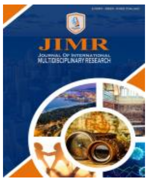

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GENETIC ENGINEERING OF TOMATO PLANTS: A NARRATIVE REVIEW OF THE UTILIZATION OF AGROBACTERIUM AND CRISPR-CAS9

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Abstract: *Global population growth increases the demand for nutritious food, while tomato (Solanum lycopersicum L.) productivity often declines due to biotic and abiotic stress. CRISPR/Cas9 technology offers a precision approach to improving the nutritional quality, disease resistance, and metabolic efficiency of tomatoes. This review analyzes research from 2015 to 2025 on gene editing related to vitamin C enhancement, leaf morphology modification, fruit ripening, and strengthening resistance to pathogens such as TYLCV and powdery mildew, with Agrobacterium-based transformation proven effective in producing stable changes. However, challenges such as off-target risks and limited access to technology remain obstacles. Overall, CRISPR/Cas9 has the potential to accelerate tomato variety improvement for global food security, but further research is needed to enhance the precision and sustainability of its development.*

Abstrak: Pertumbuhan penduduk global meningkatkan kebutuhan pangan bergizi, sementara produktivitas tomat (Solanum lycopersicum L.) sering menurun akibat stres biotik dan abiotik. Teknologi CRISPR/Cas9 menawarkan pendekatan presisi untuk meningkatkan kualitas gizi, ketahanan penyakit, dan efisiensi metabolik tomat. Tinjauan ini menganalisis penelitian 2015-2025 mengenai pengeditan gen yang berkaitan dengan peningkatan vitamin C, modifikasi morfologi daun, pematangan buah, serta penguatan ketahanan terhadap patogen seperti TYLCV dan embun tepung, dengan transformasi berbasis Agrobacterium yang terbukti efektif menghasilkan perubahan stabil. Meskipun demikian, tantangan seperti risiko off-target dan keterbatasan akses teknologi masih menjadi hambatan. Secara keseluruhan, CRISPR/Cas9 berpotensi mempercepat perbaikan varietas tomat untuk ketahanan pangan global, namun penelitian lebih lanjut diperlukan untuk meningkatkan presisi dan keberlanjutan pengembangannya.

INTRODUCTION

Rapid global population growth has created major challenges in meeting the world's food needs. One of the main challenges is the increasing demand for highly nutritious foods, amid various problems that threaten agricultural production, such as declining crop quality and pest attacks. In this regard, tomatoes (*Solanum lycopersicum* L.) play an important role as a major food source rich in vitamins and antioxidants, such as vitamin C, lycopene, and flavonoids, which have many benefits for human health (Hasfikasari & Amin, 2024). However, even though tomatoes are an important commodity in the food industry, their quality and productivity are often hampered by unpredictable environmental factors, such as climate change, disease outbreaks, and water shortages. This results in a decline in fruit quality, such as overly watery texture, short shelf life, and susceptibility to pests and diseases. Therefore, efforts to increase the nutritional content, especially vitamin C, in tomatoes are very important (Astuti & Achamar, 2022).

Genetic engineering is a potential solution to this problem. According to Yulianti *et al.,* (2024), genetic engineering is a technique of deliberately modifying DNA through a recombinant DNA process (in vitro recombination), which involves artificially combining genes (with the help of vectors/plasmids, restriction enzymes, and ligase enzymes) and then inserting the engineered DNA into host cells to produce organisms with new traits or to produce specific proteins. One widely used approach is genetic transformation *using Agrobacterium tumefaciens*, which functions as a vector to insert desired genes into tomato plants. This process enables more efficient plant breeding to improve desired traits, such as disease resistance and increased nutritional content. For example, the introduction of the lanceolate gene in tomatoes has shown changes in leaf morphology and increased nutrient concentrations, particularly vitamin C, which is focused on fruit development (Advenita *et al.,* 2023).

On the other hand, genome editing technologies such as CRISPR-Cas9 now offer a more precise and efficient way to modify tomato genes. Using the CRISPR-Cas9 system, genetic modifications can be made with highly specific targets, enabling faster and more cost-effective plant breeding compared to traditional breeding techniques. This technology has been used to edit various genes that affect tomato quality, such as genes related to disease resistance, drought tolerance, and increased antioxidant content (Tiwari & Singh, 2023). With the advancement of CRISPR-Cas9 technology, the future of genetically engineered tomatoes promises more opportunities to not only improve nutritional quality but also address the challenges of sustainable agricultural production. The integration of this technology is expected to accelerate the improvement of tomato plants, making them more resistant to climate change and more nutritious, thereby meeting the growing global food demand (Rustan *et al.,* 2025).

METHOD

This study used a literature review approach to analyze the application of CRISPR-Cas9 technology in tomato (*Solanum lycopersicum* L.) genetic engineering, focusing on editing the lanceolate gene to increase vitamin C content. The articles analyzed were obtained through searches in the Google Scholar and Frontiers databases with publications ranging from 2023 to 2025. The CRISPR-Cas9 method was applied to edit the lanceolate gene in tomatoes, which plays a role in changing leaf morphology to be simpler and diverting the flow of nutrients to the fruit. The editing process was carried out by designing single guide RNA (sgRNA) directed at specific locations in the tomato genome, using the Cas9 enzyme to cut the DNA at the target site, while *Agrobacterium tumefaciens* was used as a vector to insert the gene into the plant.

RESULTS AND DISCUSSION

Results

Table 1. Comparison of research results on the application of the CRISPR-Cas9 method using *Agrobacterium tumefaciens* in tomato plants.

No.	Author & Year	Research Object	Method/Approach	Key Findings	Implications for Cell and Molecular Biology
1.	Advenita *et al.,* (2023)	Tomato plant (Solanum lycopersicum L.)	Genetic engineering using *Agrobacterium tumefaciens* and CRISPR-Cas9 (target gene) *lanceolate*)	There was a morphological change in the leaves, which became narrower (lanceolate), and an increase in vitamin C content in the tomatoes.	Gene transfer via *Agro bacterium* causes chan ges in target gene expr ession; genome editing affects the transcriptio nal regulation of genes involved in photosynt hesis and secondary metabolite allocation.
2.	Tiwari *et al.,* (2023)	Tomato plant	CRISPR-Cas9-based genome editing with specific guide RNA.	Gene editing is precise, improving fruit quality, disease resistance, and ripening control.	Cas9 cuts target DNA through the HNH and RuvC domains; the cell's DNA repair mec hanisms (NHEJ/HDR) produce directed muta tions that affect gene expression regulation.
3.	Rustan *et al.,* (2025)	Food microorganisms *(Saccharomyces*	Genetic engineering of microorganisms for	Improved fermentation efficiency, food	Modification of metab olic pathways alters the expression of

		cerevisiae, Lactobacillus spp., Escherichia coli).	metabolic pathway optimization (fermentation, vitamin, protein, and enzyme production).	nutrient content, functional foods, and alternative protein production.	enzymatic genes; increased enzyme activity and synthesis of primary-secondary metabolites at the cellular and molecular levels.

Review of the literature for the period 2023-2025 indicates that the application of CRISPR/Cas9 plays a significant role in improving the quality, resilience, and metabolic efficiency of tomato plants through various increasingly precise and effective gene editing strategies.

Genetic Editing to Improve Tomato Fruit Quality

The use of CRISPR/Cas9 in tomato genetic modification shows great potential for improving fruit quality. In a study conducted by Tiwari & Singh (2023), CRISPR/Cas9 technology was used to edit various genes in tomatoes, with the aim of improving fruit quality, including higher vitamin C content. One of the main targets was genes that affect photosynthesis and energy activation in the fruit, thereby increasing the nutritional content of tomatoes. This modification not only improves the nutritional quality of the fruit but also extends its shelf life, making it more commercially valuable. Furthermore, research by Advenita *et al.,* (2023), shows that gene editing in tomatoes using CRISPR/Cas9 can increase photosynthetic efficiency by allocating more energy to fruit development. This results in tomatoes with higher vitamin C content. This approach enables more controlled tomato ripening, more consistent fruit quality, and meets increasingly stringent market standards (Sari *et al.,* 2021).

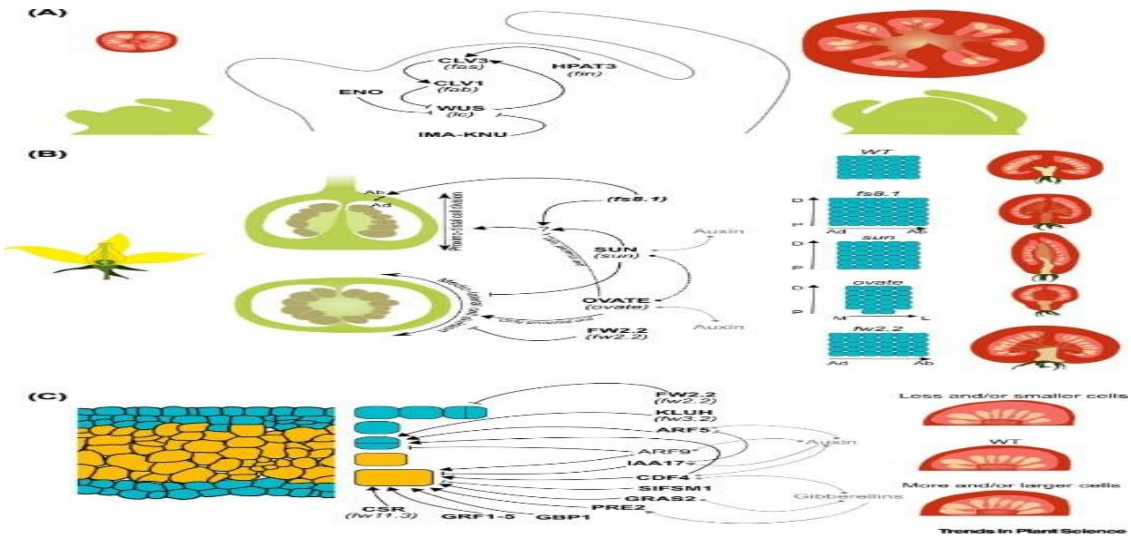

Figure 1. Tomato fruit structure (Advenita *et al.,* 2023)

Figure 1 shows the results of research conducted by Advenita *et al.*, (2023), stating that the shape and content of tomatoes are controlled by the lanceolate gene. The lanceolate gene acts as a regulator of miR319, which can contribute to growth and vitamin production as well as fruit content through the *Lanceolate* (La) allele in TGRC (Calvaro *et al.,* 2024).

Disease And Stress Resistance

In addition to improving fruit quality, CRISPR/Cas9 also plays a role in increasing tomato resistance to various diseases and environmental stressors. Tiwari & Singh, (2023), revealed that the gene editing process using CRISPR/Cas9 successfully produced tomatoes with increased resistance to diseases such as tomato yellow leaf curl virus (TYLCV) and powdery mildew. This was achieved through the editing of genes involved in plant maintenance mechanisms, such as those affecting the tomato's immune system. By optimizing these genes, tomatoes can survive longer against pathogen attacks, thereby reducing crop losses.

Furthermore, research by Rustan *et al.,* (2025) also emphasizes the importance of CRISPR/Cas9 in increasing tomato tolerance to abiotic stressors such as drought, heat, and salinity. Gene editing that affects plant metabolic pathways allows tomatoes to survive better in adverse environmental conditions, a major challenge in global agriculture. This research shows that CRISPR/Cas9 not only focuses on increasing crop yields but also helps address the challenges of plant resilience to climate change and other stressors.

Leaf Morphological Changes and Energy Transfer to Fruit

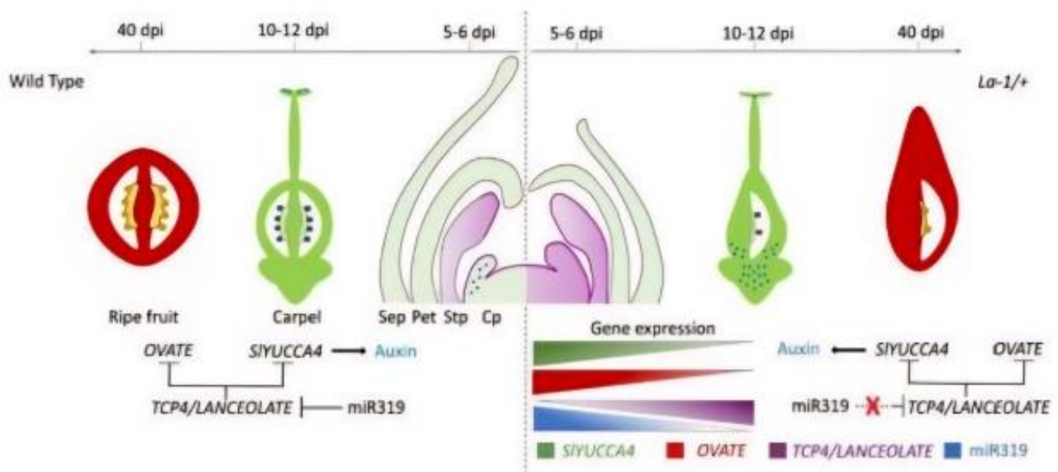

Figure 2. Gene expression scheme (Advenita et al., 2023)

Classically, the results of genetic engineering on partial dominant *lanceolate* (La) mutations in large compound leaves of tomatoes (*Solanum lycopersicum*). The La gene encodes a transcription factor from the TCP family that contains a miR319 binding site, as shown in Figure 2. Modifying tomato leaf morphology through CRISPR/Cas9 lanceolate gene editing is crucial for improving fruit yield and quality. According to Advenita *et al.,* (2023), narrower (*lanceolate*) leaves allow plants to allocate more energy to fruit development rather than maintaining excessive leaf growth. This process maximizes the production of vitamins and other nutrients in the fruit, while smaller leaves reduce the energy required for vegetative growth. This aims to increase fruit yield that is richer in vitamins, especially vitamin C. In another study conducted by Tiwari & Singh (2023), leaf modification through lancet gene editing was also proven effective in increasing fruit yield by reducing competition between vegetative and generative growth. The resulting tomatoes had fewer large leaves and more energy was diverted to fruit development, producing tomatoes with higher nutritional quality and larger fruit.

The Use of *Agrobacterium Tumefaciens* in Tomato Genetic Transformation

Agrobacterium tumefaciens has been proven to be a highly effective vector for tomato genetic transformation using CRISPR/Cas9 technology. Research by Advenita *et al.,* (2023) shows that *Agrobacterium tumefaciens* can efficiently deliver CRISPR/Cas9 constructs into tomato cells, enabling stable and heritable genetic editing. The advantage of *Agrobacterium* as a vector lies in its ability to transfer genes into the plant genome with high precision, making it the primary choice in plant biotechnology. Biotechnology is not a means to replace the principles or objectives of agriculture as a food producer, but rather is used to improve the quality of agricultural products (Roviati, 2022). The success of using *Agrobacterium* bacteria in tomato genetic transformation is also explained by Tiwari & Singh (2023), who note that by utilizing this technology, edited genes can be inserted into the tomato genome without disrupting the plant's genetic balance. Furthermore, this transformation allows for more efficient modifications compared to conventional methods, such as transgenesis, which are often more time-consuming and expensive.

Another advantage of tomatoes engineered with *Agrobacterium tumefaciens* is that they increase the nutritional content of tomato plants and improve the plants' ability to adapt to the environment, for example, to live in extreme conditions, such as land with high acidity and salt content. Genetically engineered tomatoes have the advantage of high vitamin content through plant improvement and modification. Gene editing technology will make one beneficial trait more efficient and precise because the genetic structure of tomatoes and *Agrobacterium tumefaciens* has been studied previously (Santoso, 2018).

Innovation in Gene Editing Techniques with Cas9 Variants

Cas9 is a nuclease enzyme that cuts target DNA at sequences located near the protospacer adjacent motif (PAM). This is because the Cas9 protein has two domains homologous to the RuvC and HNH nucleases, each of which plays a role in cutting one of the DNA duplexes and the blunt-cut target tomato (*Solanum lycopersicum* L.) DNA sequence (Kurniawati, 2020). Further research by Tiwari & Singh (2023), revealed that Cas9 variants, such as Cas12a, offer advantages in genetic editing precision. These variants can recognize a wider range of PAM sequences, enabling genetic editing at locations that were previously difficult to reach with conventional Cas9. This advantage allows for broader and more efficient tomato genome modification, which is crucial for improving stress tolerance and fruit yield. Furthermore, new approaches such as base editing and prime editing applied in CRISPR/Cas9 further improve the accuracy of genetic editing, reducing the risk of off-target mutations that were common in previous gene editing techniques. This opens up new opportunities for safer and more efficient genetic modification in the development of superior tomato plants (Hermantara, 2024)

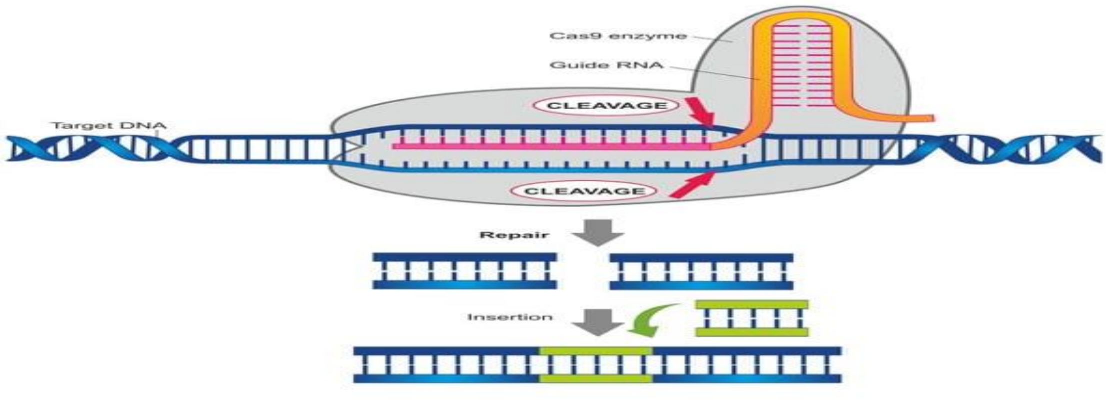

Figure 3. CRISPR-Cas9 gene editing technique (Advenita et al., 2023)

The image shows the CRISPR-Cas9 mechanism used in the journal to edit the lanceolate gene in tomato plants. In this process, guide RNA (gRNA) recognizes and attaches to the target DNA sequence, then the Cas9 enzyme cuts the DNA at that location through two nuclease domains (RuvC and HNH). After the DNA is split, the plant cells will repair it, either by closing the DNA cut or by inserting a new sequence if donor DNA is provided. This cutting and repair process enables the desired genetic changes, such as modifying the lanceolate gene to make the leaves narrower so that more photosynthesis is directed to the fruit. This mechanism forms the basis of genetic engineering in the journal, which aims to improve tomato quality, including vitamin C content, through genome editing using the CRISPR-Cas9 system.

The Potential of CRISPR/Cas9 in Improving Genes Related to Fruit Ripening

One of the main focuses of tomato genetic engineering using CRISPR/Cas9 is the editing of genes involved in the fruit ripening process, such as the RIN (Rip Inhibitor of Ripening) gene. Research by Tiwari & Singh (2023), revealed that editing the RIN gene can accelerate or slow down the tomato ripening process, depending on market demand. More controlled ripening times allow tomatoes to be harvested at peak ripeness, thereby improving quality and storage life. Conversely, research by Advenita *et al.,* (2023), shows that editing genes involved in fruit ripening also allows for the production of tomatoes with more flexible ripening properties. Modified tomatoes can have a longer shelf life, reducing post-harvest losses, which are often a problem in the distribution of horticultural products.

The Impact of CRISPR/Cas9 Technology on Tomato Resistance to Pathogens

One important application of CRISPR/Cas9 in tomatoes is to increase plant resistance to pathogens. According to Advenita *et al.,* (2023), genetic editing in tomatoes has resulted in plants that are more resistant to viruses such as Tomato Yellow Leaf Curl Virus (TYLCV) and powdery mildew. Genes involved in the plant's immune response have been edited to improve tomatoes' ability to detect and treat infections. Tiwari & Singh (2023),also note that by editing the genes that regulate the plant's immune system, tomatoes can show greater resistance to various pathogens. This reduces the need for pesticides, which in turn can lower the environmental impact of tomato cultivation.

Challenges in Implementing CRISPR/Cas9 Genetic Engineering in Tomatoes

Although CRISPR/Cas9 technology offers great potential, its application in tomatoes still faces several challenges. One of the main challenges is the risk of off-target mutations, which can cause unwanted genetic changes in plants. Research by Advenita *et al.* (2023), shows that despite the high precision of CRISPR/Cas9, there is still a small possibility of off-target mutations that can have a negative impact on plants. Furthermore, issues related to production costs and technical complexity also hinder the large-scale implementation of this technology. According to Tiwari & Singh (2023), the development of a cheaper and more efficient transformation system is still needed so that CRISPR/Cas9 can be accessed by more farmers, especially in developing countries.

The Effect of the CRISPR/Cas9 Method on Improving the Metabolic Chain of Tomatoes

Research by Tiwari & Singh (2023) and Rustan *et al.,* (2025), shows that genetic editing with CRISPR/Cas9 can affect metabolic pathways in tomatoes, increasing energy utilization efficiency and increasing the production of bioactive compounds such as vitamin C and carotenoids. These metabolic pathway modifications enable tomatoes to utilize available resources more efficiently, thereby increasing yield and fruit quality. In the future, further development in optimizing metabolic pathways using CRISPR-Cas9 technology could help produce tomatoes with better nutritional quality and higher resistance to adverse environmental conditions. This innovation has the potential to become a solution and strategy for increasing food production (Rao, 2021).

CONCLUSION

The application of CRISPR/Cas9 technology in tomato genetic engineering offers great potential for improving fruit quality, disease resistance, and tolerance to environmental stress. Genetic modifications such as editing the lanceolate gene to increase vitamin C content and genes involved in fruit ripening provide significant benefits in the production of higher quality tomatoes. Although this technology offers many advantages, challenges such as the risk of off-target mutations, high production costs, and technical complexity remain major obstacles. Therefore, further research is needed to optimize the use of CRISPR/Cas9 in the future, with a focus on cost efficiency, food safety, and consumer acceptance, so that this technology can be widely and sustainably applied in the tomato farming industry.

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