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Plant Viruses- Molecular and Biotechnological Applications

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ABSTRACT: Plant viruses until recently were seen as plant pathogenic entities causing crop losses. Consequently, they were studied from that perspective only. However, recent advances made in molecular biology enabled an entirely a new dimension of plant viruses to emerge out. Plant viruses exhibit an amazing diversity in architecture, genome makeup and reproductive strategies. These facilities have made them to use as ideal tools for biotechnological applications. Biotechnological applications of plant viruses extend in varied fields such as agriculture, horticulture, medicine, pharmacy and nanotechnology. Biotechnological applications in agriculture and horticulture involve enhancing the aesthetics of ornamental plants, cross protection, virus-induced flowering and biological control. Some plant viruses are employed both as carrier vectors and expression vectors. Expression vectors have a great role in vaccinology. Plant virus-derived vectors are developed for virus induced gene silencing and nanotechnology. Virus-based tools have been developed to deliver CRISPR/Cas9 constructs for precise genome editing. Virus-like particles (VLP) are excellent candidate for vaccine production. VLPs also meet the desirable features to be used as nanoparticles which have wide applications in the medical field.

Keywords: Plant viruses, Biotechnology, Vectors, VLPs, Virus-induced gene silencing, CRISPR/Cas9 Nanotechnology, Gene editing.

INTRODUCTION

Traditionally, the viruses for the sake of convenience have been categorized into plant viruses, animal viruses and bacteriophages. Recently, some more viruses like mycoviruses, phycoviruses, satellite viruses, virophages, giant viruses and some other virus-related particles like prions, viroids are also added. Despite the devastating and periodic appearances of the scourges caused by viruses on humans, livestock and crop plants since ancient times, the scientific investigations on these ultramicroscopic entities were initiated with plant viruses, undoubtedly with classical virus tobacco mosaic virus (TMV). This virus served as prototype model not only for plant viruses but also for all viruses. The initial studies made by Adolf Mayer, a German botanist, and later on followed by Russian botanist Dmitri Iwanovski and Dutch soil microbiologist, M.W. Beijerinck further laid the foundation for the plant virology.

Handicapped by technology and suitable instrumentation, the initial studies were concentrated on disease aspects like symptomology, epidemiology like host range, transmission, losses incurred and to a limited extent on disease control. Subsequently, investigations were directed on isolation, purification, and characterization. Between 1930-1970 both structures and chemical compositions of many plant viruses were elucidated. As many different viruses were reported during this time, some attempts were made to classify and develop a nomenclature to also plant viruses (Lwoff *et al.*, 1962: Gibbs, 1969; Mayo and Brunt 2001). Plant diseases caused by viruses still a huge problem and daunting challenges to plant pathologists. Numerous studies have been made on several pathological aspects and still efforts are ongoing. Strategies to control plant viral diseases shifted from traditional approaches to more promising genetic engineering and related biotechnological approaches like transgenic resistance (Shobha and Keshamma 2023).

Diversity of plant viruses. In 8th report of ICTV (Fauquet and Fargette 2005) 16 families, 73 genera and 977 species of plant viruses were authenticated (excluding viroids). Out of these species, 701 were true species and 276 tentative species. However, these figures are about few of all cultivated plants and many more await discovery and detailed characterization. Viruses of wild plants are still a dark matter. Plant viruses exhibit an amazing diversity in several of their

properties such as host range, transmission, expression of symptoms, host-parasite interactions, morphology, genome variations and reproductive strategies (Reddy and Reddy 2023) (Fig. 1).

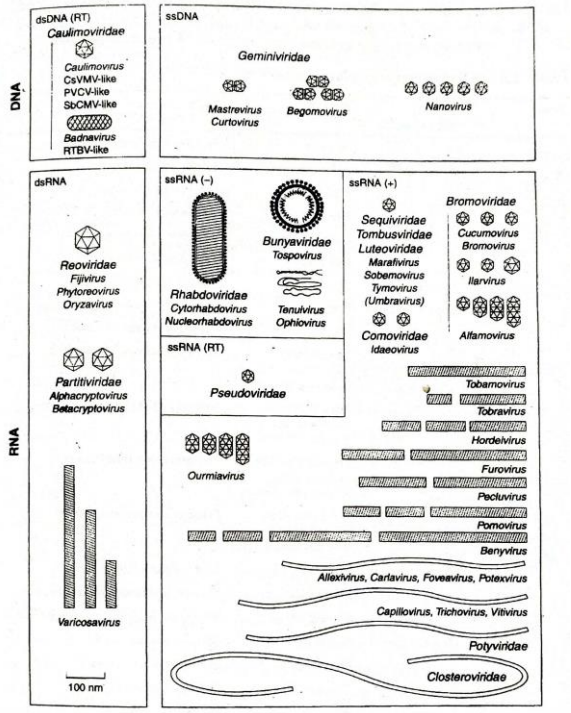

Fig. 1. Diversity of plant viruses.

Plant viruses as ideal toolkits and candidates for molecular biology and biotechnology. Incidentally, since the discovery of TMV in the late nineteenth and early 20th century, plant viruses have been largely looked upon as pathogens (Prashanth Kumar 2023). Most of the research in these 130 years has been carried out in this direction only. With advances made in molecular aspects of different viruses, the misconception about viruses as only the pathogens are slowly being erased out. Understanding of molecular architecture coupled with advances made in biochemistry, gene manipulation has opened new vistas of plant viruses hither to unimagined. Plant viruses with their great diversity have been proved to be ideal candidates for molecular and biotechnological probing as much as bacteria and yeasts. The advances made in biotechnology and next-generation sequencing technologies have accelerated novel virus discovery, identification, sequencing, and manipulation. They also revealed unique characteristics that place them as valuable tools for a wide variety of biotechnological applications. Viruses possess geometrically sophisticated architectures that make them attractive for materials for science and nanotechnology. In addition, they present an efficient machinery and a comprehensive genome structure, which make them easy to manipulate (Venkataraman and Hefferon 2021). A few viral genes were found in plant genomes and same is true with viruses harbouring plant genes, reflecting a close and long-established association between them. This provides a scope for manipulation of heterologous gene combinations for the benefit of host plant. Manipulation of genomes of viruses became possible employing the toolkits of modern molecular biology, as directed. Biotechnological potentials of engineered plant viruses and their derived products like virus –like particles (VLPs) (Nooraei *et al*., 2021) are unfolding in a range of fields like gene editing, agriculture, horticulture, medicine, pharmacy and nanotechnology.

In this review, we made an attempt to highlight several potential beneficial aspects of plant viruses (VLP) and LP their possible applications are discussed.

1. Application of plant viruses in agriculture and horticulture. Beneficial effects of viruses have been very poorly studied, and unexploited in crops. Recent studies revealed that viruses have a great potential for the benefit of agriculture. In light of climate change and global warming with increase in extreme weather conditions, water scarcity and loss of arable lands, which are concurrent with ever-increasing human populations, there is a need to employ every possible tool at our disposal without further compromising the environment. In this context, viruses hold the potential for safe, inexpensive, and non-destructive improvements to cropping practices that need to be taken seriously by horticulturists, crop scientists and plant pathologists (Roossinck, 2015).

A. Enhancing the aesthetics of ornamental plants

Plant viruses have been studied primarily because they cause disease and economic losses in agriculture and horticulture crops. Based on the plants infected by the virus, the disease shows a variety of symptoms like foliar mosaic, mottle, ring spots, necrosis, malformation, curling/rolling, yellow vein, flower and/or foliage variegation, fruit malformations, and general plant stunting. In some cases, the same type of symptoms actually improves infected host plant aesthetic look without having any adverse impact. The most famous classical example is attractive English-variegated flowers found in virus infected tulip plants. It's a familiar tale for any virology student. Several other familiar examples are the attractive mosaic leaf patterns typical of flowering maple (Abutilon pictum; Malvaceae) infected with Abutilon mosaic virus, the variegated flowers of camellia (Camellia japonica; Theaceae) infected with Camellia yellow mottle virus, and the yellow-veined Japanese honeysuckle (Lonicera japonica; Caprifoliaceae) infected with *Camellia yellow mottle virus*, and the yellow-veined Japanese honeysuckle (*Lonicera japonica*; Caprifoliaceae) infected with *Honeysuckle yellow vein mosaic virus.* A number of other plant viruses associated with distinct ornamental plant phenotypes have been reported in the past several years, and some are being sold as new plant cultivars (Valverde *et al*., 2012). Table 1 shows a range of selected viruses that improve the ornamental appearance of certain infected plants.

Table 1: List of selected viruses that enhance the aesthetics of some ornamental plants.

Virus Name	Genus	Susceptible hosts
Abutilon mosaic virus	*Begomovirus*	*Abutilon* spp., *Hibiscus* spp., *Malva* spp., *Gossypium* spp.
Camellia yellow mottle virus	*Varicosavirus*	*Camella japonica, C. sasanqua*
Clerodendron golden mosaic China virus	*Begomovirus*	*Salvia splendens, Clerodendrum cyrtophilum*
Cucumber mosaic virus	*Cucumovirus*	*Solenostemon* spp., *Viola* spp., many wild and cultivated plants
Honeysuckle yellow veinmosaic virus, Honeysuckle yellow vein virus	*Begomovirus*	*Lonicera* spp., *Solanum lycopersicum, Nicotiana tabacum, Capsicum annuum*
Nandina stem pitting virus	*Capillovirus*	*Nandina domestica*
Pelargonium flowerbreakvirus	*Carmovirus*	*Pelargonium peltatum, P.hortorum*
Pseuderanthemum yellow vein virus	*Begomovirus*	*Pseuderanthemum* sp.
Tulipbreakingvirus	*Potyvirus*	*Tulipa* spp., *Lilium* spp.

Other examples of beneficial plant viruses include several acute viruses (*Brome mosaic virus*, family Bromoviridae, *Cucumber mosaic virus*, family Bromoviridae, *Tobacco rattle virus*, family Virgaviridae, and *Tobacco mosaic virus*, family Tombusiviridae), which confer tolerance to drought and freezing temperatures in several different crops, and persistent viruses, such as White clover cryptic virus (family Partitiviridae), which can suppress nodulation in legumes when adequate nitrogen is present thus economizing the plant metabolism (Roossinck, 2015).

B. Cross protection of viral pathogens

Cross-protection is a phenomenon in which infection of a plant with a mild virus or viroid strain protects it from disease resulting from a subsequent infection with a severe strain of the same virus or viroid. Its history began around seventy years ago, when the Dutchman Thung and the Englishman Salaman independently described the phenomenon. A discovery that was initially made by several virologists in the 1930s, and it was the first means of protecting plants from virus infection. Hypothesized mechanisms for the phenomenon have included (i) antibody formation (releasing virion neutralization), (ii) rabid exhaustion of a metabolic component essential for virus multiplication (e.g., ATP, RNA, etc.) (iii) limited sites for virus multiplication (in this case generally, cell structure or (iv) specific adsorption by new cell compounds (offspring cells). However, those hypotheses were re-proposed and discussed several times later without achieving a consensus. The molecular genetics of viruses made stimulating new "theories" possible, largely based on the interference between strains of virus. This is one model that predicts that de novo progeny positive-sense RNA of the protecting strain would sequester the minus-strand RNA of the challenging strain. Other models involve a function of the coat protein, or gene recombination. While people have varying views on the topic, the real-world use of this approach has been widely appreciated. Farmers and researchers have successfully applied it to a range of crops, including tomatoes, tobacco, citrus fruits, cucurbits, grapevines, soybeans, papayas, and more. However, in the 1980s, the concept of cross-protection lost momentum as new, resistant, or more resilient crop varieties were developed, shifting the focus away from this method.

C. Virus induced flowering

Obtaining flowers in a synchronized manner and in a particular period is always desirable, however, a challenging task in horticulture. The journey of plants from their leafy, growing phase to the flowering stage is a delicate dance influenced by both their internal development and external environmental cues. Many plants spend a long time in their juvenile phase before they're ready to flower and reproduce. Traditional methods to speed up or synchronize flowering—like selective breeding or farming techniques—are often expensive, time-intensive, and tricky to pull off successfully.

Enter *florigen*, a fascinating molecule that acts as a key player in the flowering process. Belonging to the PEBP family, florigen is a mobile signal that travels through the plant, influencing when and how it flowers. It's produced in the leaves and then moves to the plant's growing tips, where it triggers the shift from leafy growth to flower formation. At its core, florigen is a protein encoded by the *FLOWERING LOCUS T (FT)* gene, a gene that's remarkably similar across many flowering plants. Scientists have found that by boosting the activity of the *FT* gene, they can speed up the flowering process, making it less dependent on factors like day length or cold temperatures.

This discovery has been a game-changer in both agriculture and horticulture. For example, in the model plant *Arabidopsis thaliana*, the *FT* gene has been well-studied, and similar genes have been identified in a wide range of plants. These genes act as master coordinators, integrating environmental signals with the plant's internal clock to kickstart flowering. Overexpressing the *FT* gene can lead to faster flowering, bypassing the usual requirements like specific light conditions or winter chilling.

However, there's a catch. While speeding up flowering sounds like a win, using genetic modification to overexpress *FT* comes with challenges. The process of genetically altering plants is labour-intensive, requires specialized skills, and doesn't work well in many species. Plus, it's often limited to certain genetic backgrounds, leaving many important crops out of

reach. For these reasons, scientists are still exploring better, more efficient ways to harness the power of florigen without running into these roadblocks.

In order to overcome these hurdles, as alternative to traditional approaches, viral vectors have been used to deliver FT orthologs to different crop plants to induce determinate growth patterns and precocious flowering**.** The principle of this strategy is: the FT gene product, florigen, is phloem mobile and naturally transported to apices to influence meristem identity. Many viruses use the phloem as a pathway to establish systemic infections. Therefore, in principle, coupling an FT ortholog with a virus-based vector that can amplify the inserted sequence and move it systemically will promote flowering. Similar to virus-induced gene silencing (VIGS) (Dommes *et al.,* 2019), the use of a virus to deliver gene sequences that promote flowering was termed VIF (McGarry and Ayre 2012). The first demonstration of virus induced flowering (VIF) used Zucchini yellow mosaic virus to deliver FT to cucurbits, stimulating flowering in short-day melon (*Cucurbita moschata*) under noninductive long days. Subsequently, it has been demonstrated in several ornamental and horticulture plants. This subject has been reviewed by McGarry *et al*. (2017); Bellinazo (2024).

D. Biological control of weeds using viruses

Biological control of plant diseases by fungi and bacteria is a well-established practice and use of viruses as bioherbicides is a novel approach. Some attempts have made in this direction. Plant viruses that cause diseases on weed and wild plants are looked upon as promising bioherbicide candidates. Invasive plant species is a common problem all over world threatening the biodiversity. Using viruses as natural herbicides to tackle invasive plant species is an innovative approach that's gaining attention. Instead of focusing on small, carefully managed areas, this method has been tested in larger, open ecosystems where invasive plants run wild. For example, in Florida, the Tobacco Mild Green Mosaic Tobamovirus has been used to control tropical soda apple (*Solanum viarum*), while in New Zealand, a mosaic virus has been deployed to manage the spread of moth plant (*Araujia hortorum*). In fact, the first viral herbicide was patented and approved by the EPA in 2015 for use in fenced pasture areas. Similarly, a virus similar to the Tobacco Rattle Virus has been suggested as a potential solution for controlling *Impatiens glandulifera*, a problematic invasive weed in central and western Europe. These efforts highlight the potential of viruses as eco-friendly tools to keep invasive species in check. Similarly, Óbuda Pepper Virus (ObPV) and Pepino Mosaic Virus (PepMV) have been proposed as biocontrol agents to keep overall populations of the weed *Solanum nigrum* under control. No instances of use of viral herbicides in control of weeds of crop plants.

PLANT VIRUSES AS VECTORS

A vector conveys different meanings in different contexts. However, in molecular biology, a vector is a DNA molecule used as a vehicle or career to carry a foreign genetic material into another cell, where it can replicate and/or expressed. The four major types of vectors are plasmids, viruses, cosmids, and chromosomes. Viral are generally genetically engineered viruses enabling them to carry modified viral DNA or RNA that has been rendered non-infectious, but still contain viral promoters and the transgene, thus facilitating the translation of the transgene.

For many years, plant viruses have been harnessed as powerful tools for a wide range of scientific and practical applications. They've become essential in both basic research and applied studies, offering a unique set of benefits. Plant virus-based vectors are particularly appealing because they are easy to work with, allow for high levels of temporary gene expression due to their ability to replicate rapidly, and can even repair themselves using their own genetic material as a template. They also spread efficiently throughout plants, leading to strong gene expression and effective gene editing. Additionally, these vectors make it possible to test different genetic constructs across various plant types, bypassing the challenges of stable plant transformation. Researchers can also control when and where genes are expressed by adjusting when the virus is introduced, and they work well in a wide range of compatible plants without the variability often seen in traditional transgenic lines (Mahmood *et al.,* 2023).

However, despite these advantages, plant viral vectors aren't without their drawbacks. One major limitation is that they typically only provide temporary gene expression, meaning the desired traits aren't passed down to future generations through seeds or breeding. This means that for annual crops, beneficial traits need to be reintroduced every growing season. Over time, the introduced genes can also be lost or mutated, especially with larger genetic inserts, which can disrupt their function. There's also the risk of unintended effects on the host plant or interactions with other viruses, as well as the potential for the virus to spread to other crops or wild plants, which could pose ecological concerns. While plant viral vectors are incredibly useful, these limitations highlight the need for careful consideration in their use. Autonomously replicating virus-based vectors provide alternative means to deliver genetic engineering (GE) reagents to plant cells (Abrahamian *et al.,* 2020; Zaidi and Mansoor 2017). Among these are the RNA viruses, which for monocots include Wheat Streak Mosaic Virus (WSMV) and Barley Stripe Mosaic Virus (BSMV), Tobacco Rattle Virus (TRV) for dicots. Single-stranded (ss) DNA viruses, like Gemini viruses, have been also widely employed as vectors for diverse crops. These viruses can be modified to carry heterologous coding sequences, and protein expression has been achieved in important crops like wheat, barley, corn, oat, and rye. Recent advancements in genetic engineering (GE) technologies have opened up exciting possibilities for scientists. They can now use viral vectors as a tool to efficiently deliver GE materials into plant cells, making the

process more effective and precise (Table 2). This breakthrough is paving the way for innovative solutions in plant science and agriculture.

Gemini viruses as vectors: Geminiviridae, the largest virus family (485 species) consists of circular, single-stranded (ss) DNA viruses infecting a wide variety of hosts ranging from staple to fiber crops such as cotton, maize, wheat cucurbits, tomato, and several ornamental and weed plants. Gemini virus genomes are remarkably compact, typically ranging from about 2.7 to 5.5 kilobases in size. Despite their small size, they encode between four to eight functional proteins, which are found on both the sense and complementary sense strands. Among the various plant vectors, Gemini viruses stand out as particularly versatile and efficient tools for genetic engineering (Lozano-Duran, 2016).

Table 2: Important viral vectors for plant genome engineering.

Virus type	Virus vector	GE platform	Plant species	Target
DNA virus	BeYDV	CRISPR and TALEN	*Solanum lycopersicum*	*ANT1*
	BeYDV	ZFN, TALEN and CRISPR	*Nicotiana tabacum*	P-*GUS:NPTII*
	BeYDV	CRISPR	*Solanum tuberosum*	*stALS1, stALS2*
	BeYDV	CRISPR and TALEN	*Solanum tuberosum*	*ALS1*
	CaLCuV	CRISPR	*Nicotiana benthamiana*	*PDS*
	WDV	CRISPR	*Triticum aestivum*	*Ubi, MLO, GFP*
	WDV	CRISPR	*Oryza sativa*	*GFP, GUS*
RNA virus	TRV	ZFN	*Nicotiana tabacum* and *Petunia hybrida*	*uidA*
	TRV	Meganuclease	*Nicotiana alata*	*DFR*
	TRV	CRISPR	*Nicotiana benthamiana*	*PDS*
	TRV	CRISPR	*Nicotiana benthamiana*	*PDS, PCNA*
	TRV	CRISPR	*Nicotiana benthamiana*	*Plant virus*
	TRV	CRISPR	*Nicotiana benthamiana*	*Plant virus*

Acetolactate synthase1 (ALS1), Solanum tuberosum acetolactate synthase1 (StALS1), green fluorescent protein (GFP), β-Glucuronidase [GUS] reporter controlling gene (uidA), Promoter of GUS and neomycin phosphotransferase (PGUS: NPTII), Bean yellow dwarf virus (BeYDV), Cabbage leaf curl virus (CaLCuV), zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALEN), clustered regularly interspaced short palindromic repeats (CRISPR), Tobacco rattle virus (TRV), genome engineering (GE), phytoene desaturase gene (PDS), prolife rating cell nuclear antigen (PCNA), ubiquitin gene (Ubi), Mildew Locus O (MLO), dihydroflavonol 4-reductase (DFR).

1. Broad Host Range: Gemini viruses have the unique ability to infect a wide variety of plant species across different families. This makes them incredibly useful as vectors, as they can target multiple hosts simultaneously.

2. Simplified Replication: These viruses require just one key protein, called Rep (or RepA in astroviruses), to kickstart replication within the host cell. This protein can be expressed naturally from the virus's own promoter located in the intergenic region, or it can be controlled by other user-defined promoters, whether they are always active or turned on by specific conditions.

3. Flexible Replication Mechanisms: Gemini viruses replicate in host cells through a combination of rolling circle replication and homologous recombination. This process also pushes the host cell into the S phase of the cell cycle, creating an ideal environment for homologous recombination—especially when paired with site-specific nucleases (SSNs) and complementary target sequences.

4. High Efficiency: Once inside the host cell, Gemini viruses replicate very efficiently, producing large quantities of replicons. This high replication rate means that if Gemini viruses are used as vectors for genome editing, they can generate plenty of SSNs and target sequences, significantly boosting the precision and effectiveness of the editing process. Gemini viruses have been engineered as vectors for the expression of heterologous proteins in plants. The cargo capacity of these viruses is quite limited; they can be converted into non-infectious replicons by replacing genes important for infection and cell-to-cell movement with heterologous sequences. To reach this goal, researchers have removed the coding sequences for the movement protein (MP) and coat protein (CP) from Gemini viruses. Yin *et al*. (2015) introduced an innovative approach called the "virus-based gRNA delivery system for CRISPR/Cas9-mediated plant genome editing," or VIGE for short Ali *et al.* (2015). This system leverages the overexpression of Cas9 in plants—specifically tested in *Nicotiana benthamiana* so far—alongside the temporary delivery of Gemini virus vectors carrying sgRNA designed to target specific genes of interest. VIGE offers a promising alternative to virus-induced gene silencing (VIGS) and can be used to create knockout libraries, opening new possibilities for plant genome editing. A number of Gemini virus-based vectors are constructed (Table 3).

Tobacco rattle virus as vector: Tobacco Rattle Virus (TRV) has emerged as a promising tool for genetic engineering in plants. Belonging to the Tobravirus genus within the Virgaviridae family, TRV is a plant pathogen with a single-stranded RNA genome that can infect more than 400 plant species across 50 different families. It spreads naturally through nematodes in the Trichodoridae family but can also be transmitted mechanically or through seeds.

What makes TRV particularly useful as a vector for gene editing is its ability to meet several key criteria for efficiency and versatility. For starters, it can systematically infect a wide range of plant species. Additionally, it's relatively easy to introduce into plants using *Agrobacterium* or by delivering it directly into the plant's growing points. Its small genome size also makes it convenient for cloning, multiplexing, and creating libraries, while its RNA genome doesn't integrate into the plant's DNA, reducing the risk of unintended genetic changes.

Table 3: Gemini virus-based vectors.

Types of vector	Virus		Viral component
Expression vector	Coding	Short peptide protein	*Tomato golden mosaic virus* (TGMV) *Beet curly top virus* (BCTV) *Bean yellow dwarf virus* (BeYDV) *Cotton leaf curl Multan virus* (CLCuV) *Bean yellow dwarf virus* (BeYDV) *Cabbage leaf curl virus* (CaLCuV) *Cabbage leaf curl virus* (CaLCuV) *Cotton leaf crumple virus* (CLCrV) *Cotton leaf crumple virus* (CLCrV) *Abutilon mosaic virus* (AbMV) *Beet curly top virus* (BCTV)
VIGS vector	Non-coding	Genome editing nuclease	*Tobacco curly shoot virus* (TCSV) *Cabbage leaf curl virus* (CbLCV)
		Genome editing repair templates	*Chilli leaf curl virus* (ChiLCV) *Cotton leaf crumple virus* (CLCrV)
		Genome editing sgRNA mi RNA RNA sponge	*Tomato yellow leaf curl China virus* (TYLCCNV) *Cotton leaf curl Multan betasatellite* (CLCuMB) *Tobacco curly shoot virus* (TbCSV)

TRV's genome is divided into two components: TRV1 (or RNA1) and TRV2 (or RNA2). TRV1 is crucial for the virus's movement within the plant and contains genes that encode proteins involved in replication and movement, as well as a cysteine-rich protein whose exact role is still unclear. TRV2, on the other hand, varies between different virus isolates and includes genes for the coat protein and non-structural proteins. While these non-structural proteins play a role in nematode transmission, they aren't necessary for lab-based infections. This means they can be replaced with multiple cloning sites, allowing researchers to insert genes or fragments of interest for experimentation.

Beyond gene editing, TRV is also an effective tool for Virus-Induced Gene Silencing (VIGS), a technique used to study gene function in a variety of plant species. Its versatility and efficiency have made it a valuable resource for advancing functional genomics research, as highlighted in studies by Senthil-Kumar and Mysore (2014); Shi *et al*. (2021). Overall, TRV's unique characteristics make it a powerful and adaptable tool for both genetic engineering and plant biology research.

A. Plant virus as expression vectors

Over the past twenty years, plants have emerged as strong contenders in the production of pharmaceuticals, rivalling traditional systems like bacteria, yeast, or mammalian cells. They are hardy, cost-effective to cultivate, and come with a lower risk of contamination. The concept of edible vaccines produced in plants once sparked significant excitement, but it has since waned due to challenges in ensuring consistent dosage and quality control without extensive purification. In response, researchers turned to transient expression using plant virus vectors, which offered a promising alternative for producing biopharmaceuticals. These virus-based systems not only speed up production and boost yields but also alleviate public concerns about genetically modified organisms (GMOs). More recently, plant viruses have carved out a role in cancer immunotherapy, where they function as nanoparticles. Today, plant virus expression vectors (Hefferon, 2017) have become a powerful and appealing tool in biopharmaceutical development.

Plant viruses have been adapted to produce vaccines, monoclonal antibodies, and other therapeutic proteins (Hefferon, 2012) (Table 4). What makes these vectors particularly appealing is their ability to bypass the lengthy process of plant transformation while still achieving high levels of gene expression on a large scale—often in just a few days. Scaling up production is as simple as increasing the number of host plants. Additionally, vaccine proteins made in plants can be purified with minimal steps, and in some cases, only partial purification is needed (Boehm, 2007; Paul and Ma 2011). These advantages make plant virus expression vectors a practical solution for tackling infectious diseases.

Table 4: Examples of plant viruses used as expression vectors for foreign proteins.

Recombinant Protein or Vaccine or VLP	Viral Vector
Cholera toxin b subunit	TMV
Human anti-non-Hodgkin's lymphoma single-chain Fv (scFv) immunoglobulins	Hybrid TMV and odontoglossum ringspot virus (ORSV)
Rice a-amylase	Hybrid TMV and tomato mosaic virus (ToMV)
Assembled full-size monoclonal antibody	Combination of non-competing viral vectors TMV and PVX
Human growth hormone	Hybrid crucifer-infecting TMV (cr-TMV) and turnip vein-clearing virus (TVCV)
Plant-produced VLP developed for drug delivery	TMV
Plant-produced chimaeric virus vaccine for influenza virus	TMV
Assembled full-size monoclonal antibody	CPMV
Plant-produced chimaeric virus vaccine for human rhinovirus 14 and human immunodeficiency virus	CPMV
Plant-produced VLP developed for encapsulation of metals	CPMV
Plant-produced chimaeric virus vaccine for hepatitis C virus	PVX
Hepatitis B core Norwalk virus capsid protein (NVCP)	BeYDV

These vectors are derived from the genomes of both positive-sense RNA viruses and single-stranded DNA viruses. Researchers have developed various strategies to design them, such as gene replacement, gene insertion using duplicated sub genomic RNA (sgRNA) promoters (SGP), heterologous SGP, complementation, gene fusions, internal ribosomal entry sites (IRES), and deconstructed viruses. Among these, gene replacement and SGP sequence insertion are the most commonly used methods.

Several plant viruses have been engineered to produce vaccines and therapeutic proteins, including Tobacco Mosaic Virus, Potato Virus X, Cucumber Mosaic Virus, Cowpea Mosaic Virus, and Alfalfa Mosaic Virus (Yusibov *et al*., 2011). Expression vectors are based on a variety of plant viruses, such as tobamoviruses, potexviruses, comoviruses, and geminiviruses. Some innovative vectors even combine genetic elements from two entirely different virus systems. For example, the Tobacco Mosaic Virus (TMV) has been used to express the genome of the Alphavirus Flock House Virus (FHV), a small insect virus that can replicate efficiently in plants without triggering cell death.

B. Applications of plant virus–derived vectors

Various uses of plant viral vectors can be broadly grouped under (1) therapeutic proteins, (2) epitope display and vaccines, (3) antimicrobial compounds, (4) virus-induced genome editing, (5) agricultural biotechnology, and (6*)* nanotechnology. These are discussed in different sections

(i) Viral vectors for plant genome engineering. Genome engineering (GE) is all about the innovative methods and strategies scientists use to precisely tweak the genetic makeup of living organisms. Think of it as a high-tech toolkit that allows researchers to target specific parts of a chromosome, making it possible to disrupt, correct, or insert genes with incredible accuracy. This precision ensures that the results are consistent and reproducible, which is a huge advantage. Over the years, GE technologies have become powerful tools for improving a wide variety of organisms, including plants.

One of the biggest challenges in plant genome engineering is figuring out the best way to deliver the necessary genetic tools into the target organisms. This is where vectors come into play. Vectors are like molecular delivery trucks, and scientists have found that viruses, with their efficient machinery and well-organized genetic structure, make excellent candidates. Specifically, plant viruses—both RNA and DNA types—have proven to be ideal for this purpose. For example, RNA viruses like Wheat Streak Mosaic Virus (WSMV) and Barley Stripe Mosaic Virus (BSMV) work well in monocots, while Tobacco Rattle Virus (TRV) is effective in dicots. On the other hand, single-stranded DNA viruses, such as Gemini viruses, have been widely used across a range of crops. These viruses can be modified to carry foreign genetic material, and they've successfully helped express proteins in important crops like wheat, barley, corn, oat, and rye.

Recent advancements in GE have led scientists to explore viral vectors even further, using them to efficiently deliver GE tools into plant cells grown in the lab. Here are a few exciting examples of what's been achieved so far:

1. Bean Yellow Dwarf Virus (BeYDV): Researchers created a streamlined version of this virus to deliver zinc finger nucleases (ZFNs) and a repair template into tobacco cells. This allowed them to target and modify a specific gene with impressive precision.

2. BeYDV Replicons for Gene Transfer: Scientists used BeYDV-based replicons to insert a strong promoter upstream of a tomato gene involved in anthocyanin production. The result? Gene targeting was 12 times more efficient than traditional methods using Agrobacterium.

3. Wheat Dwarf Virus (WDV) Replicons: A team led by Gil-Humanes *et al.* (2017) developed WDV-based replicons for precise genome editing in cereal crops like wheat, corn, and rice. These replicons not only amplified but also expressed foreign proteins in these plants.

4. CRISPR/Cas9 in Rice: Using a WDV-based replicon system, researchers achieved CRISPR/Cas9-

mediated gene targeting in rice with an impressive 19.4% efficiency in homologous-directed repair (HDR). Another ground-breaking approach is the **Virus-Induced Genome Editing (VIGE)** system, developed by Yin *et al*. (2015). This system uses Gemini virus vectors to deliver CRISPR/Cas9 components into plants like *Nicotiana benthamiana*. By overexpressing Cas9 and delivering single guide RNAs (sgRNAs) via the virus, scientists can create knockout libraries, offering an alternative to traditional gene silencing methods.

While these developments are incredibly promising, there are still some challenges to address. For instance, the recently developed TRV-mediated CRISPR/Cas9 delivery system could revolutionize the field by eliminating the need for time-consuming tissue culture processes. This could speed up the development of plants with desirable traits and help overcome regulatory hurdles that currently slow down the commercialization of genetically engineered crops.

(ii) Viral vectors for plant genome editing. When it comes to editing plant genomes, scientists often turn to CRISPR/Cas9 technology, which can be introduced into plants through methods like protoplast electroporation, biolistic bombardment of leaves, or Agrobacterium-mediated leaf infiltration. While these techniques are widely used, they come with their own set of challenges and limitations. To overcome these hurdles, researchers have developed innovative virus-based tools as an alternative way to deliver CRISPR/Cas9 components into plants.

Similar to how viruses are used for protein expression or gene silencing (VIGS), viral delivery of CRISPR/Cas9 can significantly boost functional genomics research. This approach is particularly valuable for improving crop varieties, as it sidesteps the issue of low transformation efficiency that often plagues many crop species. Over the years, several viruses have been adapted to carry Cas9 and guide RNA (gRNA) into plants. Many of these virus-induced genome editing (VIGE) systems have been tested in *Nicotiana benthamiana*, a model plant, because it's relatively easy to produce viral inoculum through leaf agroinfiltration.

While most CRISPR/Cas9 viral delivery systems rely on RNA viruses, a few DNA viruses, such as geminiviruses, have also been engineered for this purpose. These viral systems hold tremendous promise for creating crops that are more resistant to diseases, pests, and environmental stresses like drought or extreme temperatures. Another advantage is that, in many cases, the virus used for delivery isn't detected in the offspring plants unless they're propagated vegetatively. This feature helps address regulatory concerns, making the technology more practical for real-world applications.

VIRUS INDUCED GENE SILENCING

Virus-induced gene silencing, or VIGS, is a cutting-edge technique that helps scientists understand the role of specific genes in plants. It's a powerful tool that takes advantage of a plant's natural defence system against viruses. Think of it as a way to temporarily "turn off" certain genes to see what happens when they're not functioning. This process happens in the cell's cytoplasm and is known as post-transcriptional gene silencing (PTGS).

Here's how it works: scientists first modify a virus to carry a piece of the plant's own gene. This modified virus is then introduced into the plant, triggering the plant's defence system. As part of its response, the plant silences the targeted gene, allowing researchers to observe the effects of its absence. Over the years, this method has been adapted for use in a wide variety of plants, with different techniques developed to deliver the virus into the plant. For example, the virus can be introduced through Agrobacterium tumefaciens, a bacterium commonly used in plant genetic engineering, or through viral particles or RNA.

So far, around 37 VIGS systems have been created for studying gene functions in dicot plants (like tomatoes and strawberries), but fewer options are available for monocot plants (like grasses and cereals). When the modified virus infects the plant, it triggers the plant's defence mechanism, which silences the target gene. This "loss of function" helps scientists figure out what the gene does. The virus can be delivered in several ways, such as through Agrobacterium, in vitro RNA, or direct DNA inoculation. Once inside the plant, the virus replicates, creating double-stranded RNA (dsRNA), which is the key molecule that kicks off the gene-silencing process.

VIGS has become an invaluable tool in functional genomics, allowing researchers to explore gene functions by silencing them and observing the resulting changes. This has opened up new possibilities in fields where understanding gene functions was previously a challenge. For example, VIGS has been used to study biosynthetic pathways in plants like Catharanthus roseus, which produces complex compounds such as quinine and strychnine. It has also helped scientists understand symbiotic relationships and how plants interact with pathogens.

One of the most exciting applications of VIGS is its ability to silence genes in fruits like tomatoes and strawberries even after they've been picked from the plant. This is particularly useful for studying genes involved in early growth stages or metabolic processes that affect fruit development and ripening. By silencing these genes, researchers can prevent deformities or delay ripening, which has significant implications for agriculture.

VIGS has also been widely used to study how plants respond to stress, both biotic (like pests and diseases) and abiotic (like drought and salt). For instance, it has helped identify and validate genes involved in drought resistance, providing insights into how crops can be made more resilient to harsh conditions. Recent advancements in VIGS technology have expanded its use to more crop species, making it a key tool for improving stress tolerance in agriculture (Zulfiqar S *et al*. 2023).

VIRUS-LIKE PARTICLES (VLP) AND THEIR APPLICATIONS

Virus-like particles, or VLPs, are essentially synthetic versions of viruses that mimic their shape and structure but lack the genetic material needed to cause infection. This makes them safe to use while still retaining the natural appearance of the virus's proteins, which are crucial for triggering an immune response. Their size, shape, and the way they display immune-stimulating molecules on their surface make them highly effective at provoking a strong immune reaction. Once the viral proteins are produced, they often naturally come together to form these VLPs. This process can happen in various systems, whether inside living cells or in lab-based setups, allowing scientists to recreate and study these viral structures.

VLPs are particularly promising as the basis for synthetic vaccines (Roldão *et al.,* 2010). They are highly effective at stimulating the immune system, capable of triggering both antibody production and cell-based defences, which work differently than traditional inactivated vaccines. Because of this, VLPs have been extensively researched as tools for creating new vaccines, not only for infectious diseases in humans and animals but also for conditions like cancer and autoimmune disorders.

One exciting area of research involves VLPs derived from plants. These plant-based VLPs offer unique advantages, such as easier production and purification, greater stability, and a lower likelihood of interference from pre-existing immunity in patients. These qualities make plant-based VLPs a compelling alternative to those derived from animal or human sources. Beyond vaccines, VLPs are also being explored for other medical applications, such as delivering drugs to specific targets in the body or acting as imaging tools (Steinmetz, 2010 ; Chung *et al.,* 2020) to help diagnose diseases. This versatility has been well-documented in recent studies, highlighting their potential to revolutionize both preventive and therapeutic medicine (Chen and Lai 2013; Hemmati *et al.,* 2022).

ENGINEERING METABOLIC PATHWAYS

Plant genomes hold incredible potential to be engineered for producing valuable compounds that can benefit nutrition, industry, and medicine. However, this process is far from simple due to the complex biosynthetic pathways involved. Despite the challenges, researchers have begun exploring ways to modify these metabolic pathways, with some success using plant viral vectors as tools. For example, carotenoid lycopene, a compound with health benefits, is typically found in undetectable amounts in the chloroplasts of non-infected leaves.

In a ground-breaking study, introduced a new approach using a viral vector derived from the Tobacco etch virus (Wong *et al.,* 2017). This method allowed them to express an entire foreign metabolic pathway in tobacco plants, successfully producing lycopene. The pathway relied on three enzymes from the soil bacteria *Pantoea ananatis*. Remarkably, in tissues infected with the viral vector, lycopene levels rose to about 10% of the total carotenoid content.

The team also discovered that when the viral vector expressed just one of the three enzymes, *P. ananatis* phytoene synthase (crtB), it triggered a buildup of natural carotenoids in the plant. This, combined with a decrease in chlorophyll, led to a striking bright yellow color in the infected tissues across various plant-virus combinations. This innovative strategy not only highlights the potential of viral vectors in metabolic engineering but also opens new doors for enhancing the production of beneficial compounds in plants. These investigators have also shown a yellow carotenoid-based reporter can be used to visually track infection dynamics of plant viruses either alone or in combination with other visual markers.

PLANT VIRUSES AND VLPS IN NANOBIOTECHNOLOGY

Nanotechnology is rapidly emerging as a powerful tool with vast potential in biotechnology and medicine (Steele *et al*., 2017). At the heart of this innovation is nanobiotechnology, which focuses on harnessing biologically derived structures, particularly those with dimensions smaller than 100 nanometres. These tiny structures, especially nanoparticles of biological origin, are proving to be highly suitable for medical applications due to their unique properties.

Among these, viruses—specifically noninfective virus-like particles (VLPs)—stand out as ideal candidates for use as nanoparticles, often referred to as viral nanoparticles (VNPs). Plant viruses, in particular, are highly promising due to their precise size and symmetrical structure. What makes them even more appealing is their reduced risk compared to other biological materials, as many plant viruses cannot replicate in mammals. Additionally, noninfective VLPs pose minimal environmental risks, making them easier to handle, transport, and process. These advantages position plant virus-based particles as highly attractive platforms for a variety of nanobiotechnological applications.

VNPs can be used in their natural form or modified through genetic or chemical engineering to create synthetic nanoparticles tailored for specific purposes. For example, deconstructed viral vectors can be used to produce high yields of plant virus-derived VLPs directly in plants, rather than relying on other systems. A notable example of this approach is the production of VLPs based on the Cowpea mosaic virus (CPMV). One of the most exciting applications of VNPs is their ability to act as carriers for various substances, opening doors to numerous biomedical and nanotechnological uses. In medicine, VNPs are primarily utilized in three areas: delivering therapeutic cargo (Chung *et al.,* 2020), bioimaging, and metallization. The hollow interior of viral particles can be used to transport specific molecules, and there are two main strategies for loading these particles with foreign cargo. The infusion technique allows cargo to diffuse into preformed viral particles, while the caging strategy involves forming the particle around the cargo itself.

VNPs also show great promise in bioimaging. By incorporating specific cargoes into protective protein

shells and targeting them to particular tissues, VNPs can be used as imaging agents (Aljabali *et al*., 2021). For instance, plant viruses like the flexuous rod-shaped Potato virus X (PVX) and the icosahedral CPMV can be functionalized with fluorescent dyes for imaging in cell cultures or to mark blood vessels and tumor tissues in animal models. Additionally, VNPs can be loaded with metals like gadolinium for use in advanced imaging techniques such as MRI, a process known as metallization. This capability is crucial for many medical applications, including diagnostics.

With a wide variety of VNPs available—ranging from icosahedral to rod-shaped structures—and the ability to modify them for specific interactions with metals, VNPs offer a versatile platform for creating hybrid organic-inorganic materials. This versatility, combined with their low risk and high functionality, makes VNPs a key player in the future of nanotechnology and medicine.

CONCLUSIONS

Since the discovery of tobacco mosaic virus (TMV) in the 1890s, viruses have been largely viewed ne pathogens. Most of the research in these 130 years has been carried out in that direction covering discovery, detection, epidemiology, disease loses, prevention, control etc. With advances made in the molecular aspects of different viruses, the misunderstanding of viruses as only the pathogens are slowly being erased out. Understanding of molecular architecture, working system coupled with biochemistry, host parasite interaction has opened a new dimensions of plant viruses hitherto unimagined. Manipulation of genomes of viruses of became possible employing the toolkits of modern molecular biology as desired. This has opened new vistas in the field plant virology. Plant viruses are proving to be ideal candidates as much as bacteria and yeasts. Biotechnological potentials of engineered plant viruses and their derived products like virus-like particles (VLPs) are rolling out in a range of fields like genetic engineering. Agriculture, medicine and nanotechnology. In this article made an attempt to bring forth the recent biotechnological applications of plant viruses and VLP's.

FUTURE SCOPE

Future research is likely to open new vistas with regard to plant viruses.

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