provides an in-depth exploration of various genome editing techniques, including Meganucleases (MNs), Zinc-Finger Nucleases (ZFNs), Transcriptional Activator-Like Effector Nucleases (TALENs), and the CRISPR/Cas9 system. Each technique is examined for its mechanisms, efficiencies, and applications in crop improvement. The CRISPR/Cas9 system, in particular, has become the most widely adopted method due to its simplicity and versatility, enabling targeted modifications that can lead to improved disease resistance, enhanced nutritional profiles, and better adaptation to abiotic stresses. This article aims to summarize the current advancements in genome editing technologies, highlighting their potential to address the challenges of global food security and the future of sustainable agriculture.

Keywords: Genome editing, crispr/cas9, talens, ZFNS, crop improvement, agricultural biotechnology

Introduction

The global population is projected to exceed 9 billion by 2050, necessitating a significant increase in food production to meet rising demands for food, biofuels, and livestock feed (Ahmar *et al.*, 2020) ^[1]. Traditional agricultural practices are insufficient to achieve this goal, prompting the exploration of innovative biotechnological approaches such as genome editing. Genome editing techniques allow for precise modifications in the genetic makeup of crops, enabling the enhancement of desirable traits such as yield, disease resistance, and tolerance to abiotic stresses (Zhang *et al.*, 2016) ^[14].

The need for increased agricultural productivity is compounded by the challenges posed by climate change, which affects crop yields and food security. As a result, there is an urgent need for technologies that can enhance crop resilience and adaptability to changing environmental conditions. Genome editing technologies have emerged as powerful tools in this context, providing researchers with the ability to make targeted modifications to plant genomes with unprecedented precision.

This review focuses on the major genome editing technologies currently employed in crop science, including Meganucleases (MNs), Zinc-Finger Nucleases (ZFNs), Transcriptional Activator-Like Effector Nucleases (TALENs), and the CRISPR/Cas9 system. Each method's underlying principles, advantages, limitations, and applications in crop improvement will be discussed in detail.

Genome editing techniques

Meganucleases (MNs)

Meganucleases are engineered endonucleases characterized by their long recognition sites (12-40 bp), making them highly specific for target DNA sequences (Ahmar *et al.*, 2020) ^[1]. They induce Double-Strand Breaks (DSBs) at specific loci, which can be repaired through nonhomologous end joining (NHEJ) or homology-directed repair (HDR). While MNs have shown promise in various plant species, their practical application is limited due to the complexity of their design and potential off-target effects (Zhang *et al.*, 2016) ^[14]. Meganucleases function by recognizing specific DNA sequences and creating DSBs, which can lead to gene knockout or integration of new genetic material. Their specificity is attributed to the long recognition sequences, which reduce the likelihood of off-target effects.

However, the design of MNs can be challenging, and their use has declined in favor of more versatile tools. Meganucleases are often referred to as homing endonucleases due to their natural occurrence in various organisms, where they play a role in DNA repair and recombination. The ability to design MNs for specific targets has been a significant advancement in the field of genome editing, allowing for precise modifications in diverse plant species, including *Arabidopsis*, *Gossypium hirsutum* (cotton), and maize (Zhang *et al.*, 2016) ^[14]. However, the manipulation of MNs remains complex, and further research is needed to improve their efficiency and applicability in crop improvement.

Zinc-Finger Nucleases (ZFNs)

Zinc-Finger Nucleases (ZFNs) were among the first genome editing tools developed for targeted gene modification. They consist of a DNA-binding domain made up of zinc-finger motifs that recognize specific DNA triplets, coupled with a FokI nuclease domain that induces DSBs (Cermak *et al.*, 2011) ^[2]. ZFNs have been successfully employed in several crops, including *Arabidopsis*, tobacco, and maize, but their application is often hindered by the difficulty in designing specific zinc-finger arrays and their relatively low efficiency compared to newer technologies (Voytas, 2013) ^[9]. The structure of ZFNs includes two main components: the zinc-finger domain, which recognizes specific DNA sequences, and the FokI nuclease domain, which cleaves the DNA. ZFNs can target multiple sites within the genome, allowing for multiplexed editing. However, the design process is labor-intensive, and off-target effects can occur, necessitating careful validation of ZFN constructs before application in crop improvement. The development of ZFNs marked a significant advancement in the field of genome editing, as they provided the first truly targeted protein reagents for manipulating plant genomes. The ability to engineer ZFNs for specific targets has facilitated targeted gene modification in various plant species, leading to advancements in crop improvement (Liu *et al.*, 2019) ^[7]. Despite their potential, the complexity of designing ZFNs and their relatively low efficiency compared to newer technologies have limited their widespread adoption in crop science.

Transcriptional Activator-Like Effector Nucleases (TALENs)

TALENs are another class of engineered nucleases that combine the DNA-binding properties of transcriptional activator-like effectors (TALEs) with the FokI nuclease domain. Each TALE repeat specifically recognizes a single nucleotide, allowing for precise targeting of DNA sequences (Cermak *et al.*, 2011) ^[2]. TALENs have been used to edit genes in various crops, including rice and wheat, and are favored for their ease of design and lower off-target effects compared to ZFNs (Zhang *et al.*, 2016) ^[14].

The TALEN system operates by fusing the TALE DNA-binding domain with the FokI nuclease domain. Each TALE repeat recognizes a specific nucleotide, enabling the construction of TALENs that can target virtually any sequence in the genome. This flexibility, combined with their relatively low off-target effects, makes TALENs a popular choice for genome editing in plants. TALENs were developed based on the natural ability of TALE proteins to bind to specific DNA sequences in plant pathogenic bacteria

(*Xanthomonas*). The discovery of TALENs has significantly advanced the field of genome editing, allowing for precise modifications in various plant species (Liu *et al.*, 2019) ^[7]. The ability to design TALENs for specific targets has facilitated targeted gene modification in crops, leading to advancements in disease resistance, stress tolerance, and other desirable traits.

CRISPR/Cas9

The CRISPR/Cas9 system has revolutionized genome editing due to its simplicity, efficiency, and versatility. It utilizes a guide RNA (gRNA) to direct the Cas9 nuclease to specific genomic locations, where it creates DSBs. The repair process can lead to targeted mutations or the insertion of new genetic material (Gao, 2021) ^[21]. CRISPR/Cas9 has been widely adopted in crop improvement, allowing for the development of varieties with enhanced traits such as drought resistance, disease resistance, and improved nutritional content (Zhang *et al.*, 2016) ^[14]. CRISPR/Cas9 works by using a gRNA that is complementary to the target DNA sequence. The Cas9 protein binds to the gRNA and the target DNA, creating a DSB. The cell's repair mechanisms then attempt to fix the break, leading to either insertions or deletions (indels) that can disrupt gene function or the integration of new sequences if a donor template is provided. This system's ease of use and ability to target multiple genes simultaneously have made it the preferred method for genome editing in many research and agricultural settings. The discovery of the CRISPR/Cas9 system has transformed the landscape of genome editing, providing researchers with a powerful tool for precise genetic modifications in a wide range of organisms, including plants (Gao, 2021) ^[21]. The ability to easily design gRNAs for specific targets has facilitated rapid advancements in crop improvement, enabling the development of varieties with enhanced traits and better adaptability to environmental challenges.

New tools for plant genome editing

In addition to the established genome editing techniques, recent advancements have introduced new tools that enhance the capabilities of genome editing in plants. One such tool is CRISPR/Cpf1 (now known as Cas12a), which offers distinct advantages over the traditional CRISPR/Cas9 system. Cpf1 has a unique mechanism of action, recognizing a different protospacer adjacent motif (PAM) sequence and creating staggered cuts in the DNA, which can facilitate more efficient genome editing (Zhang *et al.*, 2016) ^[14]. Cpf1's ability to generate staggered ends allows for greater flexibility in the design of donor templates for HDR, making it a valuable tool for precise genome editing. Additionally, Cpf1 does not require a separate trans-activating crRNA, simplifying the design and delivery process (Zhang *et al.*, 2016) ^[14]. The introduction of Cpf1 has expanded the toolbox available for plant genome editing, providing researchers with more options for achieving specific genetic modifications. Another notable advancement is the development of base editing, which allows for the direct conversion of one DNA base into another without introducing DSBs. This technique, known as "base editing," provides a more refined approach to genome editing, enabling precise nucleotide substitutions that can enhance trait development in crops (Zhang *et al.*, 2016) ^[14]. Base editing has shown promise in improving traits such as

disease resistance and nutrient content, further expanding the potential applications of genome editing in crop science.

Applications in crop research

Genome editing technologies have vast applications in crop research, significantly enhancing agricultural productivity and sustainability. The following sections elaborate on the various applications of genome editing techniques, including Meganucleases (MNs), Zinc-Finger Nucleases (ZFNs), Transcriptional Activator-Like Effector Nucleases (TALENs), and CRISPR/Cas9, focusing on their contributions to disease resistance, nutritional quality, abiotic stress tolerance, and the acceleration of breeding programs.

Enhancing disease resistance

The development of disease-resistant crops is one of the most critical applications of genome editing technologies. Pathogen attacks can lead to significant yield losses, and traditional breeding methods often require several generations to achieve resistance. Genome editing allows for the precise modification of genes associated with disease susceptibility or resistance.

CRISPR/Cas9 applications

Rice bacterial blight resistance: The CRISPR/Cas9 system has been employed to knock out susceptibility genes in rice, such as the SWEET family genes (SWEET11, SWEET13, and SWEET14), conferring resistance to bacterial blight caused by *Xanthomonas oryzae* (Zhang *et al.*, 2016) ^[14]. By targeting these genes, researchers have successfully developed rice varieties with enhanced resistance to this devastating disease.

Wheat powdery mildew resistance

The CRISPR/Cas9 system has also been utilized to edit the MLO (mildew resistance locus O) gene in wheat, which is known to be involved in susceptibility to powdery mildew. Mutations in this gene have resulted in wheat lines exhibiting strong resistance to the disease (Wang *et al.*, 2016) ^[10].

Tomato Resistance to Viruses: In tomato, CRISPR/Cas9 has been used to introduce mutations in the ARGONAUTE 7 (SLAGO7) gene, which plays a role in the plant's immune response. The resulting knockout mutants have shown improved resistance to multiple pathogens, including bacterial and viral infections.

TALEN applications

Citrus canker resistance: TALENs have been used to target the promoter region of the CsLOB1 gene in citrus plants, leading to resistance against *Xanthomonas citri*, the causative agent of citrus canker. The resulting homozygous plants demonstrated enhanced resistance to this economically significant disease (Zhang *et al.*, 2016) ^[14].

Improving nutritional quality

Genome editing technologies also play a vital role in enhancing the nutritional profiles of crops, addressing global malnutrition and dietary deficiencies.

CRISPR/Cas9 applications

Soybean Oil composition: Researchers have utilized CRISPR/Cas9 to modify the FAD2 genes in soybean, which

are responsible for the production of polyunsaturated fatty acids. By knocking out these genes, the oleic acid content in soybean oil has been significantly increased, resulting in healthier oil with improved oxidative stability (Zhang *et al.*, 2016) ^[14].

Biofortified crops

Genome editing has been applied to enhance the levels of essential vitamins and minerals in staple crops. For instance, CRISPR/Cas9 has been used to increase the provitamin A (beta-carotene) content in rice, leading to the development of biofortified varieties that can help combat vitamin A deficiency in developing countries (Zhang *et al.*, 2016) ^[14].

Tolerance to abiotic stresses

As climate change poses significant challenges to agriculture, developing crops that can withstand abiotic stresses such as drought, salinity, and extreme temperatures is crucial. Genome editing technologies have proven effective in enhancing stress tolerance in various crops.

CRISPR/Cas9 applications

Drought tolerance in maize: Mutations in the ARGOS8 gene in maize have been induced using CRISPR/Cas9, resulting in improved drought tolerance. The modified maize lines have demonstrated enhanced grain yield under drought conditions, showcasing the potential of genome editing to develop climate-resilient crops (Zhang *et al.*, 2016) ^[14].

Salt tolerance in rice

The OsNAC041 gene, associated with salt stress tolerance, has been targeted using CRISPR/Cas9. The knockout of this gene has resulted in rice varieties with improved salinity tolerance, enabling them to thrive in saline soils (Zhang *et al.*, 2016) ^[14].

Accelerating breeding programs

Genome editing can significantly accelerate traditional breeding programs by allowing for the rapid introduction of desirable traits without the lengthy backcrossing processes typically required in conventional breeding. Techniques such as speed breeding, combined with genome editing, enable the development of new crop varieties in shorter timeframes, facilitating quicker responses to changing agricultural demands (Zhang *et al.*, 2016) ^[14].

Speed breeding and genome editing

Speed breeding utilizes controlled environments with extended photoperiods to accelerate the breeding cycle. When combined with genome editing, this approach allows for the rapid generation of new crop varieties with specific traits. For example, researchers have successfully integrated CRISPR/Cas9 with speed breeding techniques to develop new wheat varieties in a fraction of the time required by traditional methods (Zhang *et al.*, 2016) ^[14].

Development of climate-resilient crops

The CRISPR technology is widely used to develop climate-resilient crops that can withstand biotic and abiotic stresses. This application is crucial for ensuring food security in the face of changing environmental conditions.

Heat stress tolerance in tomatoes

The SIAGL6 gene has been targeted using CRISPR/Cas9 to improve heat tolerance in tomatoes. Knockout mutations in this gene have resulted in enhanced fruit setting under high-temperature conditions, demonstrating the potential of genome editing to develop crops that can thrive in a warming climate (Zhang *et al.*, 2016) [14].

Salinity tolerance in soybean

The Drb2a and Drb2b genes, which are involved in regulating salt and drought tolerance, have been edited using CRISPR/Cas9 in soybean. The resulting mutants have shown improved resistance to salinity, highlighting the role of genome editing in developing resilient crops (Zhang *et al.*, 2016) [14].

Applications in horticultural crops

Genome editing technologies have also found applications in horticultural crops, enhancing traits such as fruit quality, yield, and shelf life.

Seedless Fruits

CRISPR/Cas9 has been employed to induce parthenocarp in various fruit crops, leading to the development of seedless varieties. For example, mutations in the SIIAA9 gene in tomato have resulted in seedless fruits, which are increasingly popular among consumers (Zhang *et al.*, 2016) [14].

Improved shelf life in tomatoes

The Ripening Inhibitor (RIN) gene, which regulates fruit ripening in tomatoes, has been targeted using CRISPR/Cas9 to create mutants with delayed ripening and improved shelf life. These modifications can significantly reduce post-

harvest losses and enhance marketability (Zhang *et al.*, 2016) [14].

Future prospects and challenges

While genome editing technologies hold immense promise for crop improvement, several challenges remain. These include regulatory hurdles, public acceptance, and the need for comprehensive risk assessments. As researchers continue to innovate and refine genome editing techniques, it is crucial to address these challenges to fully realize the potential of these technologies in sustainable agriculture. In conclusion, the applications of genome editing in crop research are vast and varied, with significant implications for enhancing disease resistance, improving nutritional quality, increasing tolerance to abiotic stresses, and accelerating breeding programs. As technologies continue to advance, the integration of genome editing into crop improvement strategies will play a critical role in ensuring global food security and sustainable agricultural practices.

Conclusion

Genome editing technologies represent a powerful suite of tools for advancing crop science and addressing global food security challenges. Techniques such as CRISPR/Cas9, ZFNs, and TALENs have demonstrated their potential to enhance crop traits, improve nutritional quality, and confer resistance to diseases and environmental stresses. As research continues to evolve, the integration of these technologies into breeding programs will likely lead to the development of innovative crop varieties that can meet the demands of a growing population in a changing climate. The future of agriculture may well depend on the successful application of genome editing to create resilient, high-yielding crops that contribute to food security and sustainability.

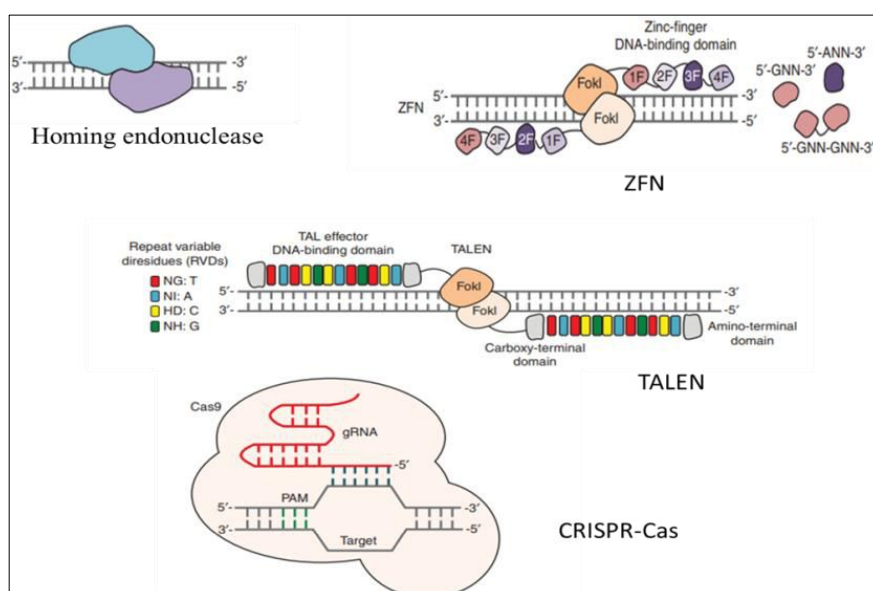


Fig 1: Different gene editing technologies

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