

Genome Editing (CRISPR) in Fruit Crops

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Open Access

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Available online at

www.sunshineagriculture.vitalbiotech.org

Article History

Received: 3. 1.2026

Revised: 7. 1.2026

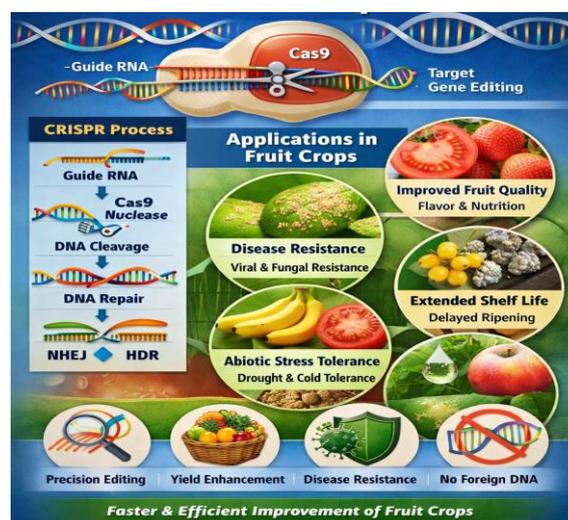
Accepted: 12. 1.2026

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INTRODUCTION

Fruit crop improvement is critical for ensuring nutritional security, enhancing farmer income, and promoting sustainable horticultural production systems. Fruits such as mango, banana, citrus, apple, grape, and strawberry contribute significantly to dietary diversity and rural livelihoods. However, improvement of fruit crops through conventional breeding is slow and inefficient due to their perennial nature, extended juvenile periods, complex genomes, vegetative propagation, and high levels of heterozygosity. Developing a new fruit variety often requires decades of selection and evaluation.

Genetic engineering has enabled the introduction of desirable traits into fruit crops, but public concerns, biosafety issues, and stringent regulatory frameworks have limited its commercial adoption. Genome editing technologies, particularly CRISPR/Cas systems, represent a paradigm shift in plant breeding by allowing precise, targeted, and predictable modifications of plant genomes. Unlike transgenic approaches, CRISPR-based editing can generate mutations indistinguishable from natural or induced mutations, making it more acceptable from regulatory and societal perspectives. As a result, CRISPR technology has emerged as a powerful tool for accelerating fruit crop improvement.



2. CRISPR Technology: An Overview

2.1 What is CRISPR?

CRISPR is a genome editing system derived from a natural adaptive immune mechanism found in bacteria and archaea. In this system, short DNA sequences from invading viruses are integrated into the bacterial genome and later transcribed into guide RNAs (gRNAs). These gRNAs guide CRISPR-associated (Cas) nucleases to recognize and cleave complementary DNA sequences in invading pathogens.

In plant genome editing, a synthetic guide RNA directs the Cas nuclease (such as Cas9 or Cas12a) to a specific target sequence in the plant genome, enabling precise genetic modification.

2.2 Mechanism of Action

The CRISPR genome editing process involves three major steps:

Target recognition: The guide RNA binds to a complementary DNA sequence adjacent to a protospacer adjacent motif (PAM).

- ✓ **DNA cleavage:** The Cas nuclease introduces a double-strand break (DSB) at the target site.
- ✓ **DNA repair:** The plant cell repairs the DSB through endogenous repair pathways:

Non-Homologous End Joining (NHEJ), resulting in insertions or deletions (indels) that often cause gene knockouts.

Homology-Directed Repair (HDR), which allows precise gene replacement or insertion when a repair template is provided. These mechanisms enable targeted gene disruption, modification, or replacement.

3. Importance of CRISPR for Fruit Crops

CRISPR technology is particularly valuable for fruit crops due to several inherent challenges:

- ✓ Long breeding cycles and extended juvenile phases
- ✓ High genetic heterozygosity and polyploidy
- ✓ Difficulty in introgressing traits without linkage drag
- ✓ Limited success of traditional hybridization in vegetatively propagated crops

CRISPR enables rapid improvement of elite cultivars by directly modifying target genes

without altering the overall genetic background, thereby preserving desirable horticultural traits.

4. Applications of CRISPR in Fruit Crops

4.1 Improvement of Fruit Quality Traits

Fruit quality attributes such as size, shape, flavor, color, texture, and nutritional composition are key determinants of consumer preference and market value. CRISPR has been successfully used to edit genes regulating these traits. Target traits include:

- ✓ Sugar and organic acid metabolism
- ✓ Carotenoid and anthocyanin biosynthesis
- ✓ Cell wall composition and firmness
- ✓ Aroma and flavor-related metabolites

Examples:

- ✓ **Tomato:** Editing genes involved in fruit ripening and carotenoid accumulation to improve shelf life and nutritional quality.
- ✓ **Strawberry:** Modification of genes associated with flavor, aroma, and fruit color.

4.2 Enhancement of Shelf Life and Post-Harvest Quality

Post-harvest losses in fruit crops are substantial due to rapid ripening, softening, and senescence. CRISPR has been used to target genes involved in ethylene biosynthesis, signaling, and cell wall degradation.

Examples:

- ✓ **Tomato:** CRISPR-mediated editing of ripening-related genes to delay softening and extend shelf life.
- ✓ **Banana:** Targeting ethylene signaling genes to reduce post-harvest losses and improve transportability.

4.3 Disease Resistance

Fruit crops are highly susceptible to bacterial, viral, and fungal diseases, leading to severe yield losses and increased pesticide use. CRISPR-based genome editing enhances disease resistance by:

- ✓ Knocking out susceptibility (S) genes
- ✓ Modifying host-pathogen interaction pathways
- ✓ Directly targeting viral genomes

Examples:

- ✓ **Citrus:** Improved resistance to citrus canker through editing of susceptibility genes.

- ✓ **Grapevine:** Enhanced resistance to powdery mildew by modifying disease-related genes.

4.4 Abiotic Stress Tolerance

Climate change-induced stresses such as drought, salinity, heat, and cold pose major challenges to fruit crop production. CRISPR enables precise modification of stress-responsive genes and regulatory networks.

Examples:

Apple and grapevine: Editing transcription factors and signaling genes associated with drought and temperature stress tolerance.

4.5 Yield and Plant Architecture Improvement

Yield-related traits such as flowering time, fruit set, and plant architecture can be optimized using CRISPR technology. Applications include:

- ✓ Regulation of flowering genes
- ✓ Improvement of fruit set and uniformity
- ✓ Modification of canopy architecture for high-density planting systems

5. Methods of CRISPR Delivery in Fruit Crops

Common methods for delivering CRISPR components into fruit crop cells include:

- ✓ Agrobacterium-mediated transformation
- ✓ Particle bombardment (biolistics)
- ✓ Protoplast transfection
- ✓ Ribonucleoprotein (RNP) delivery
- ✓ DNA-free genome editing using RNPs is particularly promising as it minimizes off-target effects and improves regulatory acceptance.

6. Advantages of CRISPR over Conventional Breeding and GM Technology

- ✓ High precision and target specificity
- ✓ Faster breeding and trait development
- ✓ No integration of foreign DNA (in many cases)
- ✓ Reduced linkage drag
- ✓ Cost-effective and scalable
- ✓ Greater public and regulatory acceptance in several countries

7. Challenges and Limitations

Despite its potential, CRISPR technology faces several challenges in fruit crops:

- ✓ Risk of off-target mutations

- ✓ Low editing and regeneration efficiency in perennial species

- ✓ Difficulty in transformation of woody fruit crops

- ✓ Regulatory uncertainty across countries

- ✓ Ethical, biosafety, and public perception concerns

8. Regulatory and Policy Aspects

Many countries have adopted differentiated regulatory frameworks for genome-edited crops, particularly those free from foreign DNA. Such policies are expected to accelerate commercialization of CRISPR-edited fruit crops. However, lack of global regulatory harmonization remains a major bottleneck for international trade and technology deployment.

9. Future Prospects

Emerging genome editing tools such as base editing, prime editing, epigenome editing, and multiplex CRISPR systems will further expand the scope of fruit crop improvement. Integration of CRISPR with genomic selection, speed breeding, and omics technologies (genomics, transcriptomic, metabolomics) will revolutionize precision breeding in horticulture.

CONCLUSION

Genome editing using CRISPR technology represents a revolutionary advancement in fruit crop improvement. By enabling precise, rapid, and sustainable genetic modifications, CRISPR addresses long-standing challenges associated with conventional breeding of perennial fruit crops. With continued technological advancements, improved transformation protocols, and supportive regulatory frameworks, CRISPR-based genome editing is poised to play a transformative role in enhancing productivity, quality, resilience, and sustainability of fruit crops, thereby strengthening global horticulture and nutritional security.

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