Recent Scenario of Development of Gene Editing Tools and Their Application in Crop Improvement

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Abstract

The rapid advancement of gene editing technologies has revolutionized modern agriculture and plant biotechnology. Traditional breeding methods, although successful in crop improvement, are often time-consuming and less precise. The emergence of targeted genome editing tools such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and especially Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated systems has transformed the field of crop improvement by enabling accurate and efficient genetic modifications. Recent developments including base editing, prime editing, multiplex genome editing, RNA editing, and AI-assisted CRISPR systems have further enhanced precision and minimized off-target effects. These tools are being widely utilized for developing crops with improved yield, nutritional quality, disease resistance, abiotic stress tolerance, and climate resilience. The present review discusses the recent scenario of development in gene editing technologies and highlights their significant applications in crop improvement programs. Furthermore, the review addresses regulatory concerns, biosafety issues, ethical considerations, and future prospects of genome editing in sustainable agriculture (Li, B., et. Al., 2024).

Key Words: *Gene editing, AI, CRISPR Cas-9, RNA editing, Zinc Finger Nuclease (ZFNs)*

Introduction

Global agriculture is facing tremendous challenges due to climate change, increasing population, declining arable land, and emerging pests and diseases. Conventional plant breeding methods have contributed significantly to crop development; however, they require several years to achieve desired traits because of genetic complexity and lengthy selection processes. Modern biotechnology has therefore shifted toward precise genome manipulation technologies that allow targeted modification of specific genes responsible for economically important traits.

Gene editing technologies have emerged as highly efficient molecular tools capable of introducing insertions, deletions, substitutions, or regulation of genes at specific genomic locations. The discovery of CRISPR-Cas systems marked a revolutionary milestone in plant biotechnology because of their simplicity, cost-effectiveness, and versatility compared with earlier technologies such as ZFNs and TALENs. Recent innovations like base editors and prime editors have made genome editing even more precise without causing double-stranded DNA breaks (Bhuyan, S. J. et al. 2023).

Genome editing is now extensively employed in crop plants including rice, wheat, maize, soybean, tomato, potato, cotton, and barley for developing superior cultivars with improved agronomic and nutritional characteristics. These technologies are expected to play a crucial role in achieving global food security and sustainable agriculture in the coming decades.

Evolution of Gene Editing Tools

1. Zinc Finger Nucleases (ZFNs)

ZFNs were among the earliest genome editing tools developed for targeted DNA modification. They consist of zinc finger DNA-binding proteins fused with the FokI endonuclease domain. Each zinc finger recognizes specific nucleotide triplets, allowing targeted cleavage of DNA sequences.

Although ZFNs demonstrated the feasibility of targeted genome editing, their complex design, high cost, and lower editing efficiency limited their widespread application in crop breeding. Nevertheless, they provided the foundation for the development of more advanced editing systemsBhuyan, (S. J. et al. 2023).

Applications of ZFNs

- Herbicide tolerance in maize
- Disease resistance in tobacco
- Improvement of oil quality in soybean

2. TALENs (Transcription Activator-Like Effector Nucleases)

TALENs were developed as an improvement over ZFNs. These nucleases utilize transcription activator-like effector proteins derived from *Xanthomonas* bacteria for DNA recognition coupled with FokI nuclease for DNA cleavage.

TALENs offered higher specificity and easier design than ZFNs. They became widely used in several plant species before the dominance of CRISPR systems.

Applications of TALENs

- Development of fragrant rice varieties
- Disease-resistant wheat
- Improved potato quality traits

Despite their effectiveness, TALENs require laborious protein engineering, which restricted their extensive commercial use (Bhuyan, S. J. et al. 2023).

CRISPR-Cas System: A Revolutionary Tool

Mechanism of CRISPR-Cas9

The CRISPR-Cas system originated as an adaptive immune mechanism in bacteria and archaea. In genome editing, the Cas9 nuclease is guided by a single guide RNA (sgRNA) to a specific DNA sequence where it introduces double-stranded breaks. These breaks are repaired by:

- Non-Homologous End Joining (NHEJ)
- Homology Directed Repair (HDR)

This process enables targeted gene knockout, insertion, or replacement.

Advantages of CRISPR-Cas9

- High precision
- Simplicity of design
- Cost-effectiveness
- Multiplex genome editing capability
- Broad applicability across species

CRISPR-Cas9 has become the most widely used genome editing system in agriculture.

Recent Developments in Gene Editing Technologies

1. Base Editing

Base editing is a novel CRISPR-derived technology that allows direct conversion of

one DNA base into another without creating double-stranded breaks. Two major types include:

- Cytosine Base Editors (CBEs)
- Adenine Base Editors (ABEs)

Base editing significantly reduces unwanted mutations and improves editing precision. Recent studies have shown its successful application in rice, wheat, tomato, and maize (Li, B., Sun, C., Li, J., & Gao, C. 2024).

Applications

- Herbicide resistance
- Nutritional enhancement
- Disease resistance

2. Prime Editing

Prime editing is regarded as the next generation of genome editing. It combines Cas9 nickase with reverse transcriptase and prime editing guide RNA (pegRNA) to introduce highly precise genetic modifications(Li, B., Sun, C., Li, J., & Gao, C. 2024).

Unlike traditional CRISPR systems, prime editing does not require donor DNA templates or double-strand breaks. It can perform:

- Base substitutions
- Insertions
- Deletions

Prime editing has enormous potential in crop improvement because of its flexibility and reduced off-target effects.

3. Multiplex Genome Editing

Modern CRISPR systems allow simultaneous editing of multiple genes in a single experiment. Multiplex editing is highly useful for polygenic traits such as:

- Yield
- Drought tolerance
- Nutritional quality

This approach accelerates crop improvement and reduces breeding duration considerably.

4. CRISPR-Cas12 and Cas13 Systems

New CRISPR-associated proteins such as Cas12 and Cas13 have expanded genome editing possibilities.

Cas12

- Produces staggered DNA cuts
- Suitable for multiplex editing
- Improved specificity

Cas13

- Targets RNA instead of DNA
- Useful for temporary gene regulation
- Potential applications in viral disease resistance

These systems represent significant advancements beyond conventional Cas9 editing (Chen, F. C. et al. (2024)

5. AI-Assisted Gene Editing

Artificial intelligence and machine learning are now integrated with CRISPR technologies for:

- Designing efficient guide RNAs
- Predicting off-target effects
- Optimizing editing efficiency

AI-assisted genome editing is improving the accuracy and reliability of crop genome engineering (Chen, L., et al., 2024).

Applications of Gene Editing in Crop Improvement

1. Improvement of Crop Yield

Gene editing has been used to manipulate genes controlling plant architecture, flowering, grain size, and photosynthetic efficiency.

Examples

- Increased grain yield in rice
- Enhanced wheat productivity
- Improved maize kernel development

Australian researchers recently initiated field trials for gene-edited wheat aimed at increasing yields by nearly 10%.

2. Abiotic Stress Tolerance

Climate change has intensified environmental stresses such as drought, salinity, heat, and flooding. Genome editing enables precise modification of stress-responsive genes (Mohamed, H. I., Khan, A., & Basit, A. 2024).

Achievements

- Drought-tolerant rice
- Salt-tolerant tomato
- Heat-resistant wheat

CRISPR-Cas9 mediated editing has emerged as an important tool for developing abiotic stress-resistant crops.

3. Disease Resistance

Plant diseases significantly reduce global crop productivity. Gene editing provides an effective approach for developing disease-resistant varieties without introducing foreign DNA (Dong, G., & Fan, Z. 2024).

Examples

- Bacterial blight-resistant rice
- Powdery mildew-resistant wheat
- Virus-resistant cassava

CRISPR-mediated crop protection strategies are rapidly expanding worldwide.

4. Nutritional Quality Enhancement

Genome editing has been employed to improve nutritional traits in crops.

Examples

- High-protein rice and soybean
- Vitamin-enriched tomato
- Reduced anti-nutritional factors in legumes

Recent studies demonstrated CRISPR-mediated enhancement of protein content in rice and soybean.

5. Improvement of Fruit Quality

Gene editing has been used for modifying fruit color, sweetness, shelf life, and texture.

Example

Scientists successfully edited tomato genes responsible for sugar metabolism, producing sweeter tomatoes while maintaining yield.

6. Food Safety Improvement

Genome editing is also being utilized to improve food quality and reduce harmful compounds.

Example

Researchers developed gene-edited wheat with significantly lower acrylamide formation during bread toasting, improving food safety.

Regulatory and Ethical Concerns

Despite enormous potential, genome editing technologies face several regulatory and ethical challenges.

Major Concerns

- Off-target mutations
- Environmental biosafety
- Gene flow to wild relatives
- Public acceptance
- Intellectual property rights

Different countries have adopted varying regulatory frameworks for gene-edited crops. Some nations regulate them similarly to GMOs, while others exempt gene-edited crops lacking foreign DNA.

China, Australia, India, the United States, and the United Kingdom are increasingly supporting genome-edited crop development through revised regulatory policies.

Future Prospects

The future of genome editing in agriculture appears highly promising. Emerging technologies such as:

- Prime editing
- RNA editing
- Epigenome editing
- AI-integrated CRISPR systems
- Transgene-free editing

will likely revolutionize sustainable agriculture.

Scientists anticipate the development of climate-smart crops capable of withstanding extreme environmental conditions while maintaining productivity. Jennifer Doudna emphasized that CRISPR technology could play a major role in addressing climate-related agricultural challenges.

Further integration of computational biology, synthetic biology, and machine learning with gene editing is expected to enhance editing precision and accelerate crop breeding programs globally.

Conclusion

Gene editing technologies have transformed modern crop improvement strategies by enabling precise and efficient modification of plant genomes. The progression from ZFNs and TALENs to CRISPR-Cas systems, base editing, and prime editing has significantly improved editing accuracy, flexibility, and applicability. These tools are now widely utilized for enhancing crop yield, stress tolerance, disease resistance, nutritional quality, and food safety.

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