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REVIEW ARTICLE

Viruses unleashed: Revolutionary approaches to gene transfer in plants

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Abstract

Gene editing has become the new era for crop improvement. *Agrobacterium*-mediated gene transfer and tissue culture for that plant regeneration have become the bottleneck for the gene transfer. Plant viral vectors have emerged as a significant tool in plant genome editing. Vectors like tobacco rattle virus (TRV) and potato virus X (PVX) are particularly effective due to their broad host range, enabling their use across various plant species for crop improvement, disease resistance and functional genomics. An ideal viral vector should achieve high gene transfer efficiency while maintaining transient expression to minimize lasting genetic alterations for optimal results. Recent advancements in techniques such as virus-induced gene silencing (VIGS) and virus-induced genome editing (VIGE) showcase the potential of these vectors for precise gene modifications is also discussed VIGS leverages the plants' innate antiviral response to silence target genes, enabling rapid functional analysis without permanent changes, while VIGE uses viral vectors to deliver cas9 components for targeted genome editing, minimizing off-target effects. However, challenges such as cargo size limitations and regulatory hurdles persist. The future direction of this field is anticipated to advance genome engineering via viral vectors in more sophisticated ways, including using these vectors for genome editing and cargo capacity optimization. This progress will open up new possibilities for the scientific community in plant genome engineering. Overall, this review provides a comprehensive understanding of the current and future potential of virus-mediated gene transfer in plant biotechnology, from the selection of suitable viral vectors to the stable integration of foreign genes into the plant genome and insights into the challenges and future prospects of virus-mediated gene transfer in plants are also presented.

Keywords

agroinfiltration; viral vectors; virus-induced gene silencing (VIGS); virus-induced genome editing (VIGE)

Introduction

Genome editing is an efficient technique to alter specific DNA sequences by introducing insertions, deletions (indels), or base substitutions. Several tools have been developed for precise genetic modifications, including nucleases such as ZFNs, TALENs, RNA-guided CRISPR-Cas9 and gene targeting via homologous recombination (HR) and non-homologous end joining (NHEJ). The creation of ZFN and TALEN vectors requires significant time and effort. The challenge of CRISPR-Cas9 technology is the selection of appropriate vectors for delivering CRISPR-Cas components into plant genomes. Traditional transient delivery methods, such as

Agrobacterium-mediated transformation and biolistic approaches, are often time-intensive and laborious. The more efficient alternative could be using plant virus-derived vectors due to their complex genome structure and effective machinery, which have already been employed for various purposes, including the production of valuable recombinant proteins (1, 2). Plant viral vectors have emerged as a crucial tool in genome editing, offering a versatile and efficient platform for delivering gene-editing reagents into plant cells.

Viruses are sub-microscopic, transmissible, intercellular obligate parasites composed of nucleic acid (RNA or DNA) surrounded by a protein coat with segmented genomes. Viruses exhibit characteristics of both living and non-living entities. Individual protein subunits, known as capsomers, are arranged in a spiral in elongated viruses and on the sides of polyhedral particles in spherical viruses (3). It may be present as a single continuous strand in one particle, known as monopartite, or it can be divided into two or more pieces, either in the same particle (bipartite) or in different particles (multipartite), with segmented genomes referred to as split genome viruses (4, 5). Notable examples of RNA viruses are *Tobacco rattle virus* (TRV) and *potato virus X* (PVX) for dicots, *Wheat streak mosaic virus* (WSMV) and *Barley stripe mosaic virus* (BSMV) for monocots (6). Furthermore, *Geminivirus* and other single-stranded DNA viruses have become popular vectors in various crops, like wheat, barley and corn to express their proteins successfully. These vectors, derived from plant viruses, offer several advantages, including the ability to infect a wide range of plant species, ease of introduction into plants and high levels of gene expression (7-9).

Viral vectors are virus-derived tools used as delivery vehicles to transport genetic material into cells for various applications such as crop improvement, disease resistance vaccine production and genome editing. Viral vectors have been utilized in plant genome engineering to deliver repair templates and sequence-specific nucleases into plant cells, allowing for precise DNA modifications and developing plants with desired traits (10, 11). Virus-induced gene Silencing (VIGS) is a simple transient transformation method used in reverse genetics to overexpress or suppress gene expression via efficient viral vectors. Virus-Induced Genome Editing (VIGE) is a technique that utilizes viral vectors to deliver CRISPR-Cas9 components into plants for genome editing transiently (12).

This review explores the use of plant viruses as vectors for gene transfer, focusing on key systems such as Tobacco Rattle Virus (TRV), Potato Virus X (PVX) and *Geminiviruses*. These viral vectors are examined for their unique mechanisms, advantages and limitations in delivering genetic material into plant cells highlighting their critical roles in virus-induced gene silencing (VIGS) and virus-induced genome editing (VIGE), two efficient techniques that have revolutionized functional genomics and genome modification in plants. By assessing recent advancements in viral vector technology, it demonstrates how these tools enhance gene function studies, improve crop traits and enable precise genetic modifications. Despite their many benefits, such as efficient delivery and transient expression, these vectors face challenges like limited payload capacity and host specificity.

Viral Vectors

***An ideal viral vector* :** An ideal viral vector should be capable of infecting a wide range of plant species and be applicable across various crops and plants. Techniques like *Agrobacterium*-mediated transformation or direct inoculation should facilitate the efficient delivery of the viral vector into plant cells without causing extensive damage. A compact genome ensures a higher success rate in gene transfer experiments. The vector should function transiently within the plant cell, fulfilling its role in gene transfer without leaving a lasting genetic footprint. The viral vector demonstrates high efficiency in facilitating genome engineering techniques such as gene targeting and Virus-Induced Gene Silencing (VIGS) (11). These methods rely on the vector's ability to deliver genetic material accurately and induce targeted modifications or gene silencing within the host plant's genome, which ensures reliable results and enables researchers to manipulate gene expression and study gene function (13, 14). Some of the important viral vectors that have been used in recent studies and its characteristics are as follows

***Important Viral Vectors* :** Some of the notable important viral vectors are as follows:-

Tobacco rattle virus (TRV): TRV is a member of the *Tobravirus* genus, which belongs to the Virgaviridae family. The virus has a positive-sense, single-stranded RNA genome that consists of two components, known as RNA 1 and RNA 2, where RNA 1with a size of 6.8kb, helps the virus replication and cell movement and RNA 2, with the size of 1.9kb-4.3kb encodes the coat protein and some nonessential proteins that can be removed to insert foreign genetic material. An RNA virus extensively employed as a vector for genome editing in dicot plants offers several advantages. TRV can infect a wide range of plant species, is quickly introduced via *Agrobacterium* and is efficiently delivered into the growing points of plants. TRV has been utilized for VIGS and as a vector for providing ZFNs and CRISPR/Cas9 reagents for plant genetic modification. For example, a recent study used TRV to silence the expression of the protoporphyrin IX magnesium chelatase subunit H (ChlH) gene, which encodes key transcriptional regulators of terpenoid indole alkaloid (TIA) biosynthesis. This study used cotyledon-based VIGS to look into gene overexpression, upregulation and downregulation in plant species that are non-model species (15). An example study that successfully edited the three target genes by delivering gRNA via tobacco rattle virus in RPS5A promoter-driven SpCas9-expressing *Nicotiana attenuata*, this editing method produced monoallelic mutations which were heritable in 2%-6% of the seeds in the T1 generation (16). A gene expression study on *Hibiscus mutabilis* using TRV vector silences the *Cloroplastos alterados 1* (CLA1) gene encoding the protein for chloroplast development, which helps in transcriptomic and functional studies of this crop (17). The present study on virus-induced gene silencing using TRV 1 and TRV 2 vectors, which induces silencing in *Crocus sativus* (Saffron), a complex genetic makeup of this crop, leads to difficulty in conventional breeding. In this study, the mutation efficiency is 33.33%, in which *pds* is used as a reporter gene, which provides functional genomic studies of this crop (18). On improving the editing efficiency of the TRV vector, a recent study states that the virus-inoculated tomato cotyledons were

exposed to heat treatment at a temperature of 37°C, which induces the editing efficiency of TRV-2 vector as 36%-46.5% in the tomato cultivar microtome using *PDS* as a reporter gene (19).

***Potato virus X (PVX)*:** PVX belongs to the genus *Potexvirus* within the family Alphaflexiviridae. PVX is a single-stranded positive-sense RNA genome with a size of 6.3kb, which affects solanaceous crops like potato, tomato, tobacco, etc; it is mechanically transmitted (20) and has a high expression level of proteins. PVX is an effective vector for the concurrent delivery of several sgRNAs within a single viral genome and sgRNAs designed in tandem within the PVX genome can achieve efficient gene editing, even when spacers and processing signals are absent. For example, (12) successfully conducted a methodological study on VIGE, targeting the genes *Phytoene desaturase* (PDS) and *Staygreen* (SGR). They delivered sgRNA using PVX in Cas9-expressing tomato plants, achieving an indel frequency of 80%. An example study on multiplex genome editing using PVX vector in *Nicotiana benthamiana* targeting *pds* gene, producing nearly 80% indel frequencies with the cas9 expression of unspaced sgRNA arrays, which also exhibit a high rate of heritable biallelic mutations (21). There is also an experimental study conducted on using PVX vector-mediated targeted genome editing in *Nicotiana bentamiana* using *PDS* as a reporter gene in which the efficient mutagenesis induced by giving heat stress to the inoculated explants for 4 days that increase 4-8% of mutation efficiency (22). A highly efficient targeted mutagenesis method for *Nicotiana benthamiana* was developed using a potato virus X (PVX) vector. It was also utilized to express a base editor, which combined modified Cas9 with cytidine deaminase, enabling targeted nucleotide substitutions in the regenerated shoots. Over 60% of regenerated shoots showed targeted mutations without antibiotic selection, while 18% contained T-DNA (20).

***Geminiviruses*:** It belongs to the *genus Geminivirus* within the family Geminiviridae, having circular single-stranded DNA with the size of 3.2-5.2kb and can be engineered to carry heterologous coding sequences. The replication of geminiviruses relies primarily on the replication-associated protein (Rep), which is essential for initiating rolling-circle replication. This process allows for high viral genome copy numbers within infected cells. Geminivirus vectors can be engineered using either an entire virus strategy, where most viral features are retained, or a deconstructed approach that removes non-essential components, allowing for the insertion of foreign DNA sequences. In the host cell nucleus, the single-stranded DNA (ssDNA) genome of geminiviruses undergoes replication through a rolling-circle mechanism, utilizing double -stranded DNA (dsDNA) intermediates, similar to the replication process of ssDNA bacteriophages. The International Committee on Virus Taxonomy (ICTV) classifies the *Geminiviridae* family into fourteen genera: *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus*, *Turncurtovirus*, *Citlodavirus*, *Maldovirus*, *Mulcrilevirus*, *Opunvirus* and *Topilevirus*. This classification is based on factors such as vector (insect pest), host range, genome organization and sequence identity.

They are highly suitable for creating viral vectors in genetic engineering due to their ease of manipulation, ability to replicate without integrating into the plant genome and capacity to deliver DNA repair templates. However, their use is constrained by limited carrying capacity and a restricted host range (23,24). Example: Wheat Dwarf India Virus (WDIV), The detection of WDIV in naturally infected wheat, barley and sugarcane in the field-along with its demonstrated ability to systemically infect wheat, oat, barley, corn, soybean and tobacco under laboratory conditions-provides strong evidence that WDIV is the first geminivirus capable of infecting both monocot and dicot plant species by using *PDS* as a reporter gene which results in 80-90% of plants shows symptoms (23).

***Foxtail mosaic virus (FoMV)*:** FoMV belongs to the genus *Potexvirus* within the family Virgaviridae, a large group of flexuous, filamentous plant viruses characterized by a single-stranded, positive-sense RNA genome. When a viral RNA silencing suppressor is present, these vectors achieve high levels of protein expression at the inoculation site, but the recombinant virus cannot spread systemically (25, 26). For example, the FoMV was used to silence *ZmPP2C-A10* (*Zea mays* 2C protein phosphatases) in maize, a negative regulator of ABA signaling. This FoMV-based approach successfully reduced the endogenous expression of *ZmPP2C-A10*, improving maize drought tolerance (26).

***Pea early browning virus (PEBV)*:** PEBV belongs to the genus *Tobravirus* and the family Virgaviridae and is a bipartite, rod-shaped virus. Its genome consists of two single-stranded RNA molecules: RNA1, which is approximately 6.8 kb in length and encodes all the proteins necessary for viral replication and movement and RNA2, which ranges from 1.8 to 4.5 kb in size and encodes the coat protein as well as proteins required for transmission by nematodes. PEBV can infect over 30 dicotyledonous species across ten different plant families, including important crops like peas, faba beans and alfalfa. It can expand its host range under laboratory conditions to include additional species like tomatoes, potatoes and rapeseed (27, 28).

***Tomato spotted wilt virus (TSWV)*:** Tomato spotted wilt virus (TSWV) belongs to the genus *orthotospovirus* and *Tospoviridae* family within the *Bunyavirales* order and holds potential as an engineered delivery platform. Naturally transmitted by polyphagous thrips, TSWV has the broadest host range among plant viruses, infecting around 1,090 species of dicotyledons and monocotyledons, including key legumes, vegetables, ornamental crops and their wild relatives. TSWV is a negative-stranded RNA virus with a genome consisting of three segments: large (L), medium (M) and small (S). The L segment encodes the RNA-dependent RNA polymerase (RdRp), while the M and S segments encode proteins essential for viral replication and movement within the host plant. Structurally, TSWV is enveloped with a helical nucleocapsid, allowing it to accommodate larger genetic payloads than icosahedral viruses, making it an appealing tool for genetic delivery systems(29).

Therefore, the effective viral vector for plant gene transfer would be a powerful tool for advancing agricultural research, crop improvement and biotechnological applications to enhance plant traits, resilience and productivity. Some notable studies are given in Table 1. RNA silencing suppressors

(RSSs) are incorporated into vector designs to improve desired gene expression. RSSs are essential for inhibiting the innate immune system of plants and preventing RNA interference at both the transcriptional and post-transcriptional levels. The viral RNA sequences produce plant gene terminators, AT-rich matrix attachment regions (MARs) and translational enhancers to maximize target gene translation efficiency (30, 31). The vector performance during impaired serial passage, insert size management and stability are employed to solve cargo size limits(14).

***Key Strategies Leveraging Viral Vectors*:** Using plant viruses as vectors for genome editing and gene insertion in plants offers a versatile strategy with several tactical benefits. Plant viruses are highly adaptable, which enables delivering to infect a diverse range of plant species and provides a range of gene transfer strategies. For precise control of gene expression in genetic engineering attempts, plant viruses' genetic components-such as sequences that regulate translational regulation and RNA-dependent RNA synthesis-are leveraged (32). Plant genomes can be efficiently edited and expressed by designed viral vectors, for example, Tobacco rattle virus (TRV) made possible by the temporary introduction of RNAs and CRISPR-Cas reagents into plant cells, the effectiveness of TRV in Virus-Induced Gene Silencing (VIGS) facilitates the investigation of functional genomics in a variety of plant species. RNA viruses, such as the Tobacco rattle virus (TRV), are preferable to DNA viruses for plant genome editing because they do not transfer foreign DNA into the plant genome, eliminating ethical and regulatory problems. With up to 80% editing efficiency, sgRNAs have been introduced into plant cells using TRV as a vector. The RNA genome of TRV does not merge with the plant genome. Thus, it has a short genome size and a broad host range (33). Modified TRV can be used as a vector for fast reverse genetics screening, investigation of plant gene function, delivery of CRISPR/Cas reagents and dual heterologous gene expression. An example study on modified TRV vector as TRV-GFP by attaching green fluorescent protein (GFP) in the 3' terminus of the TRV 2 vector which was used to identify the mutation in UV lamp itself; this method has been used in commercially essential plants like Rose, Chrysanthemum and strawberry using PDS as a reporter gene which 75-80% plants show photobleached leaves (34). All things considered, TRV is an effective and adaptable vector for functional genomics research and plant genome editing. Other than TRV, vectors like PVX, WDV, TMV and ToMV are also adaptable for genome editing.

Most remarkably, plant viruses can enter meristematic cells and allow the development of components that modify genes, leading to the creation of mutant seeds (35). Together, these methods highlight the potential of using plant viruses as efficient instruments for precise gene insertion and genome editing, opening the door to novel developments in plant biotechnology and agricultural enhancement initiatives. VIGS and VIGE open new avenues for discovery and innovation by harnessing plants' natural viral defences, driving progress in crop enhancement, biotechnology and our understanding of fundamental biological processes. Using viral vectors for gene transfer presents the exciting potential for rapid and efficient editing of target genes.

***Agroinfiltration*:** Agroinfiltration is a transient gene expression method in plants. It uses *Agrobacterium* to deliver genes without integration into the genome and CRISPR/Cas9 can be used with this technique for targeted genome editing. It allows for efficient transcription and translation. It is a potent and adaptable method for temporary gene expression that entails the direct transport of recombinant *Agrobacterium* tumefaciens into plant tissues, usually leaves (36). *Agrobacterium* tumefaciens is used as an infection medium in virus-mediated investigations to introduce viral genetic material into plant cells to study virus-host interactions, viral replication and disease development, known as Agroinfiltration. The *Agrobacterium* cells have transformed. They are prepared for infiltration into plant tissues to express their virulence genes before invasion. The virus's genetic material must be transferred into plant cells via this induction. Chemical inducers like acetosyringone are frequently employed to activate the virulence genes. To guarantee effective viral distribution, this can be accomplished in several

Table 1. Some of the notable important viral vectors used for gene transfer in plants

S.No.	Virus name	Name of the plant species	Mode of transmission	References
1.	Tobacco rattle virus (TRV)	*Nicotiana tobaccum Petunia hybrida*, *Nicotiana alata* and *Nicotiana benthamiana*	Nematode transmission	(28,53,58-61)
2.	Wheat dwarf virus (WDV)	*Triticum aestivum*, *Oryza sativa*	Leafhopper	(62-63)
3.	Bean yellow dwarf virus (BeYDV)	*Solanum lycopersicum*, *Nicotiana tabacum*, *Solanum tuberosum*, *Tomato CV microtom*	Aphid	(64-68)
4.	Potato virus X (PVX)	*Nicotiana benthamiana*, *Solanum lycopersicum*	Mechanical transmission	(20,12, 69)
5.	Tomato spotted wilt virus(TSWV)	*Nicotiana benthamiana*, *Solanum lycopersicum*, bell pepper	Thrips	(29)
6.	Pea early browning virus (PEBV)	*Nicotiana benthamiana*, *Arabidopsis thaliana*	Nematode	(28)
7.	Tobacco mosaic virus (TMV)	*Nicotiana benthamiana*	Mechanical transmission	(70)
8.	Tomato mosaic virus (ToMV)	*Nicotiana benthamiana*	Mechanical transmission	(71)
9.	Barley yellow striate mosaic virus (BYSMV)	*Nicotiana benthamiana*	Plant hopper	(72)

ways, such as syringe infiltration (a widely used method where a needleless syringe introduces *Agrobacterium* into plant leaves through a small nick in the epidermis, allowing the bacteria to enter the intercellular space), vacuum infiltration (Plant leaves are submerged in a medium containing *Agrobacterium* with the target recombinant DNA in vacuum infiltration. When a vacuum is applied and released, the air is drawn out of the leaf's interstitial spaces and replaced by the *Agrobacterium* solution, achieving infiltration), petiole injection (a method used for delivering viral vectors and RNA molecules directly into the vascular system of plants, facilitating efficient gene transfer and expression. This technique involves using a syringe to inject solutions, such as viral particles or RNA, into the petiole, which allows for systemic distribution throughout the plant), or leaf dipping(a method where leaves are immersed in a solution containing *Agrobacterium tumefaciens* to introduce foreign DNA into plant cells) (14). For example, Agroinfiltration was employed in the tomato Torrado virus (ToTV) to introduce infectious virus clones into *Nicotiana benthamiana* and *Solanum lycopersicum* plants, causing the development of classic ToTV disease (37). Using this method, we were able to look into the altered virus's contagiousness examine how the virus interacts with plants and analyze viral causes of disease

Virus-induced gene silencing (VIGS): VIGS is a cornerstone strategy offering a powerful method to dissect plant gene function. The term "virus-induced gene silencing" (VIGS), first introduced by A. van Kammen in 1997, was initially used to describe the recovery process from viral infections. It has since become widely associated with a technique that utilizes recombinant viruses to reduce the expression of endogenous genes. The first virus-induced gene silencing (VIGS) vector was developed using the tobacco mosaic virus (TMV). This vector successfully silenced the NbPds gene in *Nicotiana benthamiana* by inoculating RNA transcripts *in vitro*, which produced plants with an albino phenotype. Since its inception, VIGS has come to describe any technique that utilizes recombinant viruses to suppress the expression of endogenous genes. Initially applied to field and horticultural crops, this gene-silencing approach is also being explored in forest trees, including *Hevea brasiliensis* (38, 39).VIGS represents a paradigm shift in plant molecular biology to unravel the complexities of gene function with unprecedented speed and precision (40). VIGS harnesses the intricate machinery of plant defence mechanisms against viruses for targeted gene knockdown experiments by modified viral constructs carrying fragments of the plant's genes exploiting the plant's innate antiviral response to trigger gene silencing, which initiates mRNA degradation (41). This degradation effectively blocks the targeted gene expression.

VIGS vector systems, derived from RNA and DNA plant viral sources, have been meticulously developed to silence target genes, enhancing precision and selective efficiency. The viral RNA is targeted for degradation either through post-transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS) mechanisms. RNA silencing targets viral transcripts and can influence host gene expression to enhance antiviral defence mechanisms(31, 42). A cDNA fragment from a target plant gene is cloned into a DNA copy of the RNA virus genome and the plant is then transfected with this construct using *Agrobacterium*. During viral replication, double-stranded RNA (dsRNA) is produced, including sequences from the interest gene. The plant's Dicer-like enzymes then degrade these dsRNA molecules into small interfering RNAs (siRNA) or microRNAs (miRNA), initiating gene silencing. Dicer is an RNase III-like ribonuclease enzyme that targets explicitly double-stranded RNA (dsRNA) and functions as a dimer. It plays a crucial role in initiating RNA interference (RNAi) by cleaving dsRNA into uniformly sized small RNAs, such as small interfering RNAs (siRNAs) and microRNAs (miRNAs). Members of the Dicer family are ATP-dependent nucleases, requiring ATP for their activity. The double-stranded RNA molecules are degraded into uniformly sized small interfering RNAs (siRNAs) molecules by the plant dicer-like enzymes. Then, the small RNAs are transferred to the second enzyme complex as RISC (RNAi-induced silencing complex). RNA-inducing silencing complex (RISC) is a large RNA-multi-protein complex that induces the degradation of mRNA into small interfering RNAs (siRNA) or micro RNAs(miRNA) with two overhanging ends. These small RNAs (SiRNA) help the RNA-inducing silencing complex (RISC) to induce the degradation of target mRNA (43, 44).

For siRNAs, Dicer processes double-stranded RNA (dsRNA) into small interfering RNA (siRNA), which is then incorporated into the RNA-induced silencing complex (RISC). AGO2, a critical component of RISC, cuts the passenger strand of the siRNA, leaving the guide strand intact. This guide strand leads the active RISC to the target mRNA, as shown in Fig. 1 and when there is complete complementarity between the guide strand and the mRNA, the mRNA cleaves, resulting in gene silencing, which inhibits protein formation (45).

For miRNAs, the transcription of miRNA genes in the nucleus by RNA polymerase II results in the formation of pri-miRNA, which is subsequently cleaved by Drosha to produce pre-miRNA. This pre-miRNA is transported to the cytoplasm by Exportin 5, where it is processed into mature miRNA by Dicer. Once formed, the miRNA is incorporated into the RNA-induced silencing complex (RISC), discarding the passenger strand. The remaining guide strand then directs the miRISC to the target mRNA through partial complementary interactions, ultimately resulting in gene silencing through mechanisms such as translational repression, degradation, or mRNA cleavage (46).

It offers several advantages over gene silencing techniques, such as RNA interference (RNAi). It enables transient and localized gene knockdown to observe phenotypic changes without permanently altering the plant's genome and is valuable for functional genomic studies, essential genes or genes with complex functions, where complete knockout may lead to lethality or pleiotropic effects (47). Some of the notable example studies are given in Table 2. For example, the study on Petiole injection is an effective technique for infiltration and gene silencing without necrotic lesions using phytoene desaturase (PDS) as a reporter gene (45). Tea leaves exhibit chlorosis symptoms with significant silencing efficiency using tobacco rattle virus vector (45). An improved study on another vector Tomato-spotted wilt virus (TSWV)-based viral delivery system for nontransgenic genome editing across various crop species and genotypes. TSWV will act as a viral vector to carry large genetic cargoes for genome

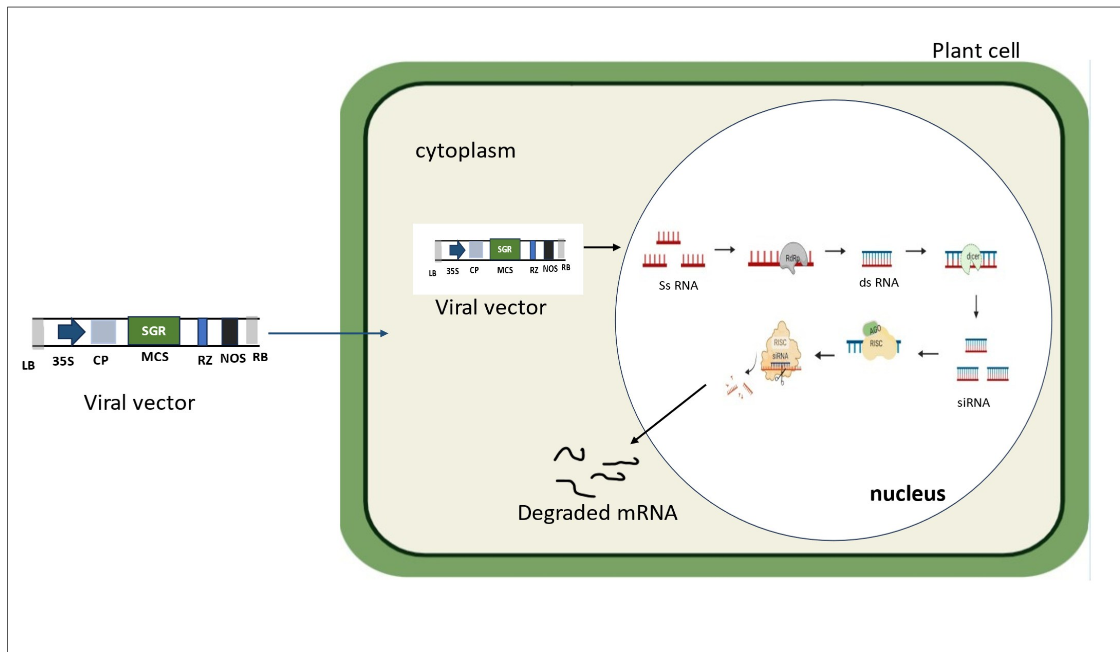

Fig. 1. Mechanism of Virus-induced gene silencing - viral vector is used to deliver the gRNA into plant cells for mRNA degradation, inhibiting protein formation. LB- left border, RB- Right border, Cp- coat protein, MCS- multiple cloning site, Rz- ribozyme, NOS- nopaline synthase.

Table 2. Studies on using viral vectors for virus-induced gene silencing

S.No	Vectors used	Name of the species	Target Genes	References
1	Tobacco rattle virus (TRV)	*Nicotiana benthamiana, Solanum lycopersicum, Arabidopsis thaliana, Gossypium spp., Catharanthus roseus, Camellia sinensis, Ilex dabieshanensis*	*Phytoene desatuarase (pds), Green fluroscent protein (GFP), chloroplastos alterados 1 (CLA1), protoporphyrin IX magnesium chelatase subunit H (ChlH)*	(15, 73-77)
2	potato virus X (PVX)	*Solanum lycopersicum, Solanum tuberosum, Nicotiana benthamiana*		(78-80)
3	Tomato spotted wilt virus (TSWV)	*Capsicum annum, Solanum lycopersicum, Nicotiana benthamiana*		(81)
4	Barley strip mosaic virus (BSMV)	*Hordeum vulgare, Nicotiana benthamiana*		(82)
5	Cassava common mosaic virus (CsCMV)	Cassava		(83)
6	Cucumber green mottle mosaic virus (CGMMV)	Cucurbits		(84)
7	Cabbage leaf curl virus (CaLCuV)	Brassicas		(85)
8	Foxtail mosaic virus (FoMV)	*Panicum virgatum*		(86)
9	Brome mosaic virus (BMV)	*Triticum aestivum*		(87)
10	Tobacco mosaic virus (TMV)	*Nicotiana benthamiana*	GFP and PDS	(39)

editing. Plant regeneration is impacted by heritable mutations when virus-infected tissues are grown in vitro without antibiotic selection (29). An example study on the overexpression of the gene was done using comparative analysis in *Catharanthus roseus* using an improved efficient cotyledon-based VIGS method. The cotyledon-based VIGS method is highly innovative and advantageous over the traditional VIGS method, including rapid, higher efficiency and enabling up and down-regulation with transient gene overexpression (15). Controlled expression is achieved through inducible promoters, which allow for regulated gene expression in response to external stimuli. Inducible vectors facilitate rapid and controllable gene expression, bolstering safety and compliance with regulatory standards.

***Virus-induced genome editing*:** Virus-induced genome editing (VIGE) is a cutting-edge technology that uses plant viruses as vectors to deliver CRISPR-cas9 components into plant cells. This innovative approach offers several advantages over traditional plant-genome editing methods, such as *Agrobacterium*-mediated transformation, often relying on tissue culture (48). Using viral vectors, VIGE offers a promising alternative by utilizing plant viruses to express Cas proteins and guide RNA throughout plant tissues, including meristematic regions. This approach can reduce off-target effects and cross-contamination issues associated with conventional methods. Compared to the positive strand of RNA viral vectors, a negative strand of RNA viral vectors shows less frequency of editing efficiency in plant genomes.

The modified viral genome is introduced into plant cells, typically through methods like Agroinfiltration or mechanical inoculation. Once inside the plant cell, the virus starts replicating and expressing the CRISPR components Cas proteins and guide RNAs into the plant cell. The guide RNA directs the Cas protein to the desired location in the plant genome, creating a double-strand break in the DNA. The double-strand break (DSB) activates the plants' innate DNA repair pathways, resulting in either gene disruption or precise gene editing. This can occur through two main mechanisms:

non-homologous end joining (NHEJ), which typically introduces small insertions or deletions (indels) leading to gene knockout, or homology-directed repair (HDR), which enables more precise modifications by incorporating a donor DNA template (49-51).

In some cases, the engineered virus can move from the initially infected cells to the meristematic cells of the plant. Meristematic cells are responsible for growth and development; editing these cells can result in heritable changes in further generations. It continues to refine VIGE techniques to enhance the efficiency of virus delivery, increase the expression levels of active guide RNAs (52) and develop species-specific virus vectors. These optimizations aim to improve the targeted genome editing success rate in plants. The development of a Tobacco rattle virus (TRV)-mediated genome editing system that allows for multiplexed and highly efficient editing of target genes in *Nicotiana benthamiana* plants to overcome the challenges associated with traditional genome editing methods by utilizing the CRISPR/Cas9 system delivered via a virus, which can bypass the need for plant transformation and regeneration and also explores the potential for germinal transmission of the edited genes (51). It highlights the broad applicability of the virus-mediated genome editing system across different plant species and its potential for accelerating trait discovery and development in agriculture (48). Two virus-induced genome editing (VIGE) approaches have been developed for solanaceous plants: eggplant, potato and tomato. Some of the notable example studies are given in Table 3. The first technique delivers single guide RNAs (sgRNAs) into a transgenic tomato line expressing Cas9 using the *Tobacco rattle virus* (TRV) vector.

In contrast, the second way uses the potato virus X (PVX) vector to carry both Cas9 and sgRNAs (50). This example study achieved mutation rates of 40.3% and 36.5% for TRV- and PVX-mediated VIGE, respectively, by optimizing the VIGE systems and assessing their effectiveness by deep sequencing additional solanaceous crops, such as potatoes and eggplant, could be grown using the PVX-mediated VIGE technique. An example approach applying an RNA virus vector PVX delivered sgRNA into cas9 expressing lines resulted in efficient somatic editing with indel frequencies of 58% by knockout phytoene desaturase (PDS) gene and Staygreen (SGR1) gene (12). The foreign gene may sometimes integrate into the plant's genome, resulting in stable integrating changes. This integration can lead to a long-lasting expression of the introduced genes in subsequent generations. *Arabidopsis FLOWERING LOCUS T* (FT) mRNA was fused to the sgRNA to enhance the heritability of TRV-based VIGE, facilitating the transfer of sgRNAs into reproductive tissues. This approach, which utilized sgRNAs enhanced with mobile RNA sequences, achieved a high editing efficiency of 90-100% in infected tissues. Notably, the efficiency of inheritable genome edits was also significantly improved, reaching 65-100% (53). An improved study on delivering TRV vectors directly to axillary meristematic cells by targeting specific genes, such as *Phytoene desaturase* (PDS), *Chl1*, *Hairless*, *Hairplus* and *Anthocyanin 2* (An2), in Cas9-expressing transgenic tomato plants, resulting in efficient gene-edited shoots that are heritable. The cytokinin biosynthesis gene *isopentenyl transferase* (*ipt*) was also delivered to promote shoot formation, though it caused some developmental abnormalities. Lower temperatures improved viral accumulation, while higher temperatures helped reduce the harmful effects of *ipt*. This method shows potential for tissue culture-free gene editing in tomatoes and other crops, enabling faster improvements (54). With its efficiency, scalability and adaptability, this approach holds great potential for advancing agricultural research, crop improvement and functional genomics.

***Applications of Viral Vectors*:** The viral vectors are easy to manipulate, which is helpful for various genome engineering applications. Compared to alternative gene transfer techniques like *Agrobacterium*-mediated transformation or particle bombardment, this strategy has several benefits in planta methods. Furthermore, viruses have evolved sophisticated mechanisms to replicate and spread within their host organisms efficiently, making them ideal delivery vehicles for introducing foreign genes into plants and ensuring a systemic viral infection, increasing the single guide RNA concentration for faster acquisition of edited genotypes, as shown in Fig. 2.

Table 3. An example study on delivering CAS9 and gRNA using viral vectors for genome editing

S.No.	Vectors used	Name of the species	Target genes	References
1	Tobacco rattle virus (TRV)	*Nicotiana benthamiana*, *Solanum lycopersicum*, *Arabidopsis thaliana*, *Gossypium* spp.		(16,53,54,88)
2	Potato virus X (PVX)	*Nicotiana benthamiana*, *Solanum lycopersicum*, *Solanum tuberosum*		(20,89)
3	Tobacco mosaic virus (TMV)	*Nicotiana benthamiana*		(70)
4	Sonchus yellow net rhabdovirus (SYNV)	*Nicotiana benthamiana*		(90)
5	Pea early browning virus (PEBV)	*Nicotiana benthamiana*	*Phytoene desatuarase (pds)*, *Staygreen 1(SGR1)*, *Green fluroscent protein (GFP)*, *chloroplastos alterados 1 (CLA1)*, *protoporphyrin IX magnesium chelatase subunit H (ChlH)*, *Flowering locus T (FT)*	(28)
6	Bean yellow dwarf virus (BeYDV)	*Solanum lycopersicum*, *Solanum tuberosum*, *Vitis vinifera*		(91,92)
7	Foxtail mosaic virus (FoMV)	*Nicotiana benthamiana*, *Zea mays*, *Setaria virdis*		(93)
8	Barley stripe mosaic virus (BSMV)	*Triticum aestivum*		(94)
9	Tomato spotted wilt virus (TSWV)	*Capsicum* spp., *Solanum lycopersicum*		(95)
10	Cassava common mosaic virus (CsCMV)	Cassava spp.,		(83)

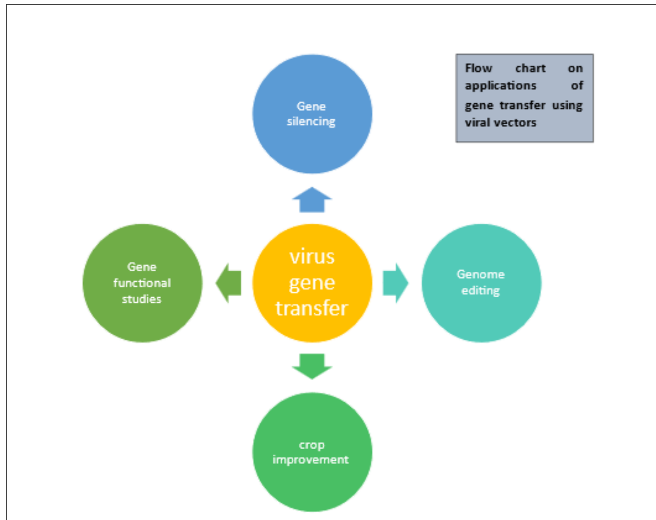

Fig. 2. Applications of gene transfer using plant viral vectors.

Virus-induced gene silencing is an excellent reverse genetic tool for gene function studies for specific sequences on genome engineering in plants via efficient transcript expression. It does not require stable transformants that allow for phenotype characterization that will be lethal in stable lines. Virus-induced gene silencing has valuable insights into various gene functions for silencing individual or multiple genes. This leads to the development new stable genotypes with desired traits by inducing heritable epigenetic modifications in plants (55). Virus-induced genome editing has emerged as a powerful method with high editing efficiency. VIGE presents a promising alternative by employing plant viruses to deliver Cas proteins and guide RNA across plant tissues, including meristematic regions, with viral vectors. This method helps minimize off-target effects and the risk of cross-contamination commonly seen with traditional approaches (53, 56).

Challenges and Future Prospects: Plant viruses can serve as viral vectors for the temporary production of RNAs and heterologous proteins. Easy modification, more temporary expression and systemic plant dispersion are only some of these vectors' benefits. The CRISPR system for gene editing has become the new era for crop improvement. Some viral vectors, particularly RNA viruses, have limited carrying capacities, which restricts the size of CRISPR-Cas components that can be delivered, posing a challenge when larger constructs are needed. Despite their high transduction efficiency, viral vectors risk off-target mutations, predominantly when sgRNAs are expressed at high levels for prolonged periods. Here, *Agrobacterium*-mediated gene transfer and tissue culture for that regeneration have become the bottleneck for gene editing.

Viral vectors such as tobacco rattle virus (TRV), potato virus X (PVX) and *Geminiviruses* enable efficient gene delivery by allowing high expression levels of sgRNAs across various plant tissues, including meristematic regions, leading to widespread and effective genome editing. These vectors have a broad host range and can infect many plant species, from model organisms like *Nicotiana benthamiana* to economically significant crops such as tomato, maize and wheat, making them highly versatile for genome editing applications. The limited genome size of Gemini viruses, which makes it difficult and impotent to transmit large DNA segments, is one of the drawbacks of plant viral vectors (14, 52). *Geminivirus* replicons are disassembled and limited to containing only the replication -associated proteins (Rep/RepA) and intergenic regions (IRs), which are effective cargo carriers. Over time, mutations, deletions and unfavourable impacts might cause the implanted genes to disappear (50, 57), including the efficient delivery of genome engineering components to plant cells and challenges such as intellectual property rights and regulatory barriers necessitate careful consideration for practical and commercial application.

VIGS and VIGE provide new pathways for discovery and innovation by exploiting the plant's innate viral defences, propelling advances in crop improvement, biotechnology and our knowledge of fundamental biological processes in plants. Gene transfer using viral vectors offers the tantalizing possibility of rapid, efficient editing for target genes and precise and high-throughput screening of gene function in many plants will be possible if we find an efficient way of delivering Cas proteins and gRNAs into meristematic cells. Using plant viruses as viral vectors for genome editing in plants represents a remarkable advancement in biotechnology. The future trajectory of this field is expected to drive genome engineering through viral vectors in more refined ways, such as genome editing using viral vectors even with cargo capacity, thereby unlocking novel opportunities for the scientific community in plant genome engineering. Continued innovation and collaboration will undoubtedly propel the development and application of viral vectors, paving the way for transformative advancements in plant biotechnology.

Conclusion

Plant virus-mediated gene transfer has emerged as a significant strategy for gene editing, offering advantages such as high transient expression, systemic distribution and ease of manipulation. Viral vectors efficiently deliver Cas reagents into plant cells and serve as viable methods like VIGS and VIGE, successfully transferring genetic material to various plant species, utilizing techniques like Agroinfiltration and their small genome size enables cloning, multiplexing and library creation. Recent advancements in cotyledon-based VIGS methods and VIGE highlight their potential for efficient gene editing and simplified processes. However, challenges such as limited genome size, heritability, off-target effects and specificity remain. Further research is essential to refine these vectors and unlock their potential in plant biotechnology.

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Authors' contributions

BK, KKK, LA, SVA and MD undertook the concept and design of the review work. BK and KKK gathered the literature and drafted the manuscript. KKK and LA did the critical revision. SVA and MD carried out verification and supervision. BK prepared the Figure. KKK provided approval for the final manuscript.

Compliance with ethical standards

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