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Genome Editing Tools for Vegetable Improvement

*Teja S
Department of Vegetable Science, ASPEE College of Horticulture,
NAU, Navsari, Gujarat, India
*Corresponding Author's email: tejas01032001@gmail.com

Genome editing has quickly become a game-changing technology in plant science. It lets scientists change DNA sequences very precisely to make targeted mutations and better crop varieties. Genome editing is easier, more accurate and has fewer risks of affecting the wrong genes than classical mutation breeding, which is slow and often unpredictable. Researchers can now edit almost any part of the genome by using tools like CRISPR/Cas9, TALENs, and Zinc Finger Nucleases. Editing is possible even in crops with complicated genetic backgrounds and having few options for breeding. This is especially important for vegetables because they are important for human health and nutrition because they are full of vitamins, minerals, and fiber. Genome editing speeds up functional genomics research in addition to breeding. This helps scientists find out what genes do with more accuracy than ever before. In the future, genome editing could help improve vegetables in a way that is environmentally friendly, uses fewer chemicals and leads to healthier diets. However, there are still problems with regulation and public acceptance. These tools represent a new era in vegetable science, where new ideas at the genetic level led to better crops and better food for everyone.

Introduction

Vegetables are an important part of people's diets because they are full of vitamins, minerals, fiber, and bioactive compounds that keep people healthy. However, growing vegetables is still hard because of pests, diseases, climate stress, and losses after harvest that can lower yield and quality. Many better varieties have come from traditional breeding, but the process is slow, takes a lot of work, and doesn't always work for crops with complicated genomes. The advent of genome-editing technologies signifies a pivotal moment in crop science. Genome editing lets you make exact, predictable changes to specific parts of the DNA, as opposed to random changes that happen in conventional mutation breeding. Scientists can use tools like CRISPR/Cas9, TALENs, and Zinc Finger Nucleases to cut, add, or change genes with amazing precision. These new technologies not only speed up research in functional genomics, but they also make it possible to grow vegetables that are more nutritious, durable, and easy for farmers to work with. Genome editing is already showing its potential by making tomatoes that can resist viruses and cucumbers that can better handle powdery mildew. As rules change and more people learn about these technologies, they promise to change how we improve vegetables. They will provide long-term solutions that use fewer chemicals, make food safer, and lead to healthier diets.

Types of genome editing tools

Zinc Finger Nucleases (ZFNs)

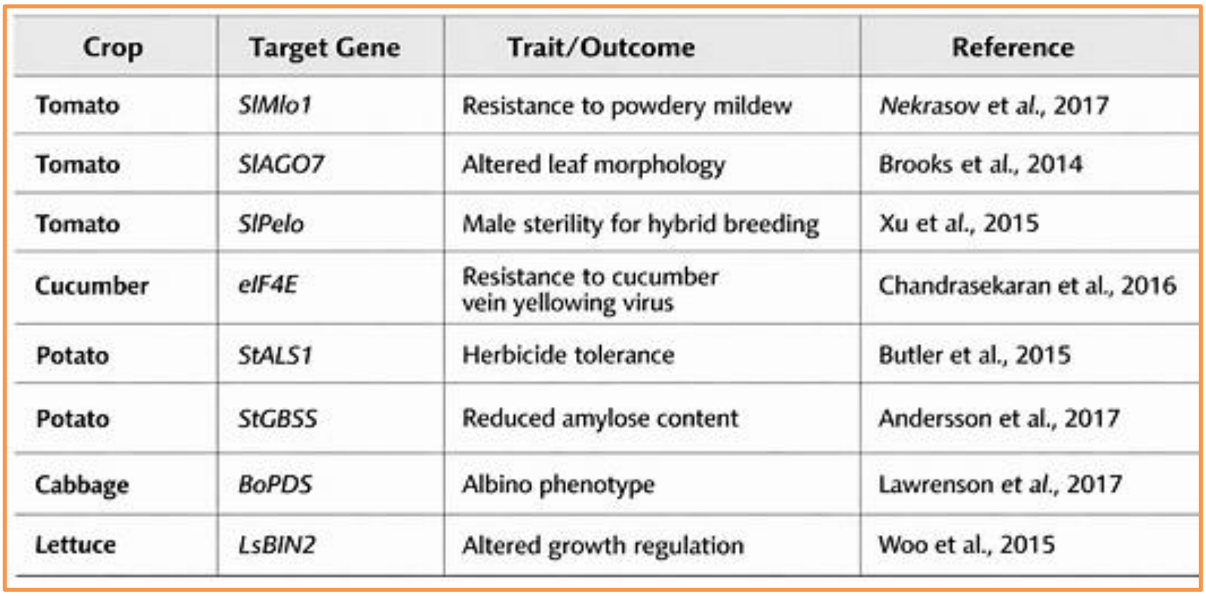
ZFNs are artificially engineered restriction enzymes that combine a zinc finger DNA-binding domain with the FokI nuclease catalytic domain. Each zinc finger recognizes a 3-base pair sequence and arrays of fingers can be designed to target specific DNA sites. When two FokI domains dimerize at the target they create a double-strand break, enabling gene editing. ZFNs are versatile but can cause off-target effects due to sequence context, leading to genome instability. They have been used experimentally in crops like wheat, maize, tobacco, and rice to introduce herbicide tolerance and site-specific gene integration, though no commercial vegetable varieties currently rely on ZFNs (Brock *et al.*, 2025). In tomato target gene NF-Y, L1L4, NF-YB6 responsible for biosynthesis of seed storage proteins and fatty acids then mutants showed varied metabolite profiles and high amounts of OA as compared to wildtype (Gago *et al*., 2017)

Transcription Activator-Like Effector Nucleases (TALENs)

TALENs are the second generation of site-specific nucleases, designed as an alternative to ZFNs. They combine a DNA-binding domain made of TALE repeats (from *Xanthomonas* bacteria) with the FokI nuclease. Each TALE repeat recognizes a single nucleotide, giving TALENs higher specificity and flexibility compared to ZFNs. By assembling repeat variable di-residues (RVDs), researchers can target virtually any DNA sequence and induce double-strand breaks. TALENs have been successfully applied in crops such as rice, soybean, wheat, and potato to improve traits like oil quality, disease resistance and storage stability. Their advantages include easier design and fewer off-target effects, though limitations such as sensitivity to cytosine methylation and sequence requirements at target sites remain (Kim). In potato **target gene** *Vinv* is edited by using TALEN(SDN1) to produce mutants with improved cold storage and processing traits. (clasen *et al.*, 2016)

CRISPR/Cas9

CRISPR/Cas9 is a powerful genome-editing system adapted from the natural bacterial immune defence against viruses. It uses two components: the Cas9 nuclease and a guide RNA (sgRNA) that directs Cas9 to a specific DNA sequence near a PAM site. Once bound, Cas9 makes a precise cut, enabling targeted mutations or gene replacements. Compared to ZFNs and TALENs, CRISPR/Cas9 is simpler, cheaper and highly versatile with the ability to edit multiple sites at once. It has been widely applied in crops, including vegetables, to improve disease resistance, stress tolerance and nutritional quality. While off-target effects remain a challenge, improved design strategies and breeding can minimize risks, making CRISPR/Cas9 the most widely adopted genome-editing tool in plant science today. (Kim *et al.*, 2021)



Crop	Target Gene	Trait/Outcome	Reference
Tomato	*SlMlo1*	Resistance to powdery mildew	Nekrasov et *al.*, 2017
Tomato	*SlAGO7*	Altered leaf morphology	Brooks et al., 2014
Tomato	*SlPelo*	Male sterility for hybrid breeding	Xu et al., 2015
Cucumber	*eIF4E*	Resistance to cucumber vein yellowing virus	Chandrasekaran et al., 2016
Potato	*StALS1*	Herbicide tolerance	Butler et al., 2015
Potato	*StGBSS*	Reduced amylose content	Andersson et al., 2017
Cabbage	*BoPDS*	Albino phenotype	Lawrenson et *al.*, 2017
Lettuce	*LsBIN2*	Altered growth regulation	Woo et al., 2015

Figure 1. Application of CRISPR-Cas9-based editing of genes in vegetables (Kim *et al.*,2021).

Base Editing

Base editing is a newer genome-editing approach that allows precise single-base changes in DNA without creating double-strand breaks. It uses a modified Cas9 nickase fused with a deaminase enzyme, guided by an sgRNA to the target site. Two main types are cytosine base editors (CBEs), which convert C-G to T-A and adenine base editors (ABEs) which convert

A-T to G-C. This technology has been applied in crops like rice, maize, wheat and potato to introduce point mutations with high efficiency. More advanced systems, such as dual base editors (STEME) can perform multiple base conversions simultaneously, offering powerful tools for fine-tuning traits and studying regulatory regions. Base editing is especially valuable for improving vegetable crops where subtle gene changes can enhance nutrition, stress tolerance, or disease resistance. (Kim *et al.*,2021)

Prime Editing

Prime editing is a recent breakthrough designed to overcome the limitations of earlier genome-editing tools. It uses a Cas9 nickase fused with a reverse transcriptase and guided by a prime editing RNA (pegRNA) to directly write new genetic information into the target site. Unlike CRISPR/Cas9, it does not rely on double-strand breaks and can introduce precise substitutions, small insertions, or deletions. Prime editing enables up to 12 types of base conversions and has been successfully demonstrated in rice and wheat, though efficiency in plants is still relatively low. Its potential lies in creating highly accurate edits without linkage drag, making it a promising tool for future vegetable improvement once technical challenges are addressed. (Kim *et al.*,2021)

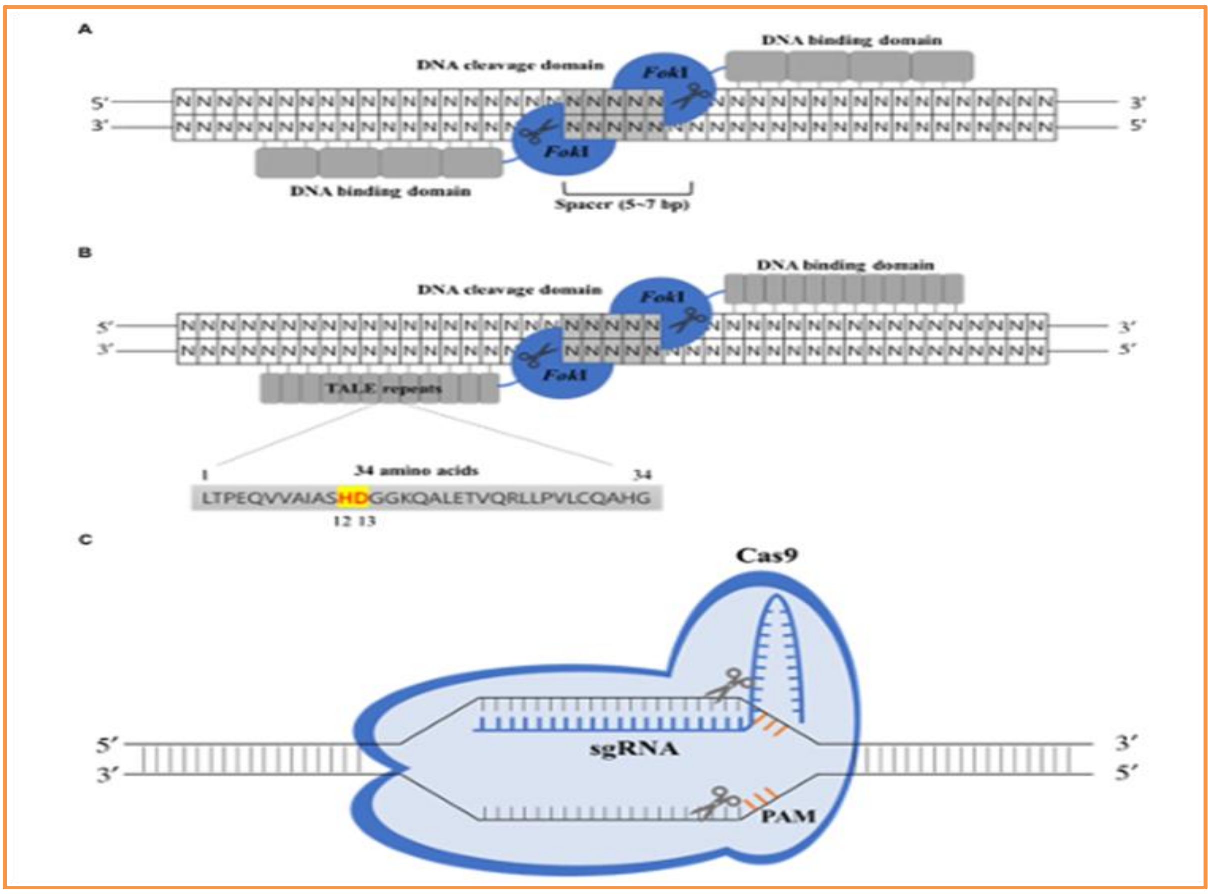


Figure 2. genome-editing technologies using site-specific nucleases include ZFNs, TALENs and CRISPR-Cas9 systems (Kim *et al.*,2021)

Challenges of Genome editing tools (Kim *et al.*, 2021)

- Limited genomic resources
- Complex and polyploid genomes
- Low transformation and regeneration efficiency
- Off-target mutations
- Delivery bottlenecks for Cas proteins and guide RNAs
- Trait complexity
- Regulatory and public acceptance barriers
- Intellectual property restrictions
- Limited field validation and trait stability

Conclusion

Genome editing tools have made it possible to make precise changes in vegetable crops that make them more resistant to disease, better for nutrition and able to handle stress. Even though there are problems like off-target effects, complicated genomes and low transformation efficiency, CRISPR/Cas and more advanced editors like base and prime editing are still very useful tools. These technologies can speed up breeding and provide strong, high-quality vegetables for sustainable agriculture if they are improved and tested in more places.

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