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REVIEW ARTICLE

Advances in Modern Biotechnological Tools for Sustainable Control of Plant Viral Diseases

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Abstract: Plant viruses pose a major threat to global food production, causing yield losses across many staple and horticultural crops. Conventional breeding for resistance is typically slow and constrained by the number of available natural resistance genes. Recent breakthroughs in biotechnology have enabled the development of accurate, flexible, and environmentally friendly technologies to confer virus resistance. This review summarizes exciting developments in RNA interference (RNAi), artificial microRNAs (amiRNAs), CRISPR/Cas genome editing, nanobiotechnology-based delivery systems, host-derived artificial microRNAs, molecular mechanisms, and coat protein-mediated resistance or antiviral technology. Both RNAi and amiRNA engineered technologies utilize the plant's own silencing machinery to degrade viral transcripts. In contrast, CRISPR/Cas systems can facilitate targeted editing of host susceptibility genes or specific cleavage of viral genomes, leading to stable, heritable resistance. Nanobiotechnology offers a strong non-transgenic delivery platform to increase the stability and uptake of dsRNA and CRISPR components in plants. Also, virus-induced gene silencing (VIGS) enables rapid functional validation of antiviral genes, and coat protein–mediated resistance (CPMR) represents a foundation for pathogen-derived protection. The combination of these molecular tools with synthetic biology and metabolic engineering facilitates the design of programmable, multi-layered defense networks. Collectively, these new biotechnological tools represent a significant departure from conventional breeding toward precision-based crop improvement. With the compound virtues of durability, broad-spectrum nature, and climate-resilient solutions for controlling plant viral diseases, it is possible to achieve sustainable agricultural productivity.

Keywords: Plant Virus Resistance, RNA Interference, CRISPR/Cas, Nanobiotechnology, Virus-Induced Gene Silencing, Transgenic Resistance

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Introduction

Among the biggest problems in agriculture worldwide are plant viruses, which threaten food security and lead to continuous yield reductions across many cropping systems [1]. Viral diseases such as papaya ringspot, rice tungro, tomato yellow leaf curl, and cassava mosaic disease account for an estimated 10–15% of annual harvest losses worldwide. These infections significantly reduce productivity in both horticultural and staple crops worldwide. [2] Unlike bacterial and fungal

pathogens, viruses are intrinsically dependent on host factors for translation, replication, and systemic transmission because they do not have their own metabolic machinery [3, 4]. This mandatory parasitism makes chemical control ineffective, which makes genetic resistance and biotechnology alternatives even more crucial.

Biotechnology is the regulated and deliberate manipulation of plants' biological processes to accomplish efficient viral disease control. From resistance breeding to transgenic introgression of novel genes, biotechnological interventions in viral disease management to protect crop yield have been enormous. The different biotechnological approaches include pathogen-derived resistance, RNA interference (RNAi), artificial microRNAs (amiRNAs), Virus-Induced Gene Silencing (VIGS), CRISPR/Cas-based genome editing, and nanotechnology-assisted delivery systems, have been developed. The mechanisms of pathogen-derived resistance depend on the expression of viral sequences that interfere with infection. In contrast, RNAi and amiRNAs exert their effects by degrading viral RNA in a sequence-specific manner. VIGS enables rapid functional analysis of host defense genes, and CRISPR/Cas systems enable direct targeting of both viral genomes and host susceptibility factors. More advanced delivery platforms have been developed using nanotechnology to enhance the stability and uptake of dsRNA molecules or genome-editing reagents, such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/CAS (CRISPR-Cas-associated protein), with a view toward improving their practical use under field conditions. Many of these methods are transgene-free, making them more acceptable for sustainable crop improvement [5]. RNA silencing-based techniques, such as the use of synthetic double-stranded RNAs or transgene-derived hairpin RNAs, exploit conserved antiviral pathways to achieve sequence-specific protection. [6-8]. More recently, CRISPR/Cas-based systems have revolutionized plant virology by allowing direct cleavage of viral genomes or disruption of host susceptibility genes [9-12]. By enabling non-integrative delivery of dsRNA and CRISPR ribonucleoproteins, nanobiotechnology now supports these tactics and closes the gap between lab innovation and field implementation [13, 14]. Nanocarriers enhance stability, uptake, and field adaptability, bridging the gap between molecular design and agricultural application [15]. The integration of RNAi, CRISPR-Cas, and nanoplatforms is empowered by synthetic biology and AI-directed engineering that represents a paradigm shift from conventional resistance breeding to precise crop protection [16, 17]. These strategies reflect a paradigm shift from classic resistance breeding to the precision engineering of resistance. By implementing RNA interference (RNAi), CRISPR/Cas, synthetic biology, and nanotechnology to develop viral transformation systems, plant scientists are developing durable and sustainable strategies for combating viral pandemics [18].

This review summarizes the current status of biotechnological tools for plant virus resistance, critically appraises their molecular basis, and discusses their potential to help secure global agricultural resilience amid population growth and climate variability.

Structural Molecular Basis of Plant–Virus Interaction and Its Role in Resistance Innovation

Plant viruses have diverse genome structures, including multipartite segmented genomes, as well as positive-sense single-stranded RNA (+ssRNA), negative-sense single-stranded RNA (-ssRNA), double-stranded RNA (dsRNA), and circular single-stranded DNA (ssDNA) [19]. Potyviridae and other +ssRNA viruses use their genomes as messenger RNAs, which are directly recruited by host ribosomes for translation [4]. To protect intermediates from RNA silencing, dsRNA viruses, including members of the Reoviridae, replicate within viral particles. Plant viruses typically enter host cells through wounds or by insect vectors that bypass the cell wall barrier. Virions decoat after entry and release their nucleic acids into the nucleus or cytoplasm, depending on their taxonomic classification. Membrane-associated viral replication complexes physically sequester viral RNA from host nucleases and spatially organize RNA synthesis during virus replication [20]. of these, viral RNA or nucleoprotein complexes could move between cells through plasmodesmata by specialized movement proteins, which the viruses use for cell-to-cell passage [2]. Phloem loading creates a systemic infection, which allows for long-distance spread to root and aerial tissues [3]. According to [4], viruses are reliant on host Susceptibility (S) factors, native cellular proteins that are usurped for viral dissemination. The eukaryotic translation initiation factors eIF4E and eIF4G are among the best-described susceptibility factors that potyviruses encode through interactions with their Viral Genome-Linked Protein (VPg) for viral RNA translation and replication [21]. Changes in these host factors have been shown to abolish VPg binding and confer recessive resistance against many economically important viruses without drastically impacting plant growth [22]. Other host proteins that are involved in membrane remodeling, intracellular trafficking, and plasmodesmata regulation also indirectly facilitate viral movement and systemic infection. Recent structural work suggests that dynamic interactions of viral replication proteins, movement proteins, and coat proteins with host membranes and cytoskeletal components are employed at sites of infection establishment [23] as well as for supporting long-distance transport in the plant. The Table 1 summarizes different biotechnological strategies for plant virus resistance, including modes of action, crops, and viruses targeted. These

approaches primarily comprise RNA interference (RNAi), artificial microRNAs (amiRNAs), CRISPR/Cas genome editing targeting viruses and hosts, Virus-Induced Gene Silencing (VIGS), and Coat Protein-Mediated Resistance (CPMR). These approaches provide precise and stable resistance by targeted degradation of viral RNAs, modification of host susceptibility factors, or direct editing of viral genomes. Another innovative application of nanobiotechnology is as an efficient delivery system that improves the field presentation and stability of these virus control tools. Insight into the structural and molecular basis of plant–virus interactions is a prerequisite to rationally designing antiviral biotechnological tools. Knowledge of viral replication proteins, movement proteins, coat proteins, and host susceptibility factors has enabled the development of RNAi constructs, artificial microRNAs, CRISPR/Cas targets, and decoy-based resistance systems. Therefore, these interactions provide a mechanistic basis for the resistance tools discussed in subsequent sections of this review.

Table 1: Biotechnological Approaches for Plant Virus Resistance

Biotechnological Approach	Mode of Action	Crops Affected	Target Viruses	References
RNA Interference (RNAi)	Expression of hairpin dsRNA triggers sequence-specific degradation of viral RNAs	Papaya, Tomato, Tobacco	Papaya ringspot virus (PRSV), Tomato yellow leaf curl virus (TYLCV), Tobacco mosaic virus (TMV)	[7]
Artificial MicroRNAs (amiRNAs)	Engineered microRNAs silence viral RNA with high specificity	Tomato, Tobacco, Cucumber	Tomato yellow leaf curl virus (TYLCV), Cucumber mosaic virus (CMV)	[24]
CRISPR/Cas Genome Editing (Virus-Targeted)	Direct cleavage of viral DNA or RNA by Cas9/Cas12 nucleases	Cucumber, Arabidopsis	Cucumber vein yellowing virus (CVYV), Geminiviruses	[25]
CRISPR/Cas Genome Editing (Host-Targeted)	Editing of host susceptibility genes to block virus replication	Cassava, Tomato	Cassava brown streak virus (CBSV), Pepper mottle virus (PepMoV)	[11]
Virus-Induced Gene Silencing (VIGS)	Viral vectors express silencing triggers targeting host susceptibility genes.	*Nicotiana benthamiana*, Tomato	Tobacco mosaic virus (TMV), Pepper veinal mottle virus (PeMoV)	[26]
Coat Protein-Mediated Resistance (CPMR)	Transgenic expression of viral coat protein to inhibit uncoating	Papaya, Tomato, Cucumber	Papaya ringspot virus (PRSV), Cucumber mosaic virus (CMV)	[27]
Nanobiotechnology for dsRNA Delivery	Nanoparticle-mediated delivery of dsRNA or CRISPR/Cas9 components	Cowpea, Tobacco, Tomato	Cucumber mosaic virus (CMV), Papaya ringspot virus (PRSV)	[7, 13]

Conceptual Framework for Biotechnological Resistance Engineering

Biotechnological resistance techniques can be classified into virus-targeted and host-targeted strategies. The engineering of resistance to plant biotechnologies is guided by a hierarchical plan, starting with determining the most amenable stage in the viral life-cycle for intervention. Resistance can develop directly by targeting viral genomes and proteins or indirectly by targeting host factors that play critical roles in infection, replication, movement, or systemic spread [28]. These strategies work through various molecular mechanisms, including disruption of susceptibility genes, transcriptional repression, post-transcriptional RNA degradation, and precision genome editing [29]. All three share common characteristics and explain the integrated defense circuitry that drives durable, sequence-specific resistance against a wide range of plant viruses. In comparison, host-targeted strategies limit the viral life cycle without directly modulating viral genomes through modification of plant susceptibility factors that are necessary for viral infection (e.g., translation initiation factors or replication cofactors like TOM1 [4]. Virus-targeted strategies are thus designed to inhibit viral proteins or nucleic acids directly [30]. This targets viral transcripts, such as RNA interference (RNAi) or CRISPR-Cas systems, for targeting viral genomes [31]. Host-targeted strategies aim to interfere with plant determinants that viruses require for their life cycles. Such factors often comprise translation initiation proteins, membrane-associated replication cofactors, and intracellular transport components. Nevertheless, extensive alterations of crucial host genes can at times affect the normal growth and development of plants [32]. By contrast, virus-targeted approaches disrupt viral genomes, transcripts, and/or proteins involved in replication and movement. Moreover, CRISPR/Cas systems, artificial microRNAs, and RNA interference can all be directed at highly conserved viral sequences, allowing rapid and specific suppression of the infection. While these approaches often provide strong repression of viral replication, they may not work if viruses acquire escape mutations in exactly those regions to be targeted [31]. This conceptual disconnect can involve manipulation of intrinsic host susceptibility or extrinsic viral elements. Thus, genetic disruption exploits loss-of-function alleles to delete host factors required for viral replication [33]. For example,

the recessive crop resistance conferred by mutations in the eIF4E gene is well characterized [32]. Transcriptional gene Silencing (TS) involves DNA methylation and chromatin reorganization to silence viral promoters. Virus-derived small interfering RNAs (vsiRNAs) specifically degrade viral mRNA, induce Post-Transcriptional Gene Silencing (PTGS) via the RNAi mechanism, and provide broad-spectrum resistance against viruses [34]. This design serves as the basis for biotechnological resistance engineering, which is commonly used to induce specific defenses through high-tech approaches such as synthetic siRNAs and artificial miRNAs. The conceptual disconnect is whether the technique manipulated host susceptibility or viral factors. Genetic disruption uses loss-of-function alleles to knock out host factors necessary for viral replication [33]. Crop recessive resistance mediated by mutations in the eIF4E gene is a well-characterized example [32]. TS (transcriptional gene silencing) is based on DNA methylation and chromatin remodeling. To silence viral inducers. In this respect, virus-derived small interfering RNAs (vsiRNAs) have been implicated in PTGS via an RNAi mechanism, in which vsiRNAs promote the specific degradation of viral mRNA and confer broad-spectrum resistance against viruses [34].

This design is behind biotechnological resistance engineering, which aims to provide specialized defenders, and advanced methods, including synthetic siRNAs and artificial miRNAs, are frequently used. A prominent approach for generating resistance alleles has been classical mutagenesis; this method is random, time-consuming, and prone to unintentional pleiotropic effects. Directed, precise genome editing using CRISPR-Cas systems enables targeted modification of viral genomes with high specificity or targeted modification of susceptibility genes [35]. In contrast to random mutagenesis, CRISPR/Cas is multiplexed, allowing simultaneous targeting of multiple loci to extend the durability of resistance, accelerate trait deployment, and improve predictability of outcomes. This advantage has made genome editing a more powerful method than traditional resistance breeding for generating virus-resistant plants.

CRISPR/Cas-Based Approaches for Plant Virus Resistance

Plant hemibiotrophs can trigger immune responses that are great on a whole plant level but ineffective in terms of protecting specific leaf tissue, allowing pathogens to infect the plant. Genomic editing that targets host Susceptibility (S) genes is an excellent way to induce durable recessive resistance in genetically edited plants. Manipulation of intrinsic components of plants required for viral infection [36], leading to ease of biosafety concerns via CRISPR/Cas-mediated editing, unlike classical transgenic resistance, which often relies on the expression of foreign genes [37]. Two of the most well-studied S-genes are the eukaryotic translation initiation factors (eIFs) 4E and (iso) 4E needed for potyvirus replication [38]. Interactions of these components with the viral genome-linked protein (VPg) facilitate translation of viral RNA. By altering or disrupting eIF4E, which confers resistance to a wide range of viruses [21, 22], this interaction can be avoided. For example, tomato plants producing the Pepper mottle virus (PepMoV) with CRISPR/Cas9-mediated knockout of eIF4E1 were resistant to infection and exhibited no plate phenotype growth or development changes [39]. Similarly, eIF4E2-deficient cherry tomatoes exhibited robust resistance to the Pepper veinal mottle virus [40]. Rather than the total loss of function, approaches mixing more sophisticated methods, such as base editing or allele replacement, have been applied to create subtle modulation of eIF4E that obviates viral compatibility but retains normal translation [41]. This approach reduces the fitness prices that full gene knockouts typically involve. In another example, in a case of eIF4E allele replacement in cucumber, substitutions of specific amino acids continued to allow for efficient host translation while preventing VPg binding without loss of transformations [21].

CRISPR/Cas CRISPR/Cas systems may additionally directly target viral nucleic acids. Gemini viruses, for example, are a type of DNA virus targeted by Cas9 and Cas12 nucleases, which create double-stranded breaks in the viral genome that prevent replication [25]. For RNA viruses, Cas13 nucleases can mediate cleavage of viral RNA genomes in a programmable manner that avoids any modification to the host cell DNA template altogether and allows for a reversible, transient mode of resistance [42, 43]. These two frameworks augment the utility of CRISPR technologies against DNA and RNA viruses, respectively. Besides viral sequence diversity, multiplex editing strategies that utilize an array of guide RNAs (gRNAs) to cleave diverse viral genes simultaneously have also been proposed, suggesting that mixed infections might be a target as well [44]. For example, multiplexing increased resistance and reduced viral escape in cassava during infection with African cassava mosaic virus [12]. For rice, multiplexing of gRNAs conferred durable resistance against Rice Tungro Bacilliform Virus (RTVB) and Rice Tungro Spherical Virus (RTSV) in plants [45]. After entering plant cells, viral genomes are transcribed and translated with the help of host factors. For example, Cas10 nucleases target RNA viruses such as Picornaviridae and Astroviridae, which have a structured domain that binds to the viral genome, much like a receptor. In contrast, Cas9 and Cas12 nucleases target DNA viruses (Geminiviridae) by introducing double-stranded breaks that leave them unable to replicate or transcribe. Cas13 acts on RNA viruses, degrading viral RNA genomes and conferring reversible, non-transgenic

resistance [46]. Guide RNAs (gRNAs) direct Cas nucleases to specific viral sequences, thereby blocking replication and systemic spread within plant tissues (Figure 1). In addition to classical DNA-targeting CRISPR/Cas9 systems, RNA-targeting CRISPR effectors such as Cas13a and Cas13d have emerged as powerful tools for engineered resistance against plant RNA viruses. These type VI CRISPR effectors utilize a single CRISPR RNA (crRNA) to recognize and cleave viral single-stranded RNA (ssRNA) genomes, reducing viral replication without altering host DNA. Cas13d, in particular, has been demonstrated to confer broad-spectrum resistance against multiple RNA viruses when coupled with multiplexed guide arrays in transgenic plants, underscoring its utility for durable control of diverse RNA viruses. This RNA-targeting mechanism is mechanistically distinct from DNA editing and expands the toolkit for programmable antiviral defenses [47]. In tomato, gRNAs targeting various regions of Tomato Yellow Leaf Curl Virus (TYLCV) stacked and improved the strength of resistance under field conditions [48].

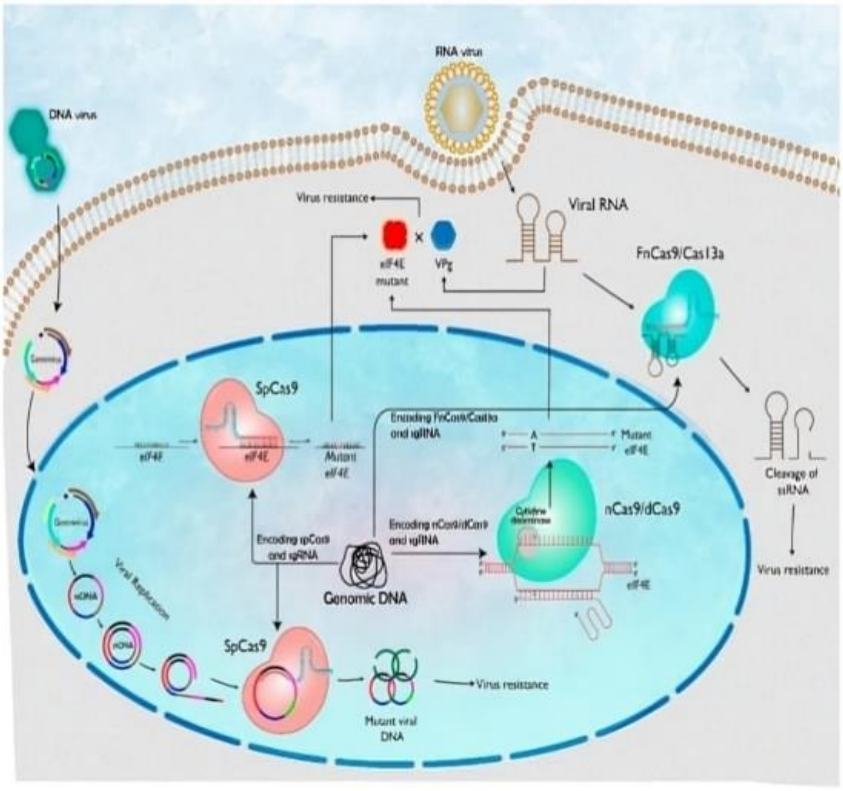

Fig. 1: Representation of CRISPR/Cas-mediated antiviral defense showing how targeted genome editing disrupts DNA and RNA virus replication, transcription, and systemic spread within plant cells [46]

RNA Interference (RNAi) Strategies

RNA interference (RNAi) is a well-conserved defense against viral infections in plants. Viral double-stranded RNA (dsRNA) is sensed and processed by Dicer-Like (DCL) enzymes into 21– 24 nucleotide long small interfering RNAs (siRNAs) [49]. These siRNAs are incorporated into the RNA-Induced Silencing Complex (RISC), in which Argonaute (AGO) proteins direct the cleavage and degradation of homologous viral RNAs [6]. Endogenous RNAi, induced during infection, together with transgene-based RNAi, whereby artificial constructs generate self-specific dsRNAs, play a role in antiviral immunity [50]. Transgenic expression of dsRNA showed a lasting resistance to viruses in a variety of crops [7]. The best studied and most advanced is that for PRSV, which constitutes a benchmark for the commercial use of RNAi-mediated resistance [51]. In common bean (Phaseolus vulgaris), strong transgenic RNAi resistance was observed under field conditions when Bean Golden Mosaic Virus (BGMV) was targeted in transgenic RNAi lines [52]. These achievements serve as models of the potential of stable dsRNA expression-mediated durable field resistance, despite the existing regulatory and public acceptance hurdles. Non-transgenic approaches to the delivery of synthetic double-stranded RNA (dsRNA) have been investigated more recently as an alternative to genetic transformation [53]. Application strategies such as foliar spraying, root drench, and nanoparticles-mediated delivery systems have been proposed to deliver virus-specific dsRNAs into plants [50, 54]. However,

persistence is becoming a challenge as naked dsRNA is quickly degraded in the environment and systemic spread is frequently limited relative to endogenous RNAi [7]. Encapsulation into clay nanosheets or in liposomes has enhanced the stability and uptake with potential for protection in the field [7]. To overcome RNAi-based immunity, viruses produce viral suppressors of RNA silencing (VSRs). For instance, potyviral HC-Pro sequesters siRNAs, tombusviral P19 binds siRNA duplexes, and begomoviral AC2/AL2 proteins antagonize transcriptional silencing [34, 55]. These VSRs dramatically decrease the durability of RNAi-based resistance and strategies, such as the use of stacked RNAi constructs targeting multiple viral genes, or in combination with CRISPR/Cas technologies, are being developed to manage these complications [50].

Transgenic Approaches for Plant Virus Resistance: Classical Foundations and Modern Innovations

The first success in this regard was reported in the 1980s with TMV-transgenic tobacco, where Coat Protein-Mediated Resistance (CPMR) resulted in delayed symptom appearance [56, 57] and established a proof of principle for pathogen-derived resistance. This mechanism was originally supposed to inhibit uncoating and is now recognized to mainly work through RNAi [58]. Applications then spread rapidly to other crops. The most successful example of CPMR, resistant papaya against PRSV, has helped revive the Hawaiian papaya industry [51]. Analogous strategies in cucumbers for resistance to CMV, in Nicotiana against tomato ringspot virus, and in oilseed rape for resistance to TuMV have been reported [27]. Nowadays, CPMR is frequently combined with RNA interference (RNAi) or multiple-gene methods, looking for an increase in durability and fewer resistance failures [59, 60]. Although it has been largely overtaken by RNAi and CRISPR-based techniques, CPMR remains an important historical landmark that influences the design of stacked-gene constructs in transgenic resistance programs. One of the first generational approaches to control viral replication was through the antisense RNA technology [61]. However, resistance was only partial and unstable because of the short half-life of antisense transcripts [50]. The construction of hairpin RNA (hpRNA) follows; they are the double-stranded RNAs and have great stability and can continuously produce siRNAs, which generate strong post-transcriptional gene silencing [8]. Progress was recently made with hpRNA constructs targeting Viral Suppressors of RNA silencing (VSRs), which are highly efficacious and have now succeeded in breaking through one of the final barriers towards effective RNAi-mediated resistance [62]. Resistance to PRSV was more robust and durable in hpRNA-induced gene silencing of PRSV genes than in conventional antisense constructs in papaya. In addition, hpRNA-mediated resistance has been effectively transferred in legumes, cereals, and solanaceae crops, with field resistance durability observed in some instances [8, 50]. Durability of virus resistance can also be improved by multigene engineering, targeting several viral genes or host susceptibility factors at once. This method lowers the possibility of resistance breakdown from viral mutation or recombination [63]. For instance, stacking of RNAi constructs targeting diverse viral open reading frames in cassava gave rise to resistance against both cassava brown streak virus and cassava mosaic virus [64].

Promoter manipulation can also be used to improve resistance by regulating tissue-specific or inducer-driven expression of antiviral transgenes. These two potential disadvantages associated with strong constitutive promoters that do not occur with phloem-specific promoters are (a) metabolic costs, versus (b) antiviral expression occurring directly at viral replication sites with no or limited nonspecific effects [59]. Synthetic promoter construction with the use of cis-regulatory motifs has also been used to improve the spatiotemporal modulation of resistance genes within crops such as rice and tomato [65]. Taken together, multi-gene stacking and promoter engineering are new advanced transgenic methods to highly enhance the durability and biosafety of virus resistance in plants [66].

Nanobiotechnology for dsRNA Delivery in Plant Virus Resistance

Molecular delivery has taken a new turn with the use of nanotechnology in plant biotechnology. It makes it possible for CRISPR-Cas ribonucleoproteins (RNPs) and double-stranded RNA (dsRNA) to be transported precisely and non-transgenically [67]. Barriers to traditional transformation techniques include transgene integration, tissue culture dependence, and biosafety concerns. Alternatively, the plant genomes and defense mechanisms can be transiently modified without DNA using NPMD (Nanoparticle-Mediated Delivery) [13, 68].

Nanoparticles (NPs) serve as protective carriers for nucleic acids, protecting them from enzymatic degradation [69]. It promotes cellular absorption via topical treatment or foliar sprays. LDH nanosheets have also been used to successfully deliver dsRNA against Tobacco Mosaic Virus (TMV) and Cucumber Mosaic Virus (CMV). This method conferred antiviral protection for up to 30 days post-treatment [7]. Collated, biodegradable nanocarriers such as chitosan and Poly (Lactic-Co-Glycolic Acid) (PLGA) nanoparticles have been proven to locally induce systemic mobility and persistence of dsRNAs in inhabiting insects, resulting in increased field-level efficacy [68].

In addition, nanomaterials facilitate non-integrative endosomal delivery of CRISPR/Cas. DNA nanostructures and Single-Walled Carbon Nanotubes (SWCNTs) deliver the CRISPR-Cas9 Complex directly into healthy cells [69]. This preserves genomic integrity, whilst allowing transient genome editing [13]. Biodegradable polymer matrices and, more recently, Virus-Like Particles (VLPs) promote nuclease stability and intracellular release. These systems connect lab advancements with juiced-up, field-ready applications [14, 70]. However, several limitations still hinder the widespread application of nanobiotechnology for managing plant viruses. Synthesizing nanoparticles with uniform size, high loading efficiency, and long-term stability is expensive, especially for large-area agricultural systems [71]. In addition, the environmental persistence of some specifically produced nanomaterials remains unknown, as in some cases particles can accumulate [72] in soil, water, and non-target species after repeated applications. In addition, factors such as rainfall, UV irradiation, and temperature changes encountered in field settings may impact nanoparticle-mediated delivery systems [13], resulting in decreased efficacy or retention on plant surfaces. In many countries, nanoparticle-based crop protection products remain under-regulated [73], particularly those that deliver RNA molecules or genome-editing reagents. Thus, additional efforts are needed to scale up implementation, minimize production costs, and establish environmental safety standards prior to broad field adoption.

Virus-Induced Gene Silencing (VIGS)

Virus-Induced Gene Silencing (VIGS) takes advantage of the plant's endogenous RNA silencing pathway by introducing a portion of the host gene into an engineered viral genome, which leads to the targeted post-transcriptional degradation of endogenous transcripts in systemic tissue upon systemic infection [74]. This makes it possible to avoid the stable transformation and the regeneration, and to make a host gene express knockdown in the whole plant temporarily and rapidly [75]. The viral vector spreads in a cell-to-cell and vasculature manner, thus gene silencing by RNA can be observed in multiple tissues after a single challenge [76].

Primarily, VIGS is used as a reverse-genetics technique to study host genes involved in different aspects of viral susceptibility (defense and developmental regulation) [74]. Its application to a wide variety of plants (including solanaceous crops, cereals, and perennials), especially those exhibiting low (transformation-incompetence) or no (transformation recalcitrance) transformation efficiency, makes it a highly valuable tool [76]. While not immediately applicable to commercial deployment of resistance, VIGS does demonstrate the feasibility of targeted gene silencing and aids in the identification of S genes as candidates for breeding durable resistance [77].

Dicer-like proteins DCL2 and DCL4 are central players of antiviral RNA silencing, producing 21nt and 22nt small interfering RNAs (siRNAs), which are required to mediate viral RNA degradation [78]. Silencing of NbDCL2 and NbDCL4 in N. benthamiana by VIGS resulted in an enhanced susceptibility to various RNA viruses such as CMV and TRV, as seen before, indicating that they are indeed critical effectors for antiviral defense [79]. RNA polymerase V synthesizes scaffold RNAs that recruit AGO-bound small RNAs to guide de novo DNA methylation through the RNA-directed DNA methylation (RdDM) pathway [80]. This mechanism establishes stable and heritable transcriptional silencing, whereas mutations in PolV disrupt scaffold RNA synthesis, resulting in transient or non-heritable silencing (Figure 2). In addition, functional studies with VIGS and GFP reporter lines demonstrated that DCL4 is mainly involved in blocking the spread of systemic silencing, while DCL2 is required for long-distance signal amplification, indicating that they act complementarily in an RNA silencing network [7].

Host-Derived Artificial MicroRNAs (amiRNAs)

In addition, nanomaterials facilitate non-integrative endosomal delivery of CRISPR/Cas. DNA nanostructures and Single-Walled Carbon Nanotubes (SWCNTs) deliver the CRISPR-Cas9 Complex directly into healthy cells [69]. This preserves genomic integrity, whilst allowing transient genome editing [13]. Biodegradable polymer matrices and, more recently, Virus-Like Particles (VLPs) promote nuclease stability and intracellular release. These systems connect lab advancements with juiced-up, field-ready applications [14, 70]. However, several limitations still hinder the widespread application of nanobiotechnology for managing plant viruses. Synthesizing nanoparticles with uniform size, high loading efficiency, and long-term stability is expensive, especially for large-area agricultural systems [72]. In addition, the environmental persistence of some specifically produced nanomaterials remains unknown, as in some cases particles can accumulate [72]. Artificial microRNAs (amiRNAs) are an advanced technology for RNA-mediated gene silencing and offer specific, sequence-dependent inhibition of virus genes [81]. These are designed by inheriting a synthetic duplex targeting the viral target sequence into the native duplex region within a pre-miRNA. This modification preserves the precursor's natural secondary structure, thereby enabling proper recognition and processing by the host's silencing machinery. [24]. This design allows the modified predecessor to be recognized by the host's Dicer-like and Argonaute machinery for cleavage of complementary viral

RNA. AmiRNAs are only about 21 nucleotides in length and are processed by the same endogenous pathways as natural miRNAs, minimizing off-target interactions with non-target genes. Hence, they achieve greater silencing specificity than canonical long hairpin-derived RNAi constructs. [24, 82]. Recent investigations have demonstrated the efficacy of amiRNAs for providing long-lasting resistance against a wide range of plant viruses. For example, in Nicotiana benthamiana and tomato, very strong resistance was achieved with amiRNAs targeting the cucumovirus coat protein and replicase genes [83]. In a similar vein, multiplexed constructs that express multiple amiRNAs from a single transcript have afforded broad-spectrum protection against mixed viral infections [84]. Tools for computing the secondary structure of amiRNAs, such as WMD3 and P-SAMS, have further enhanced design, enabling rapid prediction of both stability and target accessibility [85]. A transgene expressing a monocistronic MIRNA precursor is processed into amiRNAs that target a single site on a viral RNA via an antiviral amiRNA pathway. In the antiviral syn-tasiRNA pathway, a transgene expressing a polycistronic TAS precursor is processed into multiple syn-tasiRNAs targeting multiple sites in one or more viral RNAs [81]. Both amiRNA and syn-tasiRNA guide strands bind AGO1 to silence viral RNAs via endonucleolytic cleavage or translational inhibition (Figure 3). In summary, the amiRNA-based technique synergizes precise targeting, stable expression, and biosafety, offering a powerful new alternative to transgene-based RNAi.

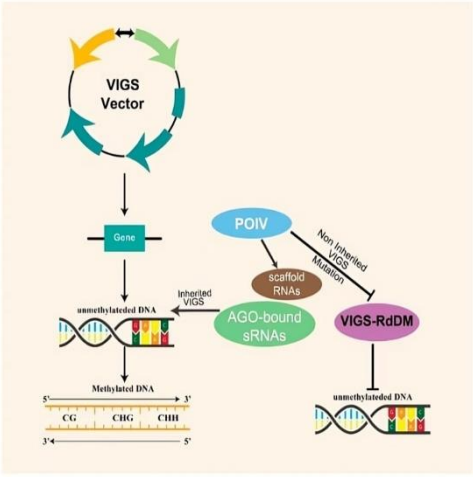

Fig. 2: Establishment of epigenetic silencing during VIGS vector insertion, where PolV-synthesized scaffold RNAs recruit AGO-bound small RNAs to direct heritable DNA methylation via the RdDM pathway, while PolV mutations abolish this process, leading to non-heritable silencing [81]

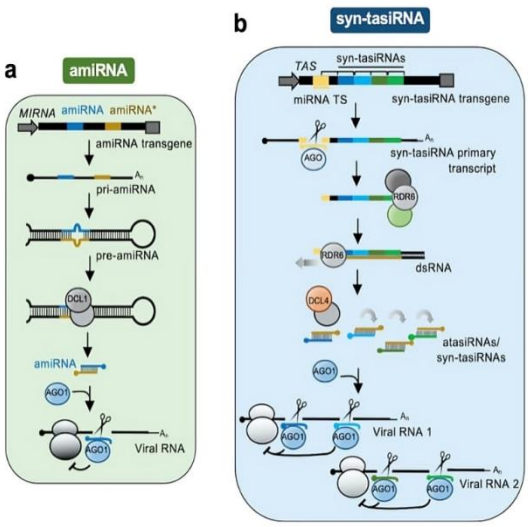

Fig. 3: Antiviral artificial small RNA (art-sRNA) pathways in plants showing amiRNA- and syn-tasiRNA-mediated mechanisms, where amiRNAs target single viral RNAs, and syn-tasiRNAs target multiple viral RNAs through AGO1-guided cleavage or translational inhibition [82]

Molecular Mechanisms Underpinning Engineered Resistance

Disruption of host Susceptibility (S) genes can be a useful approach to blocking viral infection. A case in point is the mutation of the translation initiation factors eIF4E or eIF4G, used by viruses to initiate protein synthesis [86]. This decoy strategy is relatively new and involves engineering host proteins that mimic pathogen targets. Decoy-based resistance relies on the engineering of host proteins that mimic authentic viral targets in order to activate immune responses following pathogen attack. One of the best-characterized examples involves the PBS1 protein kinase, which normally functions in effector-triggered immunity. By modifying PBS1 to contain cleavage sites recognized by viral proteases, plants can detect viral activity and initiate defense signalling. In potato, a modified PBS1 carrying the NIa protease cleavage site of Potato Virus Y (PVY) enabled recognition of viral infection and triggered strong immune responses [87]. Similar approaches have been reported in Arabidopsis, where engineered PBS1 decoys recognized NIa protease activity from Turnip mosaic virus (TuMV), leading to resistance through activation of downstream defense pathways [88, 89]. Because decoy systems exploit conserved viral protease activity, they represent a promising platform for durable and broad-spectrum resistance engineering. These engineered proteins attract pathogen effector molecules, triggering immune activation rather than facilitating infection. The PBS1 kinase gene from potato (a homolog of A. thaliana PBS1) was modified to contain a cleavage site for the NIa protease from the Potato Virus Y (PVY) [90]. Cleavage triggers immune signaling that provides strong resistance in transgenic potato lines [88]. A third example is provided by the Arabidopsis PBS1 decoy, which mediates the elicitor-mediated recognition of turnip mosaic virus (TuMV) [89]. NIa protease in the host and adaptation of its subcellular localization strategies resulted in complete resistance, indicating the potential of decoy-based recognition [88].

A promising intervention is the metabolic reprogramming for the production of more endogenous antiviral metabolites. In soybean, CRISPR/Cas9-mediated knockout of target genes of the key flavonoid biosynthesis pathway (e.g., F3H, FNSII) resulted in elevated isoflavone levels that significantly hindered Soybean Mosaic Virus (SMV) accumulation [70]. In rice plants infected with various dwarfing viruses, the heightened phenylpropanoid intermediate levels (e.g., ferulic acid and cinnamic acid) are observed. These acids upregulate lignin biosynthesis and bolster cell wall resilience, which may contribute to antiviral defense. Other work has revealed the more general antiviral function of phenylpropanoids and specialized metabolites in other species–for example, accumulation of flavonoids and phenolic compounds at sites of infection in tobacco and tomato seems to be important for defense [91]. Table 2 highlights molecular mechanisms underpinning engineered resistance in plants, including disruption of host susceptibility genes, decoy-based resistance, and metabolic reprogramming. Disruption of genes like eIF4E prevents viral replication, while decoy proteins like PBS1 trigger immune responses upon viral protease recognition. Metabolic reprogramming, such as enhancing flavonoid production, strengthens antiviral defenses. These strategies collectively offer durable and broad-spectrum resistance against various plant viruses.

Table 2: Molecular Mechanisms Underpinning Engineered Resistance to Plant Viruses

Approach	Mechanism	Example	Key Outcome	References
Disruption of Host Susceptibility Genes (eIF4E, eIF4G)	Mutation of host translation initiation factors to block viral replication	Cucumber, Tomato	Resistance to a broad range of viruses through loss-of-function mutations in host factors	[86]
Decoy-Based Resistance (PBS1)	Engineering host proteins that mimic viral protease targets to activate immunity	Potato, Arabidopsis	PBS1 modification triggers immune responses upon viral protease recognition	[88, 89]
Metabolic Reprogramming	Enhancing production of antiviral metabolites through CRISPR/Cas9 or other gene modifications	Soybean, Rice	Increased isoflavones or phenylpropanoids bolster defense against viral infections.	[31, 70]

Comparative Advantages of Biotechnological Resistance Approaches

Biotechnological strategies to engineer virus resistance have the potential for a degree of specificity that is hard to accomplish by breeding. Genome editing and RNAi-mediated approaches can be catered precisely to sequences of viruses or host susceptibility factors, leading to selectively very specific resistance with little side effects [25, 86]. This specificity is further enhanced by the ability to develop multiplexed constructs targeting all or multiple viral genes/variants, leading to increased resistance against a rapidly mutating virus population [70, 92]. RNA interference is particularly useful for targeting RNA viruses because it enables sequence-specific degradation of viral transcripts and can be rapidly adapted to newly emerging viral strains [93]. However, viral sequence variation and the emergence of escape mutants may reduce silencing efficiency over time [94]. Artificial microRNAs provide greater targeting precision and lower off-target effects than conventional

RNAi, although they are often limited by the number of targetable sequences and may provide narrower-spectrum resistance [62]. Virus-induced gene silencing is mainly valuable as a rapid functional genomics tool for identifying host genes involved in susceptibility or defense responses, but its transient nature and variability among plant species may limit its utility for long-term resistance breeding [95].

CRISPR/Cas systems provide durable and programmable resistance by targeting viral genomes or host susceptibility genes with high specificity. Multiplex editing can further improve resistance durability against mixed infections and rapidly evolving viral populations [8]. Nevertheless, limitations remain regarding efficient delivery, potential off-target mutations, and regulatory concerns associated with edited crops in some countries [96]. Nanotechnology-assisted delivery systems improve the stability, uptake, and field applicability of dsRNA molecules and genome-editing reagents [97]. Despite these advantages, large-scale production costs, nanoparticle persistence, and biosafety concerns still require further evaluation before broad agricultural deployment. Another important advantage compared to classical breeding pipelines is the swiftness of deployment. Conventional introgression of resistance genes needs years of repeated backcrossing, whereas RNAi- and CRISPR-mediated resistance can be developed and tested within a fraction of that time [96]. And this pace is crucial for crops against new viral threats, for which the conventional sources of resistance might be unavailable.

Biotechnological procedures support sustainability by minimizing the use of chemical broad-spectrum controls that often harm the environment and are ineffective against viruses [98]. Virus-based resistance is an alternative option for crop protection. For example, in PRSV-resistant papaya, engineered resistance has been shown to greatly reduce yield loss without chemical inputs [99]. Adoption of these approaches in crop protection systems has great potential to address the environmental costs of plant virus control [96].

Future Perspectives in Antiviral Crop Biotechnology

Synthetic biology provides platforms for engineering encoding programmable resistance circuits that periodically sense surfaces in response to viral infection. In modern architectures, artificial promoters combined with viral sensors enable precise activation of the antiviral RNA silencing pathway. This targeted response occurs only in the presence of the pathogen, minimizing metabolic costs and unintended side effects. [100] these programmable circuits can also include several defense layers, which makes it harder for new viruses to break through. The development of libraries of resistance genes is a forward-thinking approach that allows for the quick deployment of genetic resources against new viral challenges. Developments in high-throughput genome editing (including CRISPR) and modular cloning support generating libraries of RNAi, CRISPR, and defense regulators to become modular constructs to be used as plug-and-play in plant breeding pipelines [25]. Such libraries are especially well-suited for new viral epidemics, which require rapid resistance evolution timescales.

Gene therapy-like approaches are also emerging in plant antiviral biotechnology through the transient delivery of functional nucleic acids without permanent genome modification. Similar to medical gene therapy, these strategies aim to introduce protective molecules directly into plant cells to suppress viral replication or enhance immunity. Externally applied dsRNAs, synthetic siRNAs, messenger RNAs (mRNAs), and CRISPR ribonucleoproteins can provide temporary but highly targeted antiviral protection [7, 98]. Among these, mRNA-based technologies are gaining attention because they enable transient expression of antiviral proteins, Cas nucleases, or guide RNAs without genomic integration. Such approaches may reduce biosafety concerns associated with transgenic crops while allowing rapid deployment against newly emerging viral pathogens [71].

Nanotechnology has been proposed as a potential remedy, for example, with nano carriers, including carbon nanotubes, Layered Double Hydroxide (LDH) Nano sheets, and DNA nanostructures, enabling transgene-free delivery of RNA and CRISPR ribonucleoproteins [13]. These vector systems bypass tissue culture bottlenecks and provide avenues for accurate, point-of-need antiviral isnterventions. Table 3 synthesizes the principal limitations, possible solutions, and prospective directions for current antiviral biotechnology.

Despite substantial progress in antiviral biotechnology, several important knowledge gaps remain. The long-term stability of engineered resistance under field conditions is still uncertain, particularly when resistance depends on a single viral target or host factor. Rapid viral evolution may generate escape variants capable of overcoming RNAi constructs, artificial microRNAs, or CRISPR/Cas target sites, especially in genetically diverse virus populations [31]. Additional research is therefore needed to identify highly conserved viral regions, combine multiple resistance layers, and improve the durability of engineered resistance. Biosafety considerations also require further attention, including the potential off-target effects of genome editing, ecological impacts of nanoparticle accumulation, and the possible influence of engineered resistance on

non-target organisms [72, 96]. Addressing these issues will be essential for the safe and effective deployment of next-generation antiviral technologies in agriculture. Table 3 outlines key strategies for future antiviral crop biotechnology, focusing on synthetic biology, gene therapy-like approaches, nanotechnology, and combining multiple resistance layers. Synthetic biology enables programmable resistance circuits, while gene therapy-like strategies use transient delivery of antiviral molecules for rapid protection. Nanotechnology offers a promising transgene-free delivery system, improving the efficiency of RNA and CRISPR interventions. Challenges such as viral escape variants, biosafety concerns, and delivery efficiency remain, but solutions like multiplexing resistance layers and enhanced nanoparticle delivery offer promising directions for overcoming these hurdles.

Table 3: Challenges and Future Directions in Biotechnological Approaches for Plant Virus Resistance

Strategy	Applications	Challenges & Limitations	Potential Solutions	References
Synthetic Biology	Targeted activation of antiviral RNA silencing	High metabolic cost, off-target effects	Use of artificial promoters and modular circuits to minimize metabolic cost and improve specificity	[100]
Gene Therapy-Like Approaches	Suppression of viral replication, enhancing immunity	Short-term protection, need for repeated applications	Use of mRNA-based technologies for transient expression reduces biosafety concerns	[7]
Nanotechnology for Delivery	RNA and CRISPR delivery to plants for antiviral protection	Delivery efficiency, stability, and uptake in plants	Improve nanoparticle stability, targeted delivery, and field adaptability	[13]
Combination of Resistance Layers	Broad-spectrum, durable resistance	Viral escape variants, rapid evolution of viruses	Development of gene libraries, multiplexing of resistance layers, and targeting highly conserved viral regions	[25]
Biosafety & Ecological Considerations	Field deployment of transgenic and non-transgenic crops	Potential off-target effects, ecological impacts, and nanoparticle accumulation	Rigorous biosafety assessments, environmental monitoring, and improved delivery methods	[72, 96]

Conclusion

Innovative and long-lasting resistance techniques that go beyond the confines of traditional breeding are required due to the growing burden of plant viruses. Targeting host susceptibility genes, virus genomes, and defense pathways with previously unheard-of accuracy is now possible because of biotechnological techniques. Antiviral resistance is still largely caused by RNA interference (RNAi) and associated silencing-based processes, but CRISPR/Cas technologies provide long-lasting and adaptable genome editing that may be used on a variety of crops. Non-transgenic delivery of antiviral constructs is made possible by nanotechnology, which may hasten field deployment and regulatory approval. Among the various biotechnological tools discussed, RNAi and CRISPR/Cas systems have emerged as the most widely applied approaches because of their specificity, adaptability, and potential for durable resistance. RNAi remains particularly effective for targeting RNA viruses, whereas CRISPR/Cas platforms provide opportunities to modify both viral genomes and host susceptibility factors. Nanotechnology further complements these tools by improving delivery efficiency and supporting transgene-free applications under field conditions. Despite these developments, problems with effective distribution, stopping viral escape, and handling biosafety issues still exist. Integrated strategies that incorporate several resistance layers, use synthetic biology for programmable defenses, and leverage pan-genomic insights for broad-spectrum targets will be essential for future advancement. Successful implementation of these technologies will also depend on their integration into crop improvement programs, regulatory frameworks, and national disease management strategies. Transgene-free genome editing and nanoparticle-assisted delivery systems may improve public acceptance and simplify regulatory approval in some regions. In parallel, supportive biosafety guidelines, international harmonization of regulatory policies, and investment in field validation will be essential for translating laboratory advances into practical agricultural applications. In the end, the combination of these approaches provides a revolutionary way to manage viral infections in a way that is robust, sustainable, and ecologically friendly, enhancing global food security in the face of climate change and intensifying agriculture.

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Author's Contributions

Nabela Akter: Conducted literature review, organized and drafted the manuscript, and handled formatting.
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Md. Abdullah Al Sabbir: Contributed to data organization, reference management, and proofreading.
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Ethics

This paper is original and not submitted elsewhere. The corresponding author agrees that all authors have seen and approved the final version of the manuscript, and there is no ethical problem to disclose.

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