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A REVIEW TO COMPREHEND THE APPLICATION OF GENE SILENCING TECHNOLOGY TO OVERCOME CROP PRODUCTIVITY LOSSES

Original Research

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ABSTRACT

Background: Gene silencing represents an essential molecular mechanism by which plants regulate or suppress gene expression to combat viral infections. This process, occurring either at the transcriptional or post-transcriptional level, serves as a critical antiviral defense strategy, allowing plants to degrade or inhibit viral RNA. RNA-silencing pathways, including small interfering RNA (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA) mechanisms, play pivotal roles in protecting plants against viral replication and in maintaining genome integrity.

Objective: This review aims to comprehensively explore the molecular mechanisms, biological significance, and applications of gene silencing in plant antiviral defense, emphasizing the pathways involving siRNA, miRNA, and RNA-induced silencing complexes (RISC).

Main Discussion Points: The review discusses transcriptional and post-transcriptional gene silencing mechanisms, focusing on how dicer enzymes, Argonaute proteins, and RISC complexes mediate RNA degradation. It highlights the differentiation between siRNA and miRNA pathways and their distinct yet complementary roles in regulating viral gene expression. Additionally, the role of RNA-directed DNA methylation (RdDM) in suppressing DNA viruses is explored, alongside the importance of gene silencing in developing virus-resistant crops through RNA interference technology.

Conclusion: Gene silencing constitutes a highly adaptive and conserved defense mechanism in plants, providing an effective molecular barrier against viral pathogens. A deeper understanding of these pathways offers promising avenues for agricultural biotechnology, including the development of genetically engineered crops with enhanced viral resistance and improved yield stability.

Keywords: Gene Silencing, RNA Interference, siRNA, miRNA, Plant Viral Defense, RNA-Induced Silencing Complex.

INTRODUCTION

Gene silencing represents a natural and sophisticated defense mechanism by which plants regulate gene expression at either the transcriptional or post-transcriptional level, primarily to counter viral invasions. This biological process enables plants to suppress specific gene activity, effectively interrupting disease pathways and limiting pathogen replication. While bacterial and fungal diseases can often be mitigated through conventional chemical control measures, viral infections remain particularly challenging due to the complex dual nature of viruses—exhibiting both living and non-living characteristics (1-3). Their minimal structure, consisting in some cases of mere nucleic acid sequences without protective coat proteins, and their high mutation rates render them resistant to traditional management strategies. Furthermore, their ability to exploit host cellular machinery for replication allows them to adapt and persist, posing continuous threats to crop health and productivity (4). Viral infections in plants manifest through a broad spectrum of symptoms, including leaf mottling, puckering, vein swelling, twisting, rolling, and wrinkling, often culminating in stunted growth and a bushy canopy appearance. In severe cases, flower abortion leads to a marked reduction in fruit yield, underscoring the economic and agricultural impact of plant viral diseases (5,6). Given the vast host range and mutability of plant viruses, developing durable resistance through conventional breeding remains an ongoing challenge, thus highlighting the need for molecular approaches such as RNA-mediated gene silencing.

The discovery of RNA silencing emerged serendipitously in the early 1990s during plant transgenic studies, revealing RNA as the central molecule inducing post-transcriptional gene suppression (7). Pioneering work demonstrated that pathogen-derived RNA could trigger sequence-specific degradation of homologous RNA, establishing RNA silencing as a crucial antiviral mechanism (8). Subsequent research expanded this understanding, identifying RNA-directed DNA methylation (RdDM) and viral RNA-silencing suppressors as vital components of the plant immune response (9,10). These findings not only elucidated fundamental molecular processes but also opened new avenues for engineering virus-resistant crops through targeted gene silencing strategies. Despite these advances, the mechanistic intricacies and potential agricultural applications of gene silencing continue to be explored. The present study aims to comprehend the application of gene silencing technology as a sustainable approach to overcome crop productivity losses by developing virus-resistant plants through molecular intervention. This study is to evaluate and rationalize the application of gene silencing mechanisms in mitigating viral disease–related yield losses in crops.

Differential Interactions of Viruses in Plants

Viruses interact with plants in complex and differential ways, depending largely on their genomic composition and replication strategies. Double-stranded DNA (dsDNA) viruses replicate within the nucleus by utilizing the host cell's replication machinery, whereas RNA viruses depend primarily on the cytoplasmic environment for replication. This spatial distinction in viral replication has shaped the evolutionary dynamics of plant immune systems, which have adapted distinct molecular strategies to detect and silence foreign nucleotide sequences (1). Among these, gene silencing has emerged as a pivotal mechanism, wherein specific viral sequences are targeted and degraded, preventing the production of viral proteins and replication of the viral genome. Despite its immense potential, gene silencing as a plant defense mechanism has historically received less research attention than conventional chemical or breeding-based control strategies (2). The application of gene silencing technology has, however, revolutionized the understanding of plant-pathogen interactions and introduced a promising avenue for sustainable crop protection by leveraging the plant's intrinsic molecular defense pathways.

Plant Defense System Against DNA Viruses

Plants employ RNA-mediated silencing pathways to counter DNA viruses, operating at both transcriptional and post-transcriptional levels. At the transcriptional stage, gene silencing involves promoter methylation, which suppresses the initiation of viral RNA synthesis. Post-transcriptionally, messenger RNA (mRNA) is produced but subsequently degraded or translationally repressed, thereby halting protein synthesis (3). The activation of these silencing pathways is triggered upon the introduction of viral genes into the host genome, leading to the recruitment of small RNA molecules that mediate defense. However, viruses have concurrently evolved sophisticated counter-defense mechanisms, such as RNA silencing suppressors, to overcome these plant responses (4). The foundational concept of RNA-directed DNA methylation (RdDM) was first observed in studies involving viroid transgenes in tobacco, marking a significant

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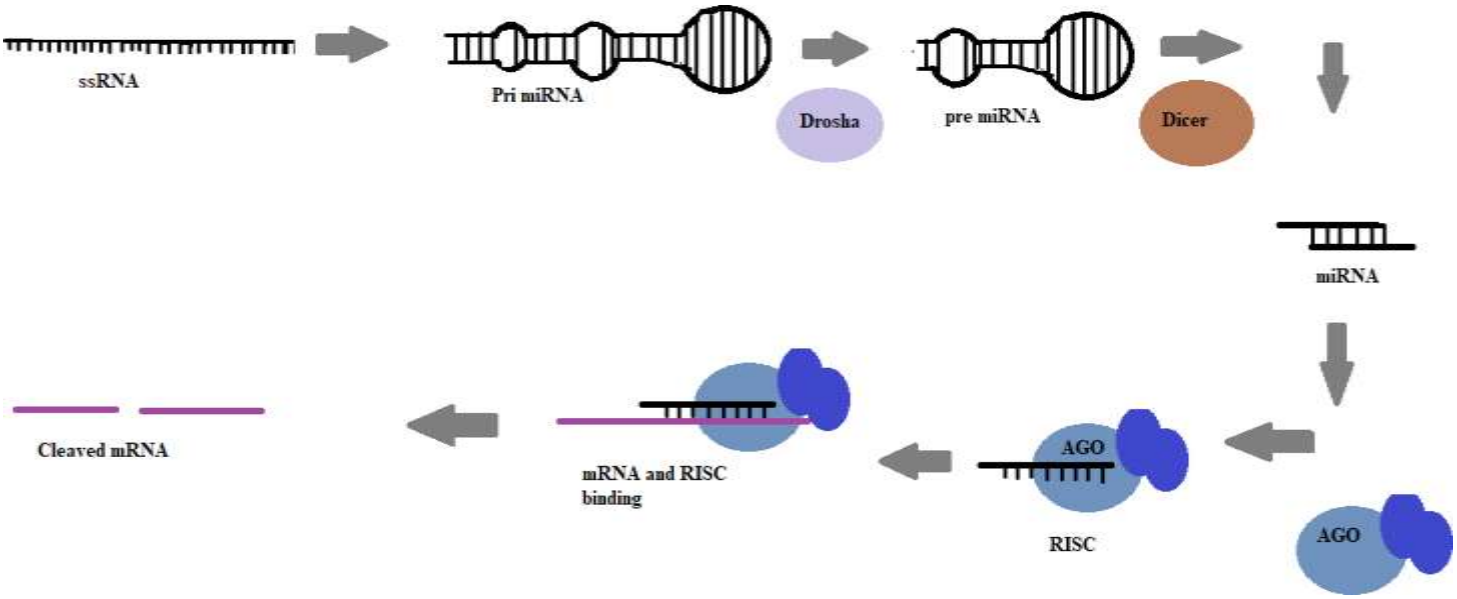
milestone in understanding the transcriptional silencing process (5). RdDM involves the conversion of double-stranded RNA into small interfering RNAs (siRNAs), which guide methylation at complementary DNA sequences, effectively silencing viral genes. This mechanism exemplifies a molecular arms race between plants and viruses—while plants evolve gene-silencing defenses, viruses develop strategies to evade or suppress them.

Post-Transcriptional Gene Silencing

Post-transcriptional gene silencing (PTGS) refers to the RNA-mediated suppression of gene expression at the mRNA level, preventing translation and thereby blocking the formation of the corresponding protein (6). The discovery of RNA interference (RNAi) demonstrated that the introduction of double-stranded RNA (dsRNA) into a cell can specifically silence genes sharing sequence homology, leading to targeted degradation of viral RNA. This process relies heavily on small RNA molecules that recognize and neutralize viral transcripts, establishing an adaptive-like immune response in plants. RNAi has become an invaluable biotechnological tool for developing virus-resistant crops. Studies have shown that the efficacy of PTGS depends on both the abundance and stability of the small RNA populations generated during infection (7). Moreover, the process serves not only as a viral defense but also as a regulatory mechanism for endogenous gene expression, underlining its dual biological significance.

miRNA

MicroRNAs (miRNAs) play a central role in the regulation of gene silencing, functioning as small, non-coding RNAs derived from longer primary transcripts (pri-miRNAs). These pri-miRNAs are processed by the Drosha-DGCR8 complex into precursor miRNAs (pre-miRNAs), which are exported from the nucleus to the cytoplasm via exportin-5. In the cytoplasm, the Dicer enzyme cleaves the pre-miRNA into a short double-stranded RNA fragment with a characteristic 3′ overhang structure (6). One strand of this miRNA duplex—known as the guide strand—is incorporated into the RNA-induced silencing complex (RISC), which includes key proteins such as Argonaute and TRN6C6. RISC identifies complementary sequences on target mRNAs and mediates their cleavage or translational repression, effectively silencing viral gene expression (7). Through this highly specific mechanism, miRNAs regulate both endogenous gene expression and defense-related responses to viral infection. The precision of miRNA-mediated regulation underscores its importance as a natural antiviral strategy and a potential tool for genetic engineering of resistant crop varieties.



siRNA

Small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) represent another crucial component of the RNA silencing pathway. Structurally, siRNAs are double-stranded molecules characterized by 5′ phosphate and 3′ hydroxyl termini, along with 2–3 nucleotide 3′ overhangs (7). They may originate from either endogenous transcriptional activity or exogenous viral RNA. The Dicer enzyme, an RNase III-like ribonuclease, processes long dsRNA into uniform siRNA fragments, which are then incorporated into RISC. Within RISC, the PAZ domain of Argonaute facilitates guide strand binding, while the passenger strand is discarded. The PIWI domain

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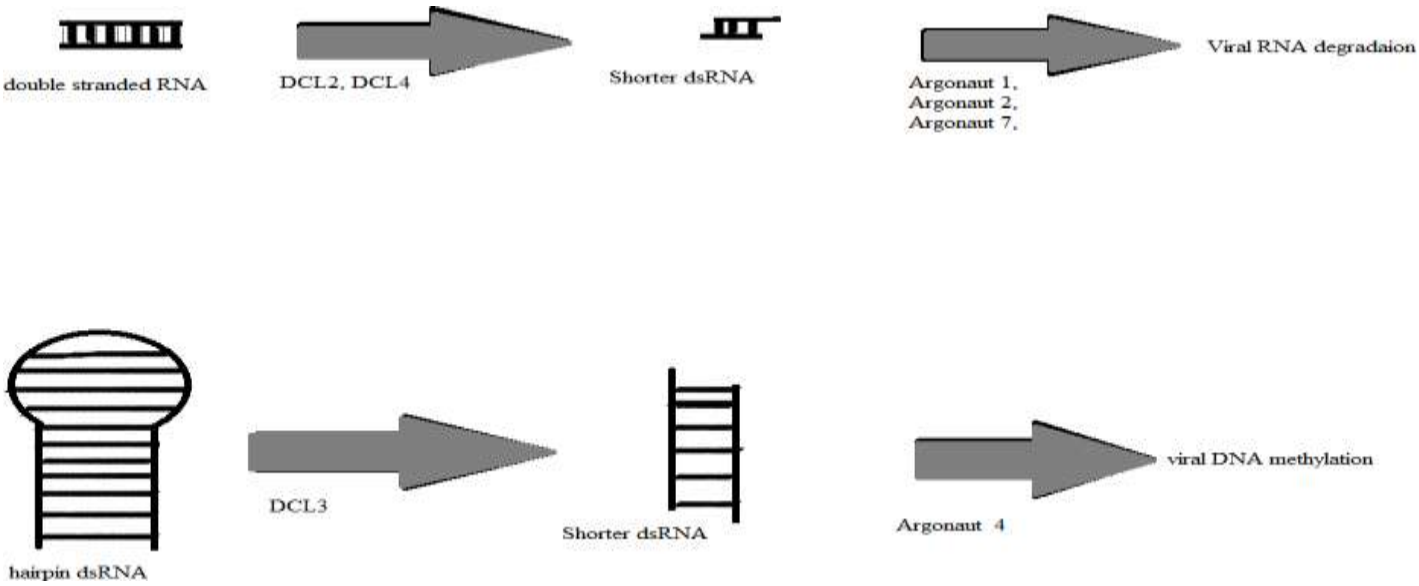
of Argonaute exhibits RNase-H-like activity, enabling the cleavage of complementary viral RNA sequences (6,7). This precise degradation prevents translation of viral proteins, effectively halting the infection process. Among the Dicer-like proteins, DCL4 and DCL2 have been identified as key contributors to antiviral defense, with DCL4-generated 21-nt siRNAs playing a more prominent role than the 22-nt siRNAs produced by DCL2 (8). Argonaute 1 (AGO1) is recognized as the principal effector in siRNA-mediated defense, forming the first line of antiviral activity, while other AGO proteins act as secondary defense layers (9). DCL3, although primarily associated with 24-nt siRNA production, contributes to DNA virus resistance through RdDM, whereas DCL1 assists in the biogenesis of 21-nt siRNAs during DNA viral infections (10). Collectively, these molecular interactions define a multi-layered antiviral defense network within the plant cell.



Plant Defense System Against DNA Viruses

The RNA-directed DNA methylation (RdDM) pathway represents a unique and highly specialized defense system in plants, primarily responsible for maintaining genomic integrity by silencing transposable elements and repetitive sequences (11). This mechanism also plays an instrumental role in defending against DNA viruses, particularly geminiviruses and pararetroviruses, which exploit host transcriptional machinery for replication. In this pathway, 24-nucleotide siRNAs generated by DCL3 guide the methylation of cytosine residues within viral DNA sequences. These siRNAs originate from double-stranded RNA produced by the coordinated activity of plant-specific enzymes such as RNA polymerase IV and RNA-dependent RNA polymerase 2 (RDR2) (12). The methylation marks established by the RdDM complex suppress viral gene transcription, preventing the accumulation of viral DNA and restricting its replication. Despite substantial progress, the complete molecular framework of RdDM remains only partially understood. Studies indicate that while RdDM efficiently suppresses DNA virus replication, certain viruses can encode suppressor proteins that interfere with siRNA biogenesis or methylation pathways, highlighting an ongoing co-evolutionary battle between plant defense and viral counter-strategies (13). Understanding and harnessing this natural mechanism could provide the foundation for the development of durable resistance in plants through targeted epigenetic engineering.

CRITICAL ANALYSIS AND LIMITATIONS

While RNA silencing technologies such as Virus-Induced Gene Silencing (VIGS), Post-Transcriptional Gene Silencing (PTGS), and RNA interference (RNAi) have demonstrated remarkable progress in developing virus-resistant crops, several critical limitations persist in the existing literature. Although VIGS has emerged as an efficient preliminary tool for identifying gene function, it primarily provides indicative rather than conclusive evidence regarding the involvement of a specific gene in plant defense mechanisms. The necessity of subsequent biochemical assays to confirm protein function underscores the methodological incompleteness of studies relying solely on VIGS (14). Furthermore, there remains uncertainty regarding the applicability of VIGS across animal systems, as current evidence

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suggesting its potential antiviral role in animals remains circumstantial (15). These conceptual gaps indicate that, while VIGS is a valuable exploratory approach, it cannot independently substantiate causal relationships between gene silencing and phenotype expression without corroborative molecular or biochemical validation. In plant biotechnology, numerous studies have successfully demonstrated RNAi-mediated virus resistance across diverse crops including papaya, tomato, potato, cassava, rice, and banana (16-18). However, most of these investigations were limited to controlled laboratory or greenhouse environments with small sample populations and short observation periods. This raises concerns regarding the reproducibility and stability of resistance under variable field conditions. The lack of large-scale randomized field trials or multi-environment evaluations limits the generalizability of these findings to real-world agricultural systems. Moreover, the majority of transgenic lines were developed in model cultivars, often neglecting local or region-specific genotypes that may exhibit different responses to RNAi constructs due to genetic and epigenetic variability. Methodological inconsistencies across studies also challenge the comparability of results. Differences in promoter efficiency, target gene selection, and transformation methods lead to variable levels of silencing efficiency and resistance expression (18,19). Additionally, many studies fail to account for potential off-target effects, gene redundancy, and the long-term epigenetic stability of silenced genes, which could confound phenotypic outcomes. The use of constitutive promoters in some constructs may also disrupt normal plant physiology, raising biosafety and yield-related concerns. Furthermore, few studies incorporate rigorous negative controls or double-blind evaluations, leaving open the possibility of experimental and performance bias.

Publication bias remains another significant limitation in the existing literature, as studies reporting successful silencing and resistance outcomes are more frequently published, while inconclusive or negative findings often remain unreported. This selective dissemination of positive results creates an overly optimistic view of RNAi efficiency and may obscure the true variability and limitations of the technology. The absence of comprehensive meta-analyses integrating both successful and unsuccessful cases further hampers objective assessment of overall efficacy. A recurring gap across the reviewed literature is the lack of long-term ecological and evolutionary studies assessing viral adaptation and resistance breakdown. Viruses exhibit high mutation rates and can potentially evolve suppressors of RNA silencing, as demonstrated in several plant–virus interaction models (20,21). The durability of RNAi-mediated resistance therefore remains questionable in the absence of multi-seasonal data. Additionally, few studies examine the potential horizontal transfer of transgenes or unintended effects on non-target organisms, particularly beneficial soil microbes and pollinators, which are essential components of sustainable ecosystems. Another limitation pertains to the variable measurement outcomes used to assess resistance efficacy. Some studies evaluate viral load through molecular assays such as qPCR, while others rely solely on phenotypic observations like symptom severity or yield recovery. The lack of standardized evaluation criteria makes cross-study comparisons difficult and may lead to inconsistent interpretations of resistance efficiency (22). Similarly, the absence of uniform criteria for quantifying RNA silencing efficiency across different gene targets creates further uncertainty in assessing success rates and reproducibility. Finally, the generalizability of RNA silencing-based approaches remains constrained by socioeconomic and regulatory factors. Most of the successful case studies, such as PRSV-resistant papaya and RNAi-based potatoes, were developed in industrialized or well-funded research environments, limiting their accessibility and scalability in resource-constrained agricultural systems (20,23). Moreover, regulatory hurdles surrounding genetically modified organisms (GMOs) continue to restrict the commercial deployment of RNAi-engineered crops in many regions, including developing countries where viral crop losses are most severe. In summary, while RNA silencing and VIGS have significantly advanced plant molecular defense research, existing literature reveals important methodological, translational, and ecological limitations. Future investigations must integrate long-term, field-based, and multidisciplinary approaches that address these constraints through standardized protocols, larger and more diverse sample sets, and transparent reporting of all outcomes—including failures—to establish a reliable foundation for the sustainable use of RNA silencing technologies in global agriculture.

IMPLICATIONS AND FUTURE DIRECTIONS

The advancement of RNA silencing technologies has transformed the understanding of plant-pathogen interactions and holds profound implications for sustainable agriculture and global food security. The reviewed literature demonstrates that Virus-Induced Gene Silencing (VIGS) and RNA interference (RNAi) can effectively suppress viral replication and enhance crop resilience through targeted post-transcriptional gene regulation. These findings emphasize the practical potential of gene silencing approaches in developing genetically stable, virus-resistant cultivars that could significantly reduce dependency on chemical pesticides and minimize crop losses, particularly in regions prone to viral epidemics (19,20). From an applied biotechnology perspective, these innovations can contribute to the establishment of climate-resilient agricultural systems and enhance yield stability—both of which are vital for meeting the nutritional

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demands of an expanding global population. Although VIGS provides valuable preliminary evidence regarding gene function, its translation into field-level applications remains limited. The methodology primarily serves as a diagnostic or exploratory tool rather than a conclusive functional assay, necessitating complementary biochemical and proteomic studies for validation (21). Understanding the cross-kingdom implications of RNA-mediated defense is another frontier of significance. Emerging evidence indicates that similar RNA-silencing components exist in animals, suggesting conserved evolutionary mechanisms that could potentially be harnessed for antiviral defense in broader biological contexts (22,23). This opens avenues for comparative molecular studies between plant and animal systems, which may ultimately inform medical virology and therapeutic RNA design. At the agricultural policy level, these findings underscore the necessity of establishing biosafety frameworks and regulatory guidelines for the deployment of RNAi-based crops. Standardized assessment criteria for environmental safety, off-target effects, and potential horizontal gene transfer must be incorporated into national and international biosafety policies before large-scale commercialization. Furthermore, international cooperation is essential to ensure equitable access to gene-silencing technologies, particularly for low-income agricultural economies where viral crop diseases have the most severe socioeconomic impact. Public awareness and transparent communication about the safety and benefits of RNA-silenced crops could also help mitigate resistance to biotechnological interventions and foster evidence-based policy-making.

Several research gaps remain to be addressed before RNA silencing can achieve its full translational potential. Long-term studies assessing durability of resistance, viral mutation dynamics, and ecological interactions are urgently needed to determine the sustainability of RNAi-based approaches. Current studies largely rely on model plants and controlled conditions, limiting the generalizability of results to field environments characterized by environmental stressors and diverse pathogen pressures (24-26). Future research should prioritize multi-season field trials across diverse climatic zones and genotypic backgrounds to evaluate real-world effectiveness. Furthermore, the molecular mechanisms underlying viral suppressors of RNA silencing remain incompletely characterized, representing a critical area for exploration to prevent resistance breakdown. Methodologically, future studies should adopt multi-omics approaches integrating transcriptomics, epigenomics, and metabolomics to better elucidate the systemic effects of gene silencing on plant physiology. CRISPR-based transcriptional regulators may also be integrated with RNAi systems to enhance specificity and stability of silencing effects. The use of inducible promoters rather than constitutive ones could minimize unintended metabolic disruptions, ensuring better biosafety and yield consistency. Moreover, comparative trials employing standardized efficacy metrics—such as viral load quantification, phenotypic scoring, and agronomic performance—will be vital for harmonizing data across studies and enabling meta-analytic assessments of RNA silencing efficiency (27,28). In conclusion, the integration of RNA silencing technologies into mainstream agricultural practice holds immense promise for sustainable crop protection, reduced pesticide dependence, and enhanced global food resilience. However, to realize this potential, future research must transcend laboratory-scale experimentation and move toward ecologically robust, ethically governed, and globally coordinated frameworks. By bridging molecular innovation with regulatory foresight and socio-agricultural inclusivity, RNA silencing can serve as a cornerstone for next-generation plant biotechnology and precision agriculture.

RNA silencing technologies have significantly advanced the development of virus-resistant crops, with several successful examples demonstrating the utility of post-transcriptional gene silencing (PTGS) and RNA interference (RNAi) in plant biotechnology. One of the earliest and most notable examples is the genetically modified papaya (Carica papaya) developed to resist Papaya Ringspot Virus (PRSV). Scientists used PTGS by inserting a gene from the PRSV virus itself, a method known as pathogen-derived resistance (PDR). This led to the creation of the 'Rainbow' and 'SunUp' papaya varieties in Hawaii, which displayed high resistance to PRSV and saved the Hawaiian papaya industry from collapse (Gonsalves, 1998). In tomatoes (Solanum lycopersicum), resistance against Tomato Yellow Leaf Curl Virus (TYLCV) has been achieved through RNAi targeting viral coat protein or replication-associated genes. Some strategies also involve silencing host genes such as *eIF4E*, which are exploited by the virus for replication (Praveen *et al*., 2010). Similarly, transgenic potatoes (*Solanum tuberosum*) engineered to silence coat protein and replicase genes of Potato Virus Y (PVY) and Potato Leaf Roll Virus (PLRV) showed significant reduction in virus accumulation and symptom expression (Missiou *et al*., 2004). In bananas (*Musa spp.*), RNAi constructs targeting Banana Bunchy Top Virus (BBTV) genes are under research and field evaluation, showing promise in developing virus-resistant lines (Shekhawat *et al*., 2012). Cassava (*Manihot esculenta*), a staple in many African countries, has been genetically engineered to resist Cassava Brown Streak Virus (CBSV) and Cassava Mosaic Virus (CMV) using RNAi constructs targeting viral coat proteins, resulting in reduced disease symptoms and viral loads (Patil *et al*., 2011). In rice (*Oryza sativa*), RNAi strategies targeting tungro virus genes or the leafhopper vector's transmission factors have yielded resistant plants with delayed or no symptom development (Tyagi *et al*., 2008). Tobacco (*Nicotiana tabacum*) served as a foundational model in RNA silencing research, where expression of the Tobacco Mosaic Virus (TMV) coat protein gene in the plant induced resistance through gene silencing, laying the groundwork for later applications in other crops (Powell-Abel *et al*., 1986). In Potato (Solanum tuberosum L.) using RNAi (RNA

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interference) modified starch composition (amylose content) targeting gene for starch branching enzyme A and B (SBE A & B) resulting in production of high amylose potatoes which are useful for processing industry and dietary use (Jobling, S. A. 2004) . Similarly targeting PPO gene family in potato resulted in silencing of PPO (polyphenol oxidase) for reducing enzymatic browning in potato tubers during processing which ultimately improved shelf life and reduced food wastage (Waltz, E. 2015). Another event of gene silencing related to cold induced sweetening in potato during cold storage for gene responsible for vacuolar invertase (VInv) to prevent accumulation of reducing sugars which resulted in reduced acrylamide formation during frying which improved quality of potato (Bhaskar, P. B. *et al*., 2010). Silencing of susceptibility genes (StDND1, StDMR6) for late blight resistance in potato against *Phytophthora infestans using RNAi showed improved disease resistance without any effects on yield (Haque et al., 2020).*

CONCLUSION

The collective evidence reviewed highlights that RNA silencing represents a central and evolutionarily conserved mechanism in antiviral defense, functioning through intricate molecular pathways that regulate gene expression and restrict viral replication in plants. Its dual role—both as a defensive response and as a target of viral counter-strategies—underscores the complexity of host–virus coevolution. Despite remarkable progress in understanding RNA-silencing components such as siRNA, miRNA, and RdDM pathways, the current literature still presents limitations in fully elucidating how these mechanisms determine host specificity and cross-kingdom interactions. Experimental evidence remains largely derived from plant models, with limited translational validation in animal or insect systems, highlighting the need for more integrative, cross-disciplinary research. Future investigations should prioritize elucidating the molecular crosstalk between viruses, insect vectors, and host plants to establish a comprehensive understanding of RNA silencing in tripartite virus–host interactions. Strengthening this knowledge will not only advance molecular virology but also pave the way for innovative biotechnological strategies to enhance viral resistance and ensure sustainable agricultural productivity.

AUTHOR CONTRIBUTION

Author	Contribution
Mubushra Sarwar	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Tariq Javaid*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Rana Aftab Iqbal	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhammad Mudassir Hussain	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Tanveer	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Maha Sarfaraz	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Rabia Kalsoom	Contributed to study concept and Data collection Has given Final Approval of the version to be published

REFERENCES

1. Sarfraz Z, Zarlashat Y, Ambreen A, Mujahid M, Iqbal MS. Advanced gene editing techniques for enhancing disease resistance and climate resilience in crops. Funct Plant Biol. 2025;52.
2. Aroca A, García I. Advances in plant molecular biology: towards new challenges. J Exp Bot. 2023;74(19):5949-54.
3. Roychowdhury R, Das SP, Das S, Biswas S, Patel MK, Kumar A, et al. Advancing vegetable genetics with gene editing: a pathway to food security and nutritional resilience in climate-shifted environments. Funct Integr Genomics. 2025;25(1):31.

4. Zaman QU, Wen C, Yuqin S, Mengyu H, Desheng M, Jacqueline B, et al. Characterization of SHATTERPROOF Homoeologs and CRISPR-Cas9-Mediated Genome Editing Enhances Pod-Shattering Resistance in Brassica napus L. Crispr j. 2021;4(3):360-70.
5. Tuncel A, Pan C, Clem JS, Liu D, Qi Y. CRISPR-Cas applications in agriculture and plant research. Nat Rev Mol Cell Biol. 2025;26(6):419-41.
6. Choudry MW, Riaz R, Nawaz P, Ashraf M, Ijaz B, Bakhsh A. CRISPR-Cas9 mediated understanding of plants' abiotic stress-responsive genes to combat changing climatic patterns. Funct Integr Genomics. 2024;24(4):132.
7. Zhang D, Zhang Z, Unver T, Zhang B. CRISPR/Cas: A powerful tool for gene function study and crop improvement. J Adv Res. 2021;29:207-21.
8. Patra S, Chatterjee D, Basak S, Sen S, Mandal A. CRISPR/Cas9 opens new horizon of crop improvement under stress condition. Biochim Biophys Acta Gen Subj. 2024;1868(10):130685.
9. Wang L, O'Conner S, Tanvir R, Zheng W, Cothron S, Towery K, et al. CRISPR/Cas9-based editing of NF-YC4 promoters yields high-protein rice and soybean. New Phytol. 2025;245(5):2103-16.
10. Rivera-Toro DM, de Folter S, Alvarez-Venegas R. CRISPR/dCas12a-mediated activation of SlPAL2 enhances tomato resistance against bacterial canker disease. PLoS One. 2025;20(3):e0320436.
11. Rasheed A, Gill RA, Hassan MU, Mahmood A, Qari S, Zaman QU, et al. A Critical Review: Recent Advancements in the Use of CRISPR/Cas9 Technology to Enhance Crops and Alleviate Global Food Crises. Curr Issues Mol Biol. 2021;43(3):1950-76.
12. Zhou J, Liu G, Zhao Y, Zhang R, Tang X, Li L, et al. An efficient CRISPR-Cas12a promoter editing system for crop improvement. Nat Plants. 2023;9(4):588-604.
13. Liu Q, Zhao C, Sun K, Deng Y, Li Z. Engineered biocontainable RNA virus vectors for non-transgenic genome editing across crop species and genotypes. Mol Plant. 2023;16(3):616-31.
14. Lou H, Li S, Shi Z, Zou Y, Zhang Y, Huang X, et al. Engineering source-sink relations by prime editing confers heat-stress resilience in tomato and rice. Cell. 2025;188(2):530-49.e20.
15. Liu L, Gallagher J, Arevalo ED, Chen R, Skopelitis T, Wu Q, et al. Enhancing grain-yield-related traits by CRISPR-Cas9 promoter editing of maize CLE genes. Nat Plants. 2021;7(3):287-94.
16. Zhang H, Zhu JK. Epigenetic gene regulation in plants and its potential applications in crop improvement. Nat Rev Mol Cell Biol. 2025;26(1):51-67.
17. Zhang D, Gan Y, Le L, Pu L. Epigenetic variation in maize agronomical traits for breeding and trait improvement. J Genet Genomics. 2025;52(3):307-18.
18. Li LL, Xiao Y, Wang B, Zhuang Y, Chen Y, Lu J, et al. A frameshift mutation in JAZ10 resolves the growth versus defense dilemma in rice. Proc Natl Acad Sci U S A. 2024;121(52):e2413564121.
19. Kang KK, Cho YG. Genetic Analysis Based on CRISPR/Cas9 Technology in Plants. Int J Mol Sci. 2023;24(22).
20. Okita TW, Delseny M. Genome editing in plants: New advances and applications in plant biology and agriculture. Plant Sci. 2023;328:111577.
21. Lv P, Su F, Chen F, Yan C, Xia D, Sun H, et al. Genome editing in rice using CRISPR/Cas12i3. Plant Biotechnol J. 2024;22(2):379-85.
22. Bhat MA, Mir RA, Kumar V, Shah AA, Zargar SM, Rahman S, et al. Mechanistic insights of CRISPR/Cas-mediated genome editing towards enhancing abiotic stress tolerance in plants. Physiol Plant. 2021;172(2):1255-68.
23. Jyotika RK, Harish S, Karthikeyan G, Kumar KK, Murugan M, Jayakanthan M, et al. Molecular approaches for the management of papaya ringspot virus infecting papaya: a comprehensive review. Mol Biol Rep. 2024;51(1):981.
24. Yan Y, Zhu X, Yu Y, Li C, Zhang Z, Wang F. Nanotechnology Strategies for Plant Genetic Engineering. Adv Mater. 2022;34(7):e2106945.
25. Nidhi S, Anand U, Oleksak P, Tripathi P, Lal JA, Thomas G, et al. Novel CRISPR-Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives. Int J Mol Sci. 2021;22(7).
26. Nuñez-Muñoz L, Vargas-Hernández B, Hinojosa-Moya J, Ruiz-Medrano R, Xoconostle-Cázares B. Plant drought tolerance provided through genome editing of the trehalase gene. Plant Signal Behav. 2021;16(4):1877005.
27. Li J, Pan W, Zhang S, Ma G, Li A, Zhang H, et al. A rapid and highly efficient sorghum transformation strategy using GRF4-GIF1/ternary vector system. Plant J. 2024;117(5):1604-13.
28. Yu H, Lin T, Meng X, Du H, Zhang J, Liu G, et al. A route to de novo domestication of wild allotetraploid rice. Cell. 2021;184(5):1156-70.e14.