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Review Article

Innovations and Uses of Gene Editing Technologies in Tomato (*Solanum Lycopersicum*) and Chili (*Capsicum spp.*)

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Abstract

Gene editing technologies have changed plant biology and crop improvement by making it possible to make precise and efficient changes to specific parts of the genome. Gene editing technologies have revolutionized the plant biology and evolution of agriculture by making it possible to easily and effectively alter specific elements of a genome. The tomato (Solanum lycopersicum) and the chili (*Capsicum spp.*) are two significant crops that belong to the Solanaceae family, and which carry significant importance to the economy, medicine, and nutrition of the globe. To advances in CRISPR/Cas systems and related platforms have improved the quality of fruits and their production, as well as resistance to biotic and abiotic stress factors. Gene editing into chili is increasingly becoming possible, as experiments have shown. The present, potential, and limitations of gene editing in tomatoes and chilies are described in this short paper, and a special emphasis is put on how these technologies could accelerate the process of plant improvement sustainability and address the problem of the global and nutritional security.

Keywords: CRISPR/Cas; TALENs; ZFNs; tomato (*Solanum lycopersicum*); chili (*Capsicum spp.*); Fruit quality; Stress resistance; Capsaicinoids; Functional genomics; Crop improvement

Introduction

Chilies and tomatoes are among the most common vegetables with economic, therapeutic and nutritional advantages. Tomatoes contain vitamins C and A, folate, lycopene, an antioxidant, and potassium, which is an essential mineral. Chili peppers contain capsaicinoids, which contain antibacterial, anti-inflammatory, and pain-killing effects [1]. The compounds have been applied in pharmacology both in the treatment of pain and in metabolic control. The importance of the two crops to the region and the global markets supports both commercial activities and small hold farmers as well as catering to global needs of nutrient-rich food products. Conventional breeding of tomatoes and chilies fail to enhance characteristics such as quality of fruits, flavors, stress resistance, disease resistance because of the linkage drag, lengthy breeding period, and genetic diversity [2]. Allelic varieties Molecular markers and genomic selection have become more efficient; they do not generate accurate genetic changes but use the existing allelic varieties. Hence, tools of accurate and direct manipulation of genes are required. Plant breeding and functional genomics have been revolutionized by the discovery of site-directed nucleases. Initial systems such as ZFNs and TALENs demonstrated that targeted DNA cleavages would result in gene knockouts or mutations. Their scalability was limited, though. CRISPR/Cas systems were introduced and transformed these disciplines, making it possible to base edit, prime edit, and control transcription [3]. Such technologies have resulted in disease resistance production, enhanced stress tolerance, altered plant structure, and enhanced nutritional content and have replaced conventional breeding with a more specific localization of the genome. The tomatoes are an ideal model system of studies of Solanaceae because tomatoes have a short life cycle, transformational potential, and huge genetic resources. They provide an avenue of functional genomics and proof of concept studies, which can be extended to other solanaceous crops such as pepper, eggplant and potatoes [4]. Nevertheless, chili peppers are also problematic because they have a complicated genome and are hard to transform and regenerate. These challenges are being overcome by advances in sequencing, transformation, and CRISPR-based methods so that pepper research can utilize tomato progress.

Evolution of Gene Editing Tools

Zinc Finger Nucleases (ZFNs)

Zinc finger nucleases (ZFNs) were created to induce a double-strand break at targeted positions in the genome of plants and enable targeted mutagenesis. Nevertheless, ZFNs have been applied infrequently in tomatoes because of such challenges as protein engineering problems, low editing efficiency, and high research expenses [5]. Such limitations notwithstanding, ZFNs came up with the idea of programmed DNA cleavage, the starting point of future technologies, though not popular in Solanaceae genome editing (Figure 1).

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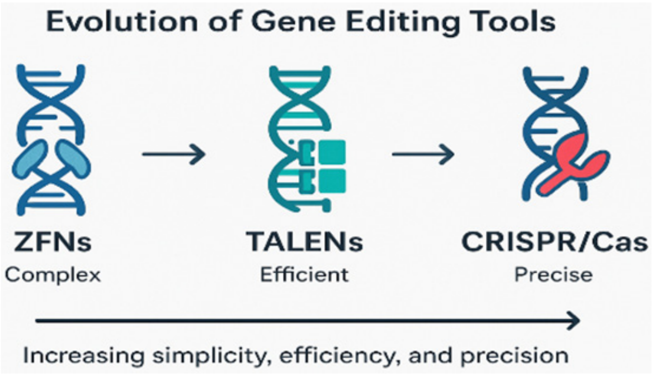

Figure 1: Schematic illustration of the development of ZFNs to TALENs to CRISPR/Cas systems, which become easier, more efficient, and more precise.

Transcription Activator-Like Effector Nucleases (TALENs)

TALENs greatly improved the ZFNs because they had a higher specificity of binding DNA and were much easier to design. TALENs are derived by the Xanthomonas species transcription activator-like effectors that contain modular repeats that can undergo modification to find nearly any DNA sequence. TALENs have been efficiently applied on tomato in both functional studies and trait improvement [6]. A good example of how TALEN-mediated editing can be applied to develop disease resistance is the targeted mutagenesis of the SlMlo1 gene that conferred powdery mildew resistance. Even with the fact that TALENs are still more labor-intensive than RNA-guided systems, they have not seen widespread use in pepper, where the problem of transformation complicates their application.

CRISPR/Cas Systems

The CRISPR/Cas technology transformed the way plants are edited, as it is more popular and widely used than ZFNs and TALENs. It targets locations in the genome using a simple 20-nucleotide RNA in an efficient and cost-effective manner. CRISPR/Cas has made mutants that have been modified to build mutants with a changed plant structure, enhanced resistance to diseases, improved fruit quality, and extended shelf life in the Solanaceae family such as tomatoes and chilies. More recent nucleases, such as Cas12a (Cpf1), have broadened the range of tools to edit with [7]. The precise replacement of a single nucleotide and precise insertions or deletions of genomes with precision tools such as base editors and prime editors eliminates the risk of undesirable mutations and broadens the range of targets that can be edited, as the process does not cause a double-strand break. CRISPR/Cas-based methods have been used to dominate functional genomics and crop enhancement in Solanaceae and tomato is the model system of choice (Figure 2).

Applications in Tomato

Fruit Quality and Yield Improvement

The production and quality of tomato fruits are also affected by many genes amongst them ripening and development. Genome editing has made it possible to fine-tune these pathways, maintaining the quality of post-harvest and extending shelf life without affecting the nutritional value. Adjustments on ripening-related regulators including SlALC, SlTAGL1 and SlFUL1 have also been done in order to enhance potential yield [8]. SlCLV3 and SlAGL6 mutations that lead to larger carpels as well as seed compartments cause large fruits and increase production [9].

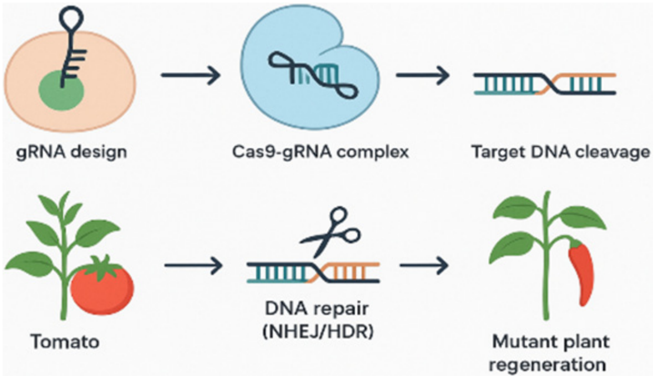

Figure 2: Overview of CRISPR/Cas9 workflow:(a) gRNA design: (b) Cas9-gRNA complex: (c) Target DNA cleavage: (d) DNA repair (NHEJ/HDR): (e) Mutant plant regeneration.

Biotic Stress Resistance

Biotic stresses such as bacterial and fungal pathogens highly limit tomato production. The editing of susceptibility genes using CRISPR has created new avenues of long-term resistance. Major breakthroughs such as knockout of SlMlo1 to make tomatoes resistant to powdery mildew and the making of SlDMR6-1 resistant to bacterial infections have been made [10]. These instances reveal that specific editing can result in general resistance, decreasing the necessity of chemical control and removing departmental flaws.

Abiotic Stress Tolerance

Abiotic factors such as salt, heat, and drought are causing a threat to tomato production when it comes to climate change. Gene editing and functional research of the stress-responsive genes are encouraging. Transcription factors such as SlDREB and SlMAPK have been edited to enhance drought tolerance and salt tolerance, perhaps by regulating osmotic balance and antioxidant defense mechanisms [11]. Such measures may be used to develop tomato varieties that are resistant to climate change (Figure 3).

Applications in Chili (*Capsicum spp.*)

Transformation Bottlenecks

The fact that Chili is not a regenerative and transformable crop makes it very difficult to study using functional genomics.

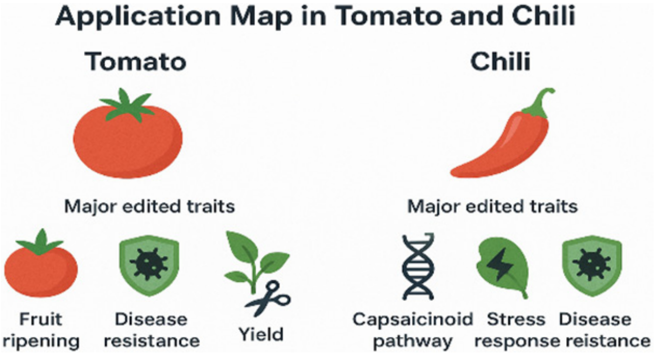

Figure 3: Dual schematic of selected traits of major interest: Tomato: Fruit ripening, resistance to disease, yield. Chili: Capsaicinoid, Stress, disease resistance.

Conventional approaches to stable genetic transformation have not been effective and the need to standardize protocols is complicated by cultivar-based responses. In the majority of studies, Agrobacterium-transformed explants are used, and regeneration is not very effective. Transformation of protoplasts and methods of temporary expression are investigated, but seldom generate stable edited lines [12]. These challenges slow down the pace of genome editing in the chili research with tissue culture and delivery techniques.

Successful Gene Editing Attempts

Editing in capsicum via CRISPR/Cas9, regardless of technical difficulties has been validated in many proof-of-concept studies. The functionality of the CRISPR system was authentic in chili as evidenced by unambiguous phenotypic indicators. Recent studies indicated a higher level of fight against fungal infections by editing the susceptibility gene CaMLO2, which implied directed mutagenesis to manage biotic stress. CaWRKY and CaNPR1 are suggested as a target to control disease resistance across the board, and CaSnRK2 is an improved resistant to abiotic stress [13].

Future Prospects

CRISPR-based technology could be used to hasten chili breeding by regulating various genes in the capsaicinoid biosynthesis pathway [14]. This enables the fine-tuning of the pungency levels, ideal shelf life, taste and nutritional value.

Genome domestication can also be facilitated with precision editing, where wild Capsicum species possessing special fruit traits, abiotic stress frequency, and resistance to disease-causing pathogens are used. Gene editing can be used to address the current barriers in chili breeding through the combination of high-throughput sequencing and improved transformation platforms to maximize the benefits of these characteristics (Table 1).

Table 1: Application of Gene Editing in Tomato and Chili.

Tool	Target Gene	Crop	Trait Modified	Key Outcome
ZFN	Pathogen-resistance gene	Tomato	Disease resistance	Proof-of-concept
TALEN	*SlMlo1*	Tomato	Powdery mildew resistance	Enhanced resistance
CRISPR/Cas9	*SlDMR6-1*	Tomato	Bacterial resistance	Broad immunity
CRISPR/Cas9	*CaPDS*	Chili	Proof-of-concept	Genome editing validation
CRISPR/Cas12a	*CaMLO2*	Chili	Fungal resistance	Improved disease tolerance

Table 2: Issues and Remedies of Gene Editing Solanaceae.

Challenge	Crop	Consequence	Potential Solution	Tool/Approach
Low transformation efficiency	Chili	Limited edited plants	RNP delivery / Viral vectors	Cas12a, CasΦ
Off-target mutations	Both	Genomic instability	High-fidelity Cas variants	SpRY, eSpCas9
Mosaicism	Both	Inconsistent phenotypes	Germline editing / tissue culture optimization	Base editors
Regulatory barriers	Both	Slow adoption	DNA-free editing	RNP-based systems

Limitations and Challenges

- It is the low transformation and regeneration efficiency of chili: It is hard to regenerate stable transgenic lines because it is resistant to tissue culture. This notwithstanding, tomato systems are established well [15]. The other issue is the risk of unwanted off-target alteration especially in the complicated genomes such as chili.

- Mosaicity further complicates the recovery of stable homozygotes mutants: of which the unprocessed and the edited alleles are found in the same plant [16]. These problems point to the necessity of the next generation editing platforms and improved guide RNA design tools.

- The challenges of genome editing in tomatoes and chili crops are limited genotype adaptability: legal constraints in commercialization, social acceptance, intellectual property and biosafety. Although there is a possibility that small CRISPR changes will be equivalent to natural mutations, national laws are not uniform, with some regions having more economic acceptability [17]. This is akin to transgenics where genome-edited crops are afforded the same treatment.

- To ensure complete use of genome editing in Solanaceae crops: transformation technologies, accuracy editing tools and common regulations are required [17] (Table 2).

Future Directions

DNA-free Obtaining

DNA-free techniques, such as RNA-based and direct delivery of ribonucleoproteins, are becoming increasingly popular to minimize regulation problems and avoid transgenic insertion. Plant viral vectors are under development to deliver CRISPR components effectively to reduce off-target integration risk, and simplify downstream regulatory clearance.

AI-based gRNA Design

AI and machine learning innovations have made predictions of gRNA efficiency and specificity in genome editing more accurate. This enables an optimized guide selection, reduction of undesirable alterations and increases repeatability among genotypes and thus off-target mutations are reduced.

Omics Integration with Higher Editing Technologies

Base editing and epigenome editing will be used as precision methods of editing, with multi-omics methods employed to refine trait development. These methods allow the use of one-nucleotide changes on regulatory factors or protein activity, which can be useful specially to increase stress resistance, capsaicinoid production, and fruit quality. Polygenic traits through CRISPR multiplex editing Polygenic traits in plants, including tomatoes and chilies, have the ability to stack, e.g., taste, yield, disease resistance, and stress tolerance. The procedure will be able to accelerate the breeding patterns and domesticate the wild capsicum species, and thus improve the quality of the two crops. These developments highlighted in tomato and chili research have the potential to boost breeding effectiveness and improve versions of desirable traits, with the possibility of transforming the future of Solanaceae crop crops in the coming decade as editing platforms and legal frameworks evolve (Figure 4).

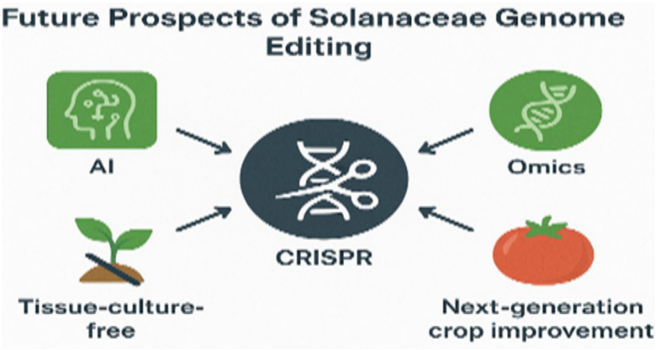

Figure 4: Conceptual figure, depicting the combination of CRISPR, AI, omics and tissue-culture-free methodologies to enhance crop improvement in the next generations.

Conclusion

The tomato has become a model system of plant genome editing in Solanaceae family where CRISPR-based techniques have been widely applied to study and improve agronomically important traits. Besides accelerating the engineering of the traits in the species, the tomato research has also provided information that can be applied in the associated crops. Still more technically challenging, however, is chili due to constraints of transformation and regeneration. Nevertheless, recent advances in CRISPR/Cas systems validation in capsicum indicate that proper genome editing can and is increasingly available. In the future, it will be necessary to add state-of-the-art editing platforms, improved technologies of transformation, and additional omics resources. These advances together can deliver specific, sustained genetic enhancement in tomatoes and chilies to satisfy the challenges of resilience, production, and nutritional quality in the entire world.

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