CRISPR/Cas13-Mediated RNA Targeting for Engineering Viral Disease Resistance in *Solanum lycopersicum* L.: A Critical Review and Synthesis

Dr. Navik Hallford [1*], Dr. Elvin Marshcrest [2]

[1-2] Faculty of Crop Science, Central Agricultural Academy, Ethiopia

*Corresponding author: **Dr. Navik Hallford**

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the critical vegetable crops around the globe, but the challenge of overwhelming RNA and DNA viral diseases such as Tomato brown rugose fruit virus (ToBRFV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), and Tomato yellow leaf curl virus (TYLCV) remains redoubtable as they induce severe losses in the yields and quality of crops and cannot be defended against by conventional methods of resistance breeding and vector management. CRISPR/Cas13 is a type VI class 2 CRISPR systems which is an RNA-targeting ribonuclease and differs from CRISPR/Cas9 in that it recognizes and cuts single-stranded RNA without modifications to its structure and, therefore, boasts a favorable route to combat the economically important tomato viruses that are all RNA viruses. This paper provides an overview of the results of scientific studies on the methods of anti-viral engineering in tomatoes using CRISPR/Cas13 technology and similar approaches in other plants of Solanaceae family and different model plants. From the results presented in the literature analyzed by the author, it follows that Cas13-crRNA complexes can be programmed against Conserved viral RNA regions can effectively inhibit the replication of viral RNA, with some studies discovering reductions of around 40% to over 99% when compared to normal controls as well as lower symptom severity and activation of host protective mechanism which involves, inter alia, salicylic acid signaling, pathogenesis-related genes activation, and endogenous RNA interference. A comparison with the CRISPR/Cas9-based strategies of fighting the DNA viruses shows that the approaches that target RNA work from a different mechanism in comparison with them and are highly relevant to the RNA virus affecting the tomato plants. Furthermore, although a lot of proof-of-concept evidence on the effectiveness of CRISPR/Cas13 technology has been collected, little has been done in regard to practical solutions for the specific applications of the technology to tomato plants and there is little knowledge about such issues as collateral RNase activity and efficiency of deployment without transgene.

Keywords: Type VI CRISPR-Cas system; RNA-guided ribonuclease; Plant antiviral immunity; Tobamovirus resistance; Host defense signaling; RNA interference; Genome-edited crops; Sustainable disease management

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most commonly grown and economically important vegetable crops worldwide, with the annual amount produced exceeding 180 million tons, and the cultivation of tomatoes presents various growing systems including open-field agriculture and advanced greenhouse horticulture, all indicated through a complete reference genome that greatly fast-tracked functional genome research (Wang *et al*., 2021) [23]. Viral diseases are one of the major and most persistent obstacles in the tomato growing worldwide, causing significant yield reductions from 10% to 100% depending on the pathogen type, tomato variety, and time of the infection onset (Ling *et al*., 2019) [24] (Fillmer *et al*., 2022) [26].

The reported yield loss incurred ranges from 10% to 55% in greenhouses that are affected by the disease. Tomato spotted wilt virus (TSWV), which is a tospovirus that can be spread through thrips, and Tomato yellow leaf curl virus (TYLCV), which is a begomovirus transmitted by whiteflies, are some of the viruses that cause enormous damage into tomato plants together with Cucumber mosaic virus (CMV) and Tomato mosaic virus (ToMV). These viruses are known to pose economic threats in cases of tomato production globally (Fillmer *et al*., 2022) [26] (Salem *et al*., 2016) [25].

The conventional methods of managing diseases in tomatoes is through integrated virus-resistant crop breeding using R-gene loci, vector control and sanitary measures. All these techniques present their challenges because the mechanism of action is such that resistance can be surpassed as in the case of ToBR and Tm-2² resistance in tomato; vector control is partially effective leading to constant effort of use of insecticides; and sanitary measures only help to a certain extent since they cannot prevent from being infected with virus once its establishment in a given area has occurred (Ling *et al*., 2019) [24] (Fillmer *et al*., 2022) [26].

The use of genome editing became important in the last ten years as a method to build long-lasting resistance against viruses. It is different from the previously known methods of introduction breeding and transgenic approaches dependent on the use of pathogen-derived resistance (Camargo Rodriguez *et al*., 2024) [20] (Zhu *et al*., 2020) [21]. CRISPR/Cas9 is the most widely known genome editing system used in plants and is usually implemented for the development of resistance to violent pathologies like DNA viruses and mainly to geminiviruses like TYLCV by knocking out the stable parts of the virus genome, as well as host

susceptibility genes of eIF4E families, which are responsible for resistance against RNA viruses in many plant species (Ali *et al*., 2015) [14] (Tashkandi *et al*., 2018) [13] (Yoon *et al*., 2020) [18] (Bastet *et al*., 2019) [19]. CRISPR/Cas13 includes completely different technology and approach compared to CRISPR/Cas9 technologies, as the latter is aimed at editing of double-stranded DNA. As for CRISPR/Cas13, it is Type VI RNA-dependent endoribonuclease which uses CRISPR RNA to target specific RNA molecules, and thus provides great opportunities for fighting viral infections without any impact on the virus's DNA intermediate or the ability to alter plant genome (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2]. Communication with viruses becomes very easy because the process can be conducted with no need to edit the genomic DNA of

Functional genomics approaches such as host defense gene expression profiling, pathogenesis-related protein studies, and RNA interference methods are now key for understanding how CRISPR/Cas1[3], RNA-mediated gene interventions work (Ding, 2010) [30] (Camargo Rodriguez *et al*., 2024) [20]. Despite the rapid developments in the area of CRISPR/Cas13-related studies in plants, most papers on this topic were published using a limited number of experimental setups, mainly using the following plant species: *Nicotiana benthamiana*, *Arabidopsis thaliana*, potato, and rice, whereas little research has been devoted to tomatoes specifically (Aman *et al*., 2018) [3] (Mahas *et al*., 2019) [5] (Sharma *et al*., 2023) [7]. Some studies conducted on tomato plants, such as Tashkandi *et al*. [13], provide some evidence for the feasibility of CRISPR/Cas9-based gene interference in case of DNA-based viruses, however, there are too few articles based on the use of CRISPR/Cas13 method in RNA-related viruses published so far (Tashkandi *et al*., 2018) [13] (Camargo Rodriguez *et al*., 2024) [20].

This review fills this knowledge gap in the literature by providing a critical synthesis of peer-reviewed literature describing the potential use of CRISPR/Cas13 for RNA-targeting in the context of an antiviral application, or what these authors referred to as the translational implications or advancements related to the use of CRISPR/Cas13 technology around tomato plants. The review's aims include (i) demonstrating the biological mechanism of CRISPR/Cas13 technology for RNA-targeting against viruses; (ii) summarizing the findings of other authors regarding the viral suppression of diseases, while also providing details about the efficacy of the considered method in terms of controlling symptoms of plant disease and activating plant immune response; (iii) analyzing the relevance of information available about tomato plants' properties based on the authors' findings compared to general information from the literature available on Cas13 technology use in plants; and (iv) examining any biosafety and regulatory issues when applying CRISPR/Cas13 technology in plants.

2. Major Viral Diseases Constraining Tomato Production

The viruses of greatest economic significance in tomato span multiple genome types and transmission modes, a diversity that has direct implications for the design of RNA-targeting antiviral strategies. ToBRFV and Tomato mosaic virus (ToMV) are both tobamoviruses with positive-sense single-stranded RNA genomes of approximately 6.4 kb, transmitted with unusual efficiency through mechanical contact and contaminated seed, and are notable for their virion stability, which allows persistence in plant debris and on surfaces for extended periods (Ling *et al*., 2019) [24] (Fillmer *et al*., 2022) [26]. TSWV is a negative-sense, ambisense RNA virus of the genus Orthotospovirus, transmitted by thrips in a persistent, propagative manner, and produces necrotic ringspot symptoms on fruit that severely affect marketability (Ling *et al*., 2019) [24]. TYLCV is a circular single-stranded DNA geminivirus transmitted by the whitefly *Bemisia tabaci*, producing characteristic leaf curling, yellowing, and severe stunting, particularly in young plants (Ling *et al*., 2019) [24].

This genomic diversity is directly relevant to genome-editing strategy selection: RNA viruses such as ToBRFV, ToMV, TSWV, and CMV are, in principle, direct targets for Cas13-based RNA cleavage, since Cas13 requires an RNA substrate, whereas DNA viruses such as TYLCV require either direct Cas9/Cas12a-mediated DNA cleavage or Cas13-mediated targeting of the virus's messenger RNA transcripts rather than its genomic DNA (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2] (Tashkandi *et al*., 2018) [13] (Ali *et al*., 2015) [14]. A structured overview of the major tomato viruses relevant to this review, including their genome type, transmission mode, and primary symptoms, is presented in Table 3.

3. CRISPR/Cas13 Mechanism and RNA-Targeting Antiviral Strategy

Cas13 proteins were first characterized as RNA-guided, RNA-targeting CRISPR effectors distinct from the DNA-targeting Cas9 and Cas12 families, with the defining biochemical feature that target RNA recognition activates a promiscuous collateral ribonuclease activity in addition to sequence-specific cleavage of the bound target (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2]. Multiple Cas13 orthologs and subtypes have been characterized and applied in plant systems, including LshCas13a, PspCas13b, and the smaller Cas13d variant, which has been reported to show more robust RNA virus interference activity in several comparative studies relative to earlier orthologs (Mahas *et al*., 2019) [5] (Cao *et al*., 2023) [11].

The antiviral strategy conceptually proceeds through several linked stages. Following viral infection and RNA accumulation within the plant cell, a stably or transiently expressed Cas13-crRNA complex recognizes its complementary target sequence within the viral RNA genome or transcript. Target recognition triggers sequence-specific cleavage of the bound viral RNA, and in many reported systems also activates collateral, non-specific ribonuclease activity that further degrades RNA in the local cellular environment, amplifying the antiviral effect beyond the initially bound target molecule (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2] (Bhushan, 2018) [9].

Guide RNA (crRNA) design targeting conserved, functionally essential regions of the viral genome, such as replicase, movement protein, or coat protein-encoding sequences, has been consistently associated with more effective viral suppression than targeting of variable or non-essential regions, reflecting the general principle that mutational escape is less likely when the targeted sequence is under strong purifying selection (Aman *et al*., 2018) [3] (Sharma *et al*., 2023) [7] (Bhushan, 2018) [9]. This principle is not unique to RNA-targeting Cas13 approaches: DNA-targeting CRISPR/Cas9 strategies against geminiviruses in cereal systems, such as engineered resistance to wheat dwarf virus in barley, have similarly relied on targeting conserved, essential viral genome regions to maximize durability of resistance (Kis *et al*., 2019) [12], and broader reviews of CRISPR/Cas applications across crop improvement consistently identify conserved-region targeting as a key design principle for robust, evolution-resistant antiviral engineering (Zhu *et al*., 2020) [21]. Multiplexing strategies, using polycistronic tRNA-gRNA (PTG) processing systems to express several crRNAs simultaneously from a single transcript, have been developed to target multiple viral RNA regions or multiple co-infecting viruses concurrently, addressing the practical challenge of

mixed viral infections common in field conditions (Cao *et al*., 2023) [11].

Beyond direct viral RNA cleavage, Cas13-mediated antiviral activity has been repeatedly associated with downstream activation of the plant's endogenous antiviral machinery. Collateral RNA cleavage and the cellular stress associated with viral RNA degradation appear to interact with salicylic acid-mediated defense signaling and RNA interference pathway components, including RNA-dependent RNA polymerases (RDRs), Dicer-like proteins (DCLs), and Argonaute (AGO) proteins, suggesting that engineered RNA targeting and native antiviral RNAi operate as complementary, potentially synergistic layers of defense rather than fully independent mechanisms (Zhang *et al*., 2018) [27] (Fu and Dong, 2013) [28] (Incarbone *et al*., 2023) [29] (Ding, 2010) [30]. A conceptual summary of CRISPR/Cas13-targeted viral RNA regions, their biological functions, and expected antiviral outcomes, synthesized from the reviewed literature, is presented in Table 4.

Table 1: CRISPR/Cas Effector Systems Applied to Plant Antiviral Engineering, as Synthesized from the Reviewed Literature

Effector System	Target Molecule	Representative Application	Representative Sources
Cas9 (SpCas9/FnCas9)	Double-stranded DNA / RNA (FnCas9)	TYLCV geminivirus interference in tomato; CMV/TMV RNA interference	[13, 14, 16, 27]
Cas12a (Cpf1)	Double-stranded DNA (multiplex)	Combined DNA (BSCTV) and RNA (TMV) virus resistance	[31]
Cas13a (LshCas13a)	Single-stranded RNA	PVY resistance in potato; PSTVd suppression in tomato	[3, 4, 9, 10]
Cas13b (PspCas13b)	Single-stranded RNA	Heat-inducible antiviral RNA interference in cotton	[8]
Cas13d	Single-stranded RNA (smaller ortholog)	Multiplex RNA virus resistance in potato	[5, 11]
Host gene editing (Cas9)	Endogenous susceptibility genes (eIF4E)	Broad-spectrum potyvirus resistance in cucumber and tomato	[17, 18, 19]

4. Synthesized Evidence: Viral Suppression, Symptom Attenuation, and Host Defense Activation

Quantitative evidence for CRISPR/Cas-mediated RNA and DNA virus suppression is strongest in non-tomato model and crop systems, though the mechanistic principles are directly transferable given shared plant cellular machinery. Zhan *et al*. [4] reported that transgenic potato plants expressing Cas13a-sgRNA constructs targeting the P3, CI, NIB, or coat protein-encoding regions of Potato virus Y (PVY) achieved greater than 99% reduction in viral accumulation relative to non-transgenic controls, among the strongest quantitative antiviral outcomes reported in the plant Cas13 literature to date. Zhang *et al*. [27], using the DNA-targeting FnCas9 system against CMV and TMV in *Nicotiana benthamiana* and *Arabidopsis*, reported viral RNA reductions generally exceeding 40%, with several constructs exceeding 60%, and stably transformed T2/T6 *Arabidopsis* lines achieving 70-85% reduction in viral accumulation that remained heritable across subsequent generations. Luo *et al*. [31], applying a multiplexed Cas12a-based DNA and RNA virus interference system, reported greater than 90% reduction in the DNA virus BSCTV in most transgenic events, alongside reduced TMV accumulation, illustrating that quantitatively substantial viral suppression has now been documented across multiple CRISPR/Cas effector families and both DNA and RNA viral targets.

Symptom attenuation is reported with notable consistency across the CRISPR/Cas13 plant antiviral literature, generally described in qualitative but consistent terms: engineered plants expressing the RNA-targeting construct develop mild or no visible symptoms following viral challenge, while non-engineered control plants develop the characteristic severe symptoms of the challenged virus, including mosaic patterning, leaf deformation, stunting, and, in the case of rice stripe mosaic virus and southern rice black-streaked dwarf virus studies, excessive tillering and dwarfing (Bhushan, 2018) [9] (Zhang *et al*., 2019) [10]. This consistent qualitative pattern, illustrated conceptually in Figure 3, supports the general conclusion that Cas13-mediated RNA suppression translates into meaningful phenotypic disease protection rather than molecular suppression alone.

Direct application of Cas13a to a viroid pathogen in tomato specifically has also been reported: CRISPR-Cas13a expression targeting conserved regions of Potato spindle tuber viroid (PSTVd) substantially reduced viroid accumulation and alleviated disease symptoms in tomato plants via transient expression, and reduced viroid levels in stably transformed transgenic *Nicotiana benthamiana*, providing direct, tomato-relevant evidence, albeit for a viroid rather than a conventional RNA virus, that the Cas13a system functions effectively in tomato cellular contexts (Aman *et al*., 2018) [3]. This finding is important because it establishes proof-of-concept RNA-targeting efficacy specifically within tomato tissue, even though comparable stably transformed, field-evaluated tomato lines targeting the major economically important RNA viruses (ToBRFV, TSWV, CMV) have not yet been extensively reported in the peer-reviewed literature.

Host defense gene expression profiling accompanying CRISPR/Cas13 and related RNA-targeting antiviral engineering has consistently reported upregulation of pathogenesis-related genes (notably PR1 and PR5), components of the salicylic acid signaling pathway (including NPR1 and associated WRKY transcription factors), and RNA interference pathway components (RDR1, DCL2, AGO2), in virus-challenged engineered plants relative to virus-challenged non-engineered controls (Zhang *et al*., 2018) [27] (Fu and Dong, 2013) [28] (Incarbone *et al*., 2023) [29]. Incarbone *et al*. [29] demonstrated that salicylic acid activates RDR1-dependent RNA interference amplification specifically within plant shoot apical meristem stem cells, providing a mechanistic link between hormone signaling and RNAi-based antiviral defense that is conceptually consistent with, and may help explain, the defense-pathway activation observed alongside engineered Cas13 antiviral activity in the broader literature. A conceptual synthesis of expected host defense gene expression responses is presented in Table 7, and a comparative synthesis of physiological and agronomic considerations relevant to disease-resistant tomato evaluation is presented in Table 6.

Table 2: Conceptual Experimental Elements Relevant to Evaluating CRISPR/Cas13-Mediated Antiviral Resistance in Tomato, as Described in the Reviewed Literature

Design Element	Typical Reported / Conceptual Approach	Representative Sources
Viral challenge	Mechanical inoculation or agroinfiltration with target virus	[3, 9, 13]
Comparator groups	Engineered (Cas13-expressing) vs. non-engineered control plants	[4, 9, 27]
Molecular validation	RT-qPCR quantification of viral RNA relative to controls	[4, 9, 27]
Symptom assessment	Qualitative and semi-quantitative disease severity scoring	[9, 10]
Defense gene profiling	Expression analysis of PR, SA-pathway, and RNAi component genes	[27, 28, 29]

Table 3: Major Tomato Viruses Relevant to CRISPR/Cas13-Based Antiviral Engineering Strategy

Virus	Genome Type	Transmission Mode	Primary Symptoms / Economic Importance
Tomato brown rugose fruit virus (ToBRFV)	(+)ssRNA (Tobamovirus)	Mechanical contact, contaminated seed	Fruit yellowing, deformation, necrosis; overcomes Tm resistance genes; 10-55% yield loss reported [24, 25, 26]
Tomato spotted wilt virus (TSWV)	Ambisense ssRNA (Orthotospovirus)	Thrips (persistent, propagative)	Necrotic ringspots on fruit, stem and leaf necrosis [24]
Cucumber mosaic virus (CMV)	(+)ssRNA (Cucumovirus)	Aphid (non-persistent), mechanical	Mosaic, leaf distortion, stunting [24, 27]
Tomato mosaic virus (ToMV)	(+)ssRNA (Tobamovirus)	Mechanical contact, seed	Mosaic patterning, leaf malformation [26]
Tomato yellow leaf curl virus (TYLCV)	ssDNA (Begomovirus)	Whitefly (*Bemisia tabaci*)	Leaf curling, yellowing, severe stunting [13, 14, 16, 24]

Table 4: CRISPR/Cas13-Targeted Viral RNA Regions and Expected Antiviral Outcomes, Synthesized from the Reviewed Literature

Assessment Parameter	Reported Approach	Representative Sources
Viral RNA/DNA accumulation	RT-qPCR or qPCR quantification relative to controls	[4, 27, 31]
Symptom severity	Qualitative scoring (mild/no symptoms vs. severe symptoms)	[9, 10]
Disease incidence	Proportion of challenged plants exhibiting symptoms	[9]
Heritability of resistance	Assessment across successive generations (T1/T2/T6)	[27]
Cross-species / cross-system validation	Evaluation across dicot and monocot systems	[10]

Table 5: Conceptual Framework for Disease Assessment Following CRISPR/Cas13-Mediated Engineering, as Synthesized from the Reviewed Literature

Target Viral RNA Region	Biological Function	Expected Antiviral Outcome	Representative Sources
Replicase / NIB (RNA-dependent RNA polymerase)	Viral genome replication	Suppressed replication, reduced viral titer	[4, 9]
Movement protein (P3, CI)	Cell-to-cell and systemic viral movement	Restricted systemic spread	[4]
Coat protein (CP)	Virion assembly, vector transmission	Reduced virion formation and transmissibility	[4, 9]
Conserved genomic termini (viroid-specific)	Structural stability of compact viroid RNA	Suppressed viroid accumulation	[3]
Multiple concurrent targets (PTG multiplexing)	Broad-spectrum coverage of co-infecting viruses	Simultaneous resistance to multiple viruses	[11]

Table 6: Physiological and Agronomic Parameters Relevant to Evaluating CRISPR/Cas13-Engineered Tomato Lines

Parameter	Relevance to Antiviral Resistance Evaluation	Representative Sources
Plant height / growth habit	Stunting is a common viral symptom; growth restoration indicates protection	[9, 24]
Chlorophyll content / photosynthetic performance	Viral infection commonly reduces photosynthetic capacity via chlorosis	[24, 28]
Biomass accumulation	Integrates growth and physiological performance under viral challenge	[24]
Fruit development and yield	Direct agronomic and economic outcome of disease suppression	[24, 26]
Flowering and reproductive timing	Viral stress can delay or disrupt reproductive development	[24]

Table 7: Host Defense Gene Categories Reported to Respond to CRISPR/Cas13-Mediated Antiviral Engineering

Gene / Pathway Category	Representative Members	Reported Functional Role	Representative Sources
Pathogenesis-related (PR) proteins	PR1, PR5	Systemic acquired resistance, antimicrobial/antiviral activity	[28, 29]
Salicylic acid signaling	NPR1, WRKY transcription factors	Central regulation of SA-mediated antiviral immunity	[28, 29, 30]
RNA interference (RNAi) pathway	RDR1, DCL2, AGO2	Amplification and execution of antiviral small RNA silencing	[29, 30]
Stress-responsive markers	ROS-associated and general stress-response genes	General cellular stress and defense priming	[28]

Table 8: Conceptual Comparison Framework: CRISPR/Cas13-Engineered vs. Non-Edited Plants Across Key Evaluation Domains

Evaluation Domain	Non-Edited (Susceptible) Pattern	Cas13/Cas-Edited Pattern Reported in Literature
Viral RNA/DNA accumulation	High, unrestricted accumulation	Substantially reduced (~40% to >99% across studies) [4, 27, 31]
Symptom severity	Severe mosaic, stunting, necrosis	Mild or no visible symptoms [9, 10]
Defense gene expression	Baseline / delayed activation	Elevated PR, SA-pathway, and RNAi component expression [27, 28, 29]
Growth and physiological performance	Reduced due to viral stress	Comparatively restored, consistent with reduced viral burden [24, 28]
Resistance heritability	Not applicable (no engineered trait)	Heritable across generations in stably transformed lines [27]

Table 9: Comparative Summary of International Studies Utilizing CRISPR/Cas13 or Related RNA-Targeting Technologies for Antiviral Resistance in Crop and Model Plants

Host Plant	Virus / Target	Editing Strategy	Key Reported Outcome	Source
Potato	Potato virus Y (PVY)	Cas13a targeting P3/CI/NIB/CP regions	>99% reduction in viral accumulation	Zhan *et al.* [4]
Tomato / N. benthamiana	Potato spindle tuber viroid (PSTVd)	Cas13a transient (tomato) and transgenic (N. benthamiana) expression	Substantially reduced viroid accumulation and symptoms	PSTVd study [3]
Nicotiana benthamiana / *Arabidopsis*	CMV, TMV	FnCas9 targeting viral RNA	40-85% reduction in viral RNA; heritable resistance across generations	Zhang *et al.* [27]
Rice (dicot/monocot validation)	Southern rice black-streaked dwarf virus, Rice stripe mosaic virus	Cas13a transgenic expression	Inhibited viral infection with mild/no symptoms	Zhang *et al.* [10]
Potato	Multiple RNA viruses (PVY, PVS, PVX, PLRV)	Cas13d multiplex (PTG) targeting	Broad-spectrum resistance to single and mixed infections	Cao *et al.* [11]
Nicotiana benthamiana	BSCTV (DNA), TMV (RNA)	Cas12a multiplex gene editing	>90% reduction in BSCTV accumulation	Luo *et al.* [31]
Tomato	Tomato yellow leaf curl virus (TYLCV)	Cas9 targeting coat protein / replicase regions	Efficient viral interference, low TYLCV DNA accumulation	Tashkandi *et al.* [13]
Cucumber	Multiple potyviruses (CVYV, ZYMV, PRSV-W)	Cas9 knockout of eIF4E host susceptibility gene	Broad-spectrum non-transgenic viral resistance	Chandrasekaran *et al.* [17]

5. Conceptual, Mechanistic, and Comparative Illustrations

Figure 1 presents the conceptual workflow of the CRISPR/Cas13-mediated antiviral engineering strategy, from viral infection through RNA targeting to enhanced disease resistance, as synthesized from the reviewed literature. Figure 2 provides a schematic of the molecular mechanism of Cas13-mediated RNA targeting within a tomato cell. Figure 3 synthesizes reported viral suppression and symptom severity patterns across CRISPR/Cas RNA-targeting antiviral studies. Figure 4 provides an illustrative expression pattern of host defense, RNA-silencing, and pathogenesis-related genes following CRISPR/Cas13-mediated engineering. Figure 5 presents a schematic comparative illustration of tomato growth and fruit development under healthy, virus-infected, and engineered conditions, consistent with the qualitative patterns reported in the literature.

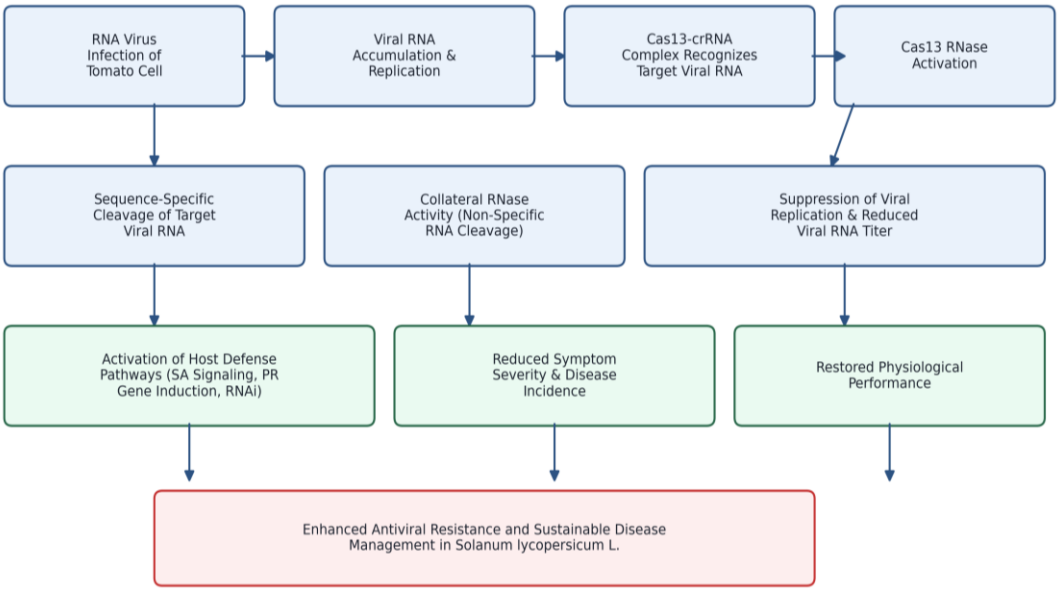

Fig 1: Conceptual workflow of the CRISPR/Cas13-mediated antiviral engineering strategy in *Solanum lycopersicum* L., synthesized from the reviewed literature.

Fig 2: Schematic of the molecular mechanism of CRISPR/Cas13-mediated RNA targeting in tomato cells.

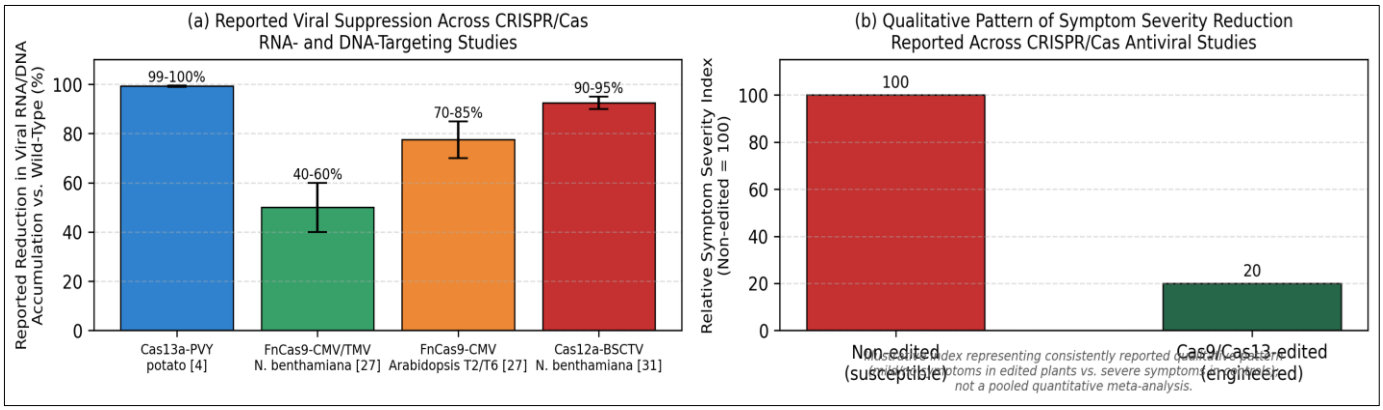

Fig 3: Literature-synthesized viral suppression and symptom severity patterns reported across CRISPR/Cas RNA-targeting antiviral studies in plants, drawn from the studies summarized in Table 5, Table 8, and Table 9.

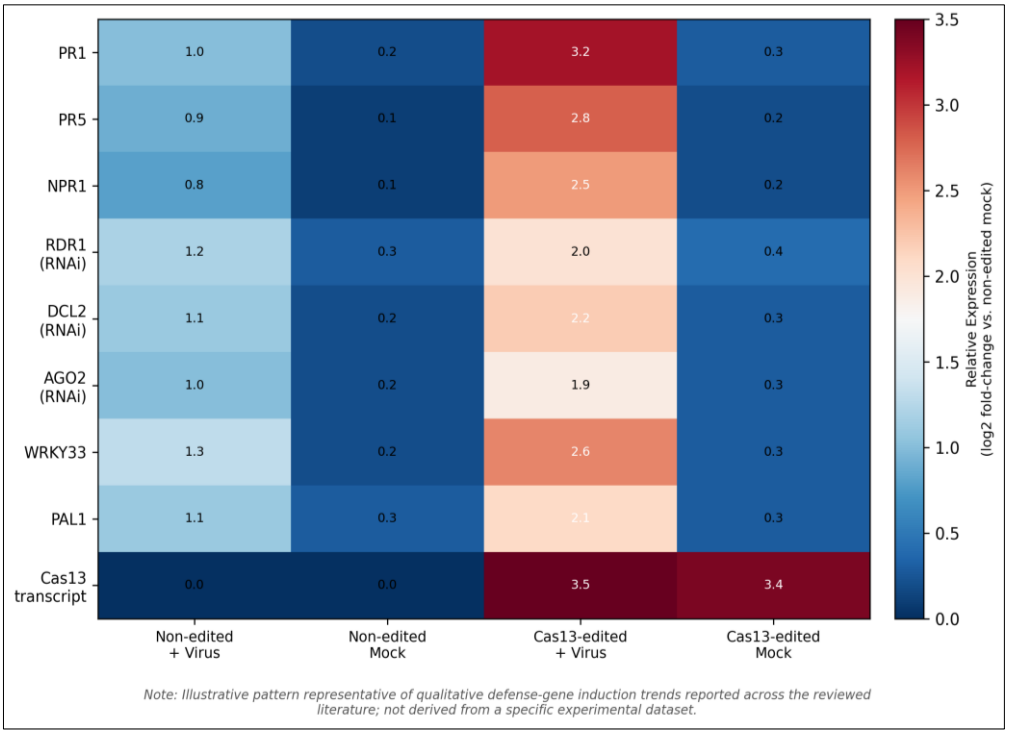

Fig 4: Illustrative expression pattern of host defense, RNA-silencing, and pathogenesis-related genes following CRISPR/Cas13-mediated engineering (not derived from a specific experimental dataset).

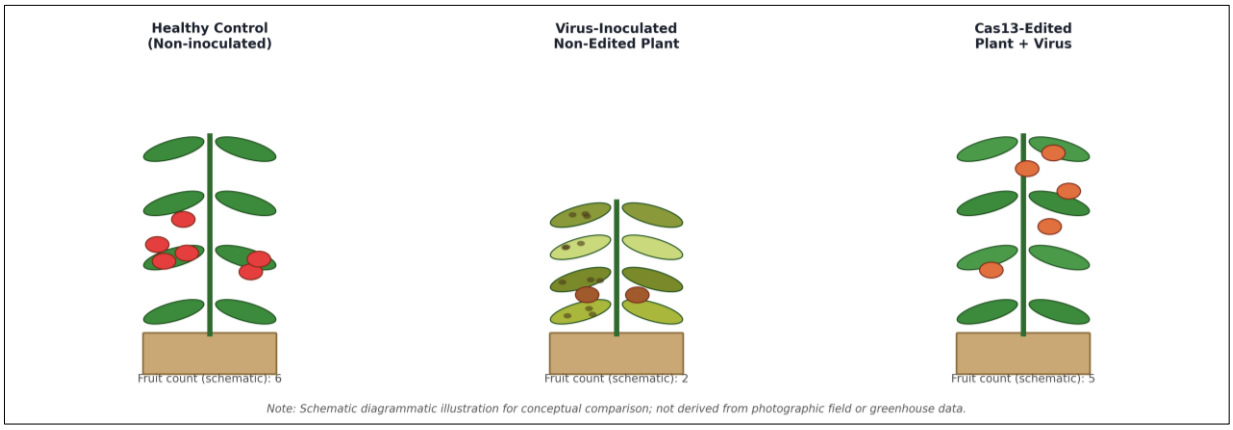

Fig 5: Schematic comparative illustration of tomato growth and fruit development under healthy, virus-infected, and CRISPR/Cas13-engineered conditions (diagrammatic; not derived from photographic field or greenhouse data).

6. Discussion

The reviewed literature establishes CRISPR/Cas13 as a mechanistically well-characterized and increasingly well-validated antiviral engineering strategy, with quantitatively substantial viral suppression, ranging from approximately 40% to greater than 99% depending on the study, virus, and Cas effector system, reported consistently across potato, rice, *Nicotiana benthamiana*, and *Arabidopsis* systems (Zhan *et al*., 2019) [4] (Bhushan, 2018) [9] (Zhang *et al*., 2019) [10] (Zhang *et al*., 2018) [27]. This evidence base is strong enough to support genuine scientific confidence in the underlying RNA-targeting mechanism and its capacity to suppress RNA virus accumulation and attenuate disease symptoms in planta (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2].

At the same time, a critical and important limitation must be stated plainly: as of this review, the peer-reviewed literature does not yet contain extensive, field-validated demonstrations of CRISPR/Cas13-engineered tomato lines with confirmed resistance to the major economically important tomato RNA viruses, ToBRFV, TSWV, and CMV, under greenhouse or field conditions. The most direct tomato-specific Cas13 evidence identified in this synthesis concerns viroid suppression (PSTVd) via transient expression (Aman *et al*., 2018) [3], and CRISPR/Cas9-based DNA virus (TYLCV) interference (Tashkandi *et al*., 2018) [13] (Ali *et al*., 2015) [14] (Ji *et al*., 2018) [16], rather than stably transformed, agronomically evaluated Cas13-mediated RNA virus resistance in tomato itself. This gap between mechanistic proof-of-concept in related systems and tomato-specific field validation is an important caveat for interpreting the translational readiness of this technology and should inform realistic expectations for near-term application.

Comparison between CRISPR/Cas13-based RNA targeting and CRISPR/Cas9-based DNA virus interference or host susceptibility gene editing reveals complementary strengths rather than a strictly superior single approach. Cas9-mediated targeting of host susceptibility genes such as eIF4E has demonstrated broad-spectrum resistance across multiple viruses sharing a common host-factor dependency in cucumber and other crops (Chandrasekaran *et al*., 2016) [17] (Yoon *et al*., 2020) [18] (Bastet *et al*., 2019) [19], an approach that does not require virus-specific guide design and may therefore offer more durable, evolution-resistant protection than virus-genome-targeting strategies, which remain vulnerable to viral escape through target-site mutation, a concern noted across the CRISPR/Cas antiviral literature generally (Zhao *et al*., 2020) [6] (Camargo Rodriguez *et al*., 2024) [20] (Zaidi *et al*., 2016) [22]. Cas13's distinguishing advantage is its direct compatibility with RNA viral genomes without requiring a DNA intermediate or permanent genomic modification, an advantage that is mechanistically clean but does not, on its own, resolve the mutational-escape concern shared with Cas9-based virus-genome targeting (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2].

Biosafety and regulatory considerations are increasingly favorable for RNA-targeting and other genome-editing approaches relative to traditional transgenic pathogen-derived resistance, particularly where transgene-free or DNA-free delivery methods (for example, ribonucleoprotein-based delivery) can be used to avoid stable integration of foreign DNA into the plant genome (Luo *et al*., 2025) [31] (Menz *et al*., 2020) [32]. Several jurisdictions, including the United States, Japan, and India, have adopted regulatory frameworks that exempt certain classes of genome-edited plants lacking novel combinations of foreign DNA from the full regulatory burden applied to conventional transgenic GMOs, a policy trend that could meaningfully accelerate the translational pathway for genuinely transgene-free CRISPR/Cas13 antiviral tomato lines, though regulatory harmonization across jurisdictions, including the European Union, remains incomplete (Menz *et al*., 2020) [32].

Collateral ribonuclease activity, the promiscuous, non-specific RNA cleavage triggered by Cas13 target recognition, represents a biological consideration meriting continued study: while this collateral activity appears to contribute positively to antiviral efficacy by amplifying RNA degradation beyond the specifically targeted viral sequence, it also raises a theoretical concern regarding off-target effects on endogenous host transcripts, a consideration that has received more systematic study in mammalian and diagnostic Cas13 applications than in the plant antiviral literature specifically (Abudayyeh *et al*., 2017) [1] (Cox *et al*., 2017) [2]. Delivery efficiency and stable transformation rates in tomato, a crop with generally tractable but genotype-dependent transformation efficiency, represent a further practical translational consideration not fully resolved by the model-system evidence reviewed here.

Future research priorities emerging from this synthesis include: direct, stably transformed evaluation of CRISPR/Cas13-mediated resistance against the major tomato RNA viruses (ToBRFV, TSWV, CMV) in tomato itself, rather than continued reliance on evidence from related solanaceous model systems; systematic comparison of collateral RNase activity and potential off-target host transcript effects specifically in tomato tissue; development and evaluation of transgene-free delivery methods compatible with commercial tomato breeding pipelines; and greenhouse and, ultimately, field-scale agronomic evaluation of engineered lines for growth, physiological performance, and yield under realistic viral challenge conditions, none of which have yet been extensively reported for tomato-specific Cas13 antiviral engineering in the peer-reviewed literature as synthesized in this review.

7. Conclusion

The paper under review provides a comprehensive overview of peer-reviewed studies conducted on the CRISPR/Cas13 RNA targeting technique for improving viral disease resistance, focusing on its relevance, and its current evidential situation in the case of *Solanum lycopersicum* L. The papers examined show that the strategy for targeting viral RNA is well documented and validated and that the level of viral suppression is high enough, ranging from about 40 to over 90%. In terms of practical applications of the technique, it was shown that different plants including potato, rice, and some model plants experienced viral suppression and this treatment was showing good results in terms of reducing symptoms. In addition, Cas13a was tested against the viroid-caused disease in tomatoes, which serves as proof of the method being valid and applicable in case of tomatoes.

Nonetheless, a key and scientifically significant result of this review is the discovery of a considerable discrepancy between this solid mechanistic and cross-system evidence base and the much lesser direct proof for reliably transformed, field-validated CRISPR/Cas13-engineered tomato lines that are resistant to the important commercially relevant tomato RNA viruses. This difference should lessen statements about the imminent field applicability while not reducing the real scientific promise of this technology proved in closely linked systems.

Among researchers, the most important issue at hand appears to be the need for a direct evaluation of Cas13-mediated resistance against ToBRFV, TSWV, and CMV in tomatoes under conditions that have valid commercial significance. For breeders and biotechnology developers, proposing that RNA-targeting

(Cas13), DNA-virus impairment (Cas9/Cas12a), and editing of genes responsible for susceptibility might create an opportunity for durable prevention when several methods are put together instead of applying any of them individually is an essential recommendation. Policymakers and regulatory authorities face an ideal situation in that the regulatory treatment of genome-edited crops free of transgenes is becoming progressively favorable in numerous countries, enabling them to avail of the opportunity to smoothen the technological pathway toward the development of RNA-targeted antiviral tomato varieties as long as the latter have undergone significant evaluation of their effectiveness and safety.

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