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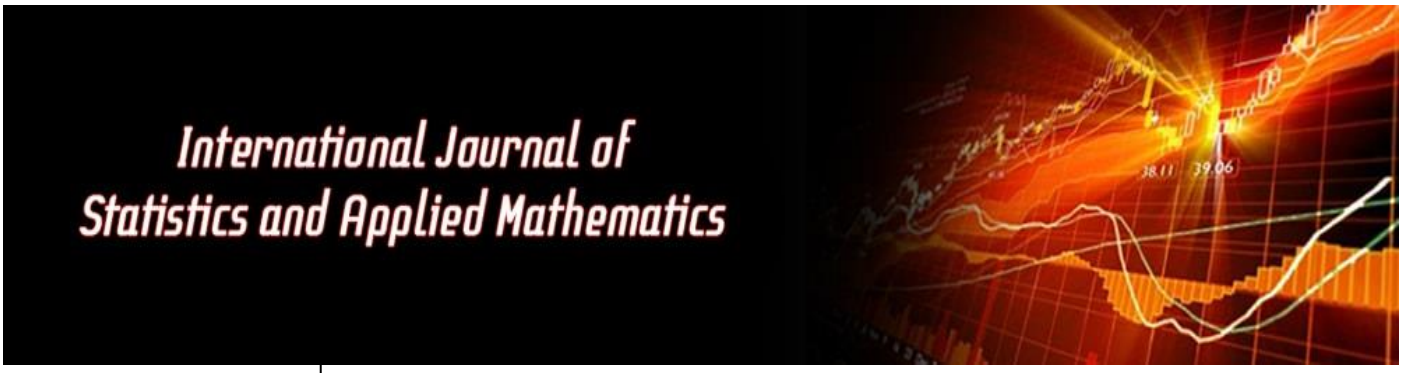

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Post-harvest loss reduction in tomato by employing genome editing technology

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Abstract
This review paper delves into the post-harvest loss of tomatoes, analyzing its implications for farmers and the supply chain. It explores gene editing tools, including TALEN, ZFN, and CRISPR/Cas9, and their advantages and disadvantages. The paper focuses on how genome editing techniques can be applied to enhance tomato post-harvest life and quality. Challenges in implementing gene editing solutions are discussed, and potential ways to overcome them are proposed. Overall, the study emphasizes the promising potential of gene editing to reduce post-harvest losses and improve tomato production efficiency.

Keywords: Genome editing, CRISPR/Cas9, tomato, post-harvest

Introduction
Tomato holds the title of the world's largest vegetable crop, renowned for its exceptional nutritional value and extensive cultivation. It is highly regarded as a protective food due to its special nutrient content and its wide availability. Being one of the essential vegetable crops, tomatoes are primarily grown for their juicy and fleshy fruits. They hold significant commercial and dietary importance, making them vital to global agriculture and diets. (Quinet *et al.*, 2019) [23]. In 2011, worldwide tomato production reached approximately 160 million tonnes, accounting for approximately 15 percent of the total vegetable production. On average, individuals globally consumed about 20 kilograms of tomatoes per year. In 2022, the global consumption of processed tomatoes amounted to 79.52 million tons. It is projected that this consumption will continue to increase, with estimates indicating it will reach 99.46 million tons by the year 2028. (Source-Research and markets, Global Tomato Industry Report- 2020: Trends and Opportunities by Country, Consumption, Production, Price Developments, Imports and Exports (2007-2025), Dublin, Feb. 14, 2020 (Globe Newswire)) Tomatoes are a versatile food item with widespread culinary applications. They are a good source of vitamin C and the phytochemical lycopene. They are consumed fresh in salads, cooked as a vegetable, and incorporated into various dishes. Additionally, a significant portion of the global tomato harvest is used for processing. Processed tomato products include canned tomatoes, tomato juice, ketchup, puree, paste, and "sun-dried" tomatoes or dehydrated pulp. Tomato plants are herbaceous annuals known for yielding edible fruit. They have a maximum height of 2 meters and possess stems covered in rough hairs, along with leaves arranged spirally. These plants produce yellow flowers, which ultimately transform into round fruits of diverse colors. Typically, tomato plants are climbing vines requiring support as they can sprawl up to 3 meters tall. Optimal growth conditions involve well-drained locations that receive abundant sunlight throughout the day and slightly acidic soil. However, excessive nitrogen may lead to lush foliage but limited fruit production. (Prasad, personal communication, July 22, 2023).

Production

Country	Total tomato production (in tonnes)	
	Year 2019-20	Year 2020-21
China	63,805,187.44	66,139,606.32
India	19,778,500	20,865,500
Turkey	13,023,002.5	13,149,636.5
USA	10,899,045.5	10,707,187

Source: FAOSTAT UN

Table 1: Major tomato-producing countries of the world

Post-harvest losses

Post-harvest losses of tomatoes can greatly affect farmers and the entire supply chain. Tomatoes are highly susceptible to such losses, which can amount to as much as 50 percent of the total harvest. These losses not only result in the wastage of saleable and consumable produce but also lead to a significant squandering of resources like land, water, fertilizers, and chemicals. On average, for each tonne of tomatoes produced, about 21 kg of fertilizers are lost due to post-harvest losses. Additionally, tomato losses also account for a substantial 86 cubic meters of water per tonne of tomatoes. (Thole *et al.*, 2021) [25]. Post-harvest losses of tomatoes take place at multiple stages, including harvesting, transportation, storage, and prior to reaching consumers. These losses have adverse consequences, resulting in reduced profits for growers, processors, and traders, and ultimately impacting the country's foreign exchange earnings. Based on the regression analysis, several factors were identified as significant determinants of the level of post-harvest losses in tomatoes. These factors include the gender of the farmer, household size, farm size, duration of storage, membership in a Farmer Based Organization (FBO), and the type of tomato variety cultivated. (Alidu *et al.*, 2016) [2]. The size of the household and the overall value of post-harvest losses were discovered to have a significant negative impact on the per-capita income and welfare of tomato farmers. As post- harvest losses increase due to inefficiencies in harvesting, transportation, storage, or other factors, the financial stability and living conditions of the farmers and their families are adversely affected. Consequently, finding effective strategies to minimize post-harvest losses and enhance the value of produce becomes crucial for improving the economic well-being and overall well fare of tomato farmers.

Post-harvest deterioration of tomatoes

Post-harvest losses of tomatoes can be attributed to various factors. These encompass mechanical losses arising from inadequate handling during harvesting and storage, microbial activity driven by microorganisms such as bacteria, fungi, and yeasts that impact perishable food crops like fruits and vegeta bles, and environmental elements like temperature and humidity. These environmental factors, particularly temperature, and humidity, stand out as the primary contributors to post-harvest losses. (Mohan *et al.*, 2023, Emana *et al.*, 2017, Goka *et al.*, 2021) [18, 8, 11].

Table 2: Post-harvest pathogens of tomato fruit

Disease	Pathogen	Symptoms
Bacterial soft rot	*Erwinia carotovora*	Water soaking brown discoloration at wounds
Sour rot	*Geotrichum candidum*	White crusty growth
Rhizopus rot	*Rhizopus stolonifer*	Gray to white mold on lesions
Black mold rot	*Alternaria alternata*	Lesions with black mold
Buckeye rot	*Phytophthora capsici*	Greyish-brown lesions, buckeye-like rings
Gray mold rot	*Botrytis cinerea*	Water-soaked light brown center

Source: Bartz JA, 1991) [3]

Physiological disorders

Physiological disorders in harvested tomato fruit refer to irregularities in fruit shape, color, or both, which are not caused by infectious diseases or insect infestations. Instead, these abnormalities stem from environmental stress experienced by the plant. Several factors contribute to these disorders, including high temperature and intense light exposure, excessively rapid fruit growth resulting in cracking, varietal differences among cultivars leading to varying susceptibility to cracking, boron deficiency (particularly in calcareous soil), and concentric cracking caused by fruit exposure to sunlight (Masarirambi *et al.*, 2009) [17].

Table 3: Physiological post- harvest disorder of tomato fruit.

Disease	Symptoms
Blossom end rot	Light tan, water-soaked lesion, expands and becomes leathery.
Catfacet	Deformed with enlarged scars and holes
Sunscald	Due to exposure to sun, whitish shin blisters, become sunken and pale yellowish
Cracking	Splitting of epidermis in circular patterns or splitting that radiates towards blossom end from the stem scar

Source: University of Illinois, Report on plant disease, RPD No 981, December 2014)

Types of gene editing tools

Genome editing, also known as gene editing, refers to a set of powerful technologies that empower scientists to modify an organism's DNA. These cutting-edge tools enable precise ma insulation of genetic material, enabling the addition, removal, or alteration of specific locations within the genome. Various methods for genome editing have been created and refined, pro-viding researchers with unprecedented capabilities to engineer DNA with high precision and accuracy.

TALEN

TALEN (Transcription Activator-Like Effector Nucleases) is a powerful genome editing tool that can target and cut DNA at specific locations. It consists of two components: A TALE domain that recognizes the target DNA sequence and a FokI nuclease domain that facilitates the DNA cleavage when two TALEN units bind in opposite orientations. The cell's natural repair mechanisms then come into play, leading to mutations or precise alterations if a repair template is provided. TALEN can be easily customized to target virtually any DNA sequence by rearranging the repeats in the TALE domain, with each repeat recognizing a single nucleotide. TALEN has found applications in genome editing across various organisms, including plants, animals, and humans, for

purposes such as enhancing crop traits, creating animal models, and potentially treating diseases.

Advantages-1-High specificity and programmability by rearranging repeat units and choosing suitable RVDs for binding to any desired DNA sequence. 2-Flexible design, adjusting length and position to match the target site. 3-Discrimination between methylated and unmethylated cytosines with some RVDs recognizing different methylation states. 4-High activity in heterochromatic regions, less affected by chromatin compared to CRISPR/Cas9. 5-Ability to edit mitochondrial and chloroplast DNA by fusing a targeting signal for these organelles. 6-Capability for creating novel genome editing tools by combining with various functional domains like base editors, trans posases, nickases, activators, recombines, and epigenetic modifiers.

Limitations-1-Higher cost and complexity in the lab compared to CRISPR/Cas9. Each edit necessitates constructing a new TALEN protein, which can be challenging and prone to errors. 2-Lower multiplexing capacity, as two TALEN monomers are required for each target site, limiting simultaneous editing. 3-Potential for unwanted off-target effects due to binding to similar genomic sequences, especially if lacking strong RVDs or using non-conventional RVDs. 4-Risk of TALE repeats being lost through recombination when delivered by viral vectors. 5-Induction of large deletions at the target site due to the NHEJ repair mechanism. 6-Potential cell toxicity when fused with a deaminase domain that acts on double-stranded DNA. (Becker and Boch, 2021) [4].

ZFN

Zinc Finger Nuclease is a gene editing tool that consists of zinc finger protein domains for specific DNA binding and a nuclease domain for DNA cleavage. They can be designed to target virtually any DNA sequence, leading to the creation of targeted DNA double-strand breaks (DSBs). The repair of these DSBs can result in genetic modifications, such as small insertions, deletions, or rearrangements through non-homologous end joining (NHEJ), or specific sequence modifications or gene integration through homology-directed repair (HDR) using investigator-designed donor DNA.

Advantages-1-They can target and create DNA double-strand breaks (DSBs) at specific locations in the genome, allowing precise modifications. 2-ZFN-mediated DSBs can lead to gene-specific mutations through error-prone repair processes like non-homologous end joining (NHEJ). 3-Specific DNA sequence modifications can be achieved through homology-directed repair (HDR) using donor DNA templates, enabling targeted mutagenesis, gene deletion, editing, and transgene integration. 4-ZFNs can enhance our understanding of plant gene function and improve crop traits like herbicide resistance, stress tolerance, yield, and quality. 5-ZFNs can be delivered to plant cells using various methods, and modified genomes can be propagated through in vitro cell and tissue culture systems. (Petolino JF, 2015) [22].

Limitations-1-ZFNs can bind to DNA sequences that are similar but not identical to the target sequence, leading to off-target effects. 2. ZFNs require the design and construction of a new protein for each target site. 3- ZFNs can cause double-strand breaks at sites other than the intended target site. 4-The design of ZFNs is limited by the requirement for two specific DNA-binding domains.

CRISPR/Cas9

Clustered regularly interspaced short palindromic repeats is a versatile genome editing technology that uses a Cas9 enzyme and guide RNA to target and cleave specific DNA sequences. The guide RNA can be a single guide (SG) RNA or a complex of crRNA and tracrRNA. The DNA break is typically 3 base pairs upstream of a protospacer adjacent motif (PAM), necessary for Cas9 binding. Repair mechanisms like NHEJ or HDR can result in indel mutations or targeted gene replacement. CRISPR/Cas9 is applicable to various organisms through delivery methods like plasmids, viruses, or nano particles. It finds diverse applications in research, biotechnology, and medicine, including gene knockouts, knockins, gene regulation, and correcting genetic diseases.

Advantages of CRISPR/Cas9 over TALEN and ZFN-CRISPR/Cas9 is simpler to design and construct, as it only re- quires a short RNA sequence to target a specific DNA sequence, while ZFNs and TALENs require engineering large modular proteins. 2-CRISPR/Cas9 is more versatile and can target a wider range of DNA sequences, as long as they are adjacent to a PAM sequence, while ZFNs and TALENs have more stringent requirements for target recognition. 3-CRISPR/Cas9 is more suitable for multiplex gene editing, as multiple sgRNAs can be co-delivered with Cas9 to the cell, enabling simultaneous editing of more than one target at the same time, while ZFNs and TALENs are less efficient for this purpose.

Limitations-CRISPR/Cas9 has potential off-target effects, leading to unintended mutations and possible harmful outcomes. Its efficiency varies depending on target sequence, cell type, delivery method, and repair mechanism, posing challenges for editing certain targets and cell types (Belhaj, *et al.*, 2015) [5].

Applications of genome editing techniques in tomato

Abbreviations- SlbZIP1- Basic region leucine zipper (bZIP) transcription factor 1 in tomato, SIRT- Sucrose-induced repression of translation, AA-Amino acids, Sl7-DR2 -7-dehydrocholesterol reductase 2, SlINVINH1- Solanum lycopersicum invertase inhibitor 1, SlVPE5- Solanum lycopersicum vacuolar processing enzyme 5, TSS- Total soluble solid, SlAPX4- Solanum lycopersicum ascorbate peroxidase 4, SlINVINH1-Solanum lycopersicum invertase inhibitor 1, SGR1-Stay-Green 1, SlATG5-Solanum lycopersicum Autophagy-related gene 5, NBS-LRR-Nucleotide-binding site–leucine-rich repeat, SlPLC2- Tomato phospholipase C2 gene, XSP10-Xylem sap protein 10, SlSAMT-Salicylic acid methyl transferase, SlXTH5- Solanum lycopersicum Xyloglu- can endotransglucosylase/hydrolase 5, SlPG-Tomato Poly-galacturonase Gene.

Table 4: Application of CRISPR/Cas9 to enhance nutritional quality and taste

Mutation type	Target gene	Target gene function	Outcome
Knockout	SlbZIP1	SIRT	Increase in sugar and AA.
Knockout	Sl7-DR2	Encodes 7-dehydrocholestrol reductase (VitD3 precursor)	Accumulation of VitD3 precursor, Decrease in α-tomatine
Knockout	SlINVINH1 & SlVPE5	Inhibits sugar accumulation	Higher glucose, fructose & TSS levels.
Knockout	SlAPX4	Mediates ascorbate decrease	Increase in ascorbate content in ripe tomato
Knockout	SGR1	Carotenoid biosynthesis	Increase in carotenoids

Table 5: Application of CRISPR/Cas9 to enhance post-harvest resistance in tomatoes

Mutation type	Target gene	Target gene function	Outcome
Knockout	SlATG5	Involved in autophagy	Inhibits autophagy, boosts *B. cinerea* susceptibility
Knockout	miR482b & miR482c	Target NBS-LRR genes involved in *P. infestans*	*P. infestans* resistant tomatoes
Knockout	SlPLC2	Involved *in B. cinerea* susceptibility	Improved resistance to *B. cinerea*
Knockout	XSP10 & SlSAMT	Negative regulator of defense against *F. oxysporum*	Strong phenotypic tolerance to *F. oxysporum*

Challenges and Solution

Genome editing is a groundbreaking technology in molecular biology that enables accurate and targeted modifications within the genome. In the field of plant breeding, it is considered a remarkable innovation as it offers the ability to create precise changes in plants' genetic makeup. This revolutionary approach allows for the rapid generation of novel plants, free from transgenes, and comparable to those achieved through traditional breeding methods. Nevertheless, employing genome editing tools to tackle post-harvest loss in tomatoes may present certain challenges. ZFNs and the TALENs system have shown promise in inducing targeted mutations (In Dels) in various horticultural crops to address and improve post-harvest storage quality concerns. (Kumari C. et al, 2022). The limited genetic diversity in tomatoes presents significant obstacles in breeding. However, the emergence of CRISPR- associated protein9 (CRISPR/Cas9) genome editing has revolutionized tomato breeding, enabling rapid and efficient advancements in the field. (Tiwari *et al.*, 2023) [26]. The CRISPR-Cas9 system is a highly effective and precise genome manipulation tool inspired by the bacterial adaptive immune system. It utilizes an endonuclease to create double-stranded breaks at specific DNA target sites, guided by a single guide RNA. These breaks can be repaired by cellular mechanisms, resulting in small indels at the cut sites. Compared to other editing tools like ZFN, TAL-ENs, and mega nucleases, CRISPR-Cas9 stands out for its simplicity, user-friendliness, and minimal off-target effects. This technology has been successfully applied in various horticultural and industrial crops to enhance stress tolerance, shelf life, nutritional quality, flavor, and metabolite content. (Sharma, *et al.*, 2023) [24].

Conclusion

In conclusion, genome editing, especially the CRISPR-Cas9 system, offers a promising solution to reduce post-harvest loss in tomatoes. By precisely modifying key genes, we can extend shelf life, enhance fruit quality, and improve stress tolerance. This technology holds the potential to revolutionize tomato breeding and contribute to sustainable agriculture and food security. However, ethical and biosafety considerations must accompany its deployment. With continued research and responsible implementation, genome editing can lead to a future where tomatoes reach consumers with improved freshness and nutritional value, minimizing food waste and maximizing agricultural productivity.

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