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Review

Understanding of Research Milestones Embedded Within the History of Plant Viruses Especially Tobacco Mosaic Virus

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Abstract: Since the discovery of plant viruses, their profound impact on plant sciences and human society has driven extensive research efforts to understand their nature and develop management strategies. This review examines the evolution of plant virus research across six distinct phases, highlighting advancements in biochemical, structural, and molecular characterization. The first phase marked the identification of plant viruses as novel pathogens, introducing them as a unique area of study. The second phase explored the biological aspects, including modes of transmission and the role of vectors in virus spread. The third phase focused on biochemical and biophysical approaches to purify and characterize plant viruses, laying the foundation for structural virology. The fourth phase introduced molecular biology techniques, elucidating viral replication, heredity, and host interactions at a molecular level. In the fifth phase, molecular genetics advanced our understanding of viral genomes, enabling gene mapping, cloning, and silencing approaches to study gene function and pathogenicity. Finally, the sixth phase emphasized modern diagnostic and management strategies, including advanced detection methods, genetic resistance, and integrated approaches for virus control. Together, these phases represent a comprehensive trajectory of plant virology research, underscoring its relevance to global agriculture, food security, and sustainable crop protection strategies. This review not only provides a historical perspective but also highlights the importance of continued innovation in combating plant viruses and mitigating their impact on crop health and productivity.

***Keywords*:** Tobacco Mosaic Virus, plant virus, history, molecular genetics, molecular mechanism, diagnostics, and management

Highlights:

- Presented the historical landmarks of plant virology since 1,800s.
- Describe the story how the plant virus was discovered and identified.
- Described the techniques utilized to unfold the mystery about viruses.
- Briefly outlined the characterization of plant viruses.
- Outlined the diagnostics and detection methods for the plant viruses.

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1. Introduction

A virus is an ultramicroscopic nucleoprotein entity that becomes active only when it enters living host cells. Plant viruses or phytoviruses are those viruses that are present within the plant systems. The areas of study that encompass plant viruses and their attributes are broadly termed plant virology. Plant viruses cause diseases in plant species including cultivated cereals, pulses, vegetables, flowers, fibers, and fruits which impact the yield and quality of production worldwide [1]. The increasing global trade, insect vector movement, and plant diversity have resulted in the spread of viruses across the planet. The rapid growth of the human population coupled with climate change also has a massive effect on the evolution of viruses and their vectors and interaction with the host plants. The evolution of plant viruses is a continuous process that leads to the evolution of new viruses. These new viruses may cause the unexpected yield loss [2-3].

Despite decades of research, the complete understanding of plant-virus interactions remains a challenge, as every stage of plant growth and every plant part is susceptible to viral infection. Therefore, plant viral diseases pose a significant threat to meeting global food demands. This review addresses critical research questions, including: What are the key stages in the evolution of plant virology as a discipline? How have technological advancements contributed to our understanding of plant viruses? And what strategies are most effective for virus detection and management?

The establishment of plant virology as a scientific discipline began with the discovery of plant viruses by Mayer, Ivanowski, and Beijernick, marking the first step in understanding the complex interactions between plants and viruses [4]. The viral disease was initially understood only by the visible symptoms on the plant which were transmitted from an infected plant to a healthy plant by various abiotic and biotic agents. The biotic agents as insect vectors were found to be the most common [5]. For many years, scientists have been studying the different viruses based on the only visible symptoms of the plants. However, this proved to be a tedious task as it was hard to predict because similar symptoms were produced due to other pathogens or also due to nutrient deficiencies [6-7]. Tobacco Mosaic Virus (TMV) was the first plant viruses which were crystallized by Stanley. The study of TMV becomes a model to understand the biochemical, genetic, and molecular aspects including the life cycle of other viruses [8]. The outcome of these studies was utilized to develop the diagnosis and detection methods for the plant virus. Routine viral diagnosis methods were developed like enzyme-linked immunosorbent assays and polymerase chain reaction and advanced methods. Some advanced methods were also developed that offer precise detection such as loop-mediated isothermal amplification, recombination polymerase amplification, biosensor-based, microarray-based technique, and next-generation sequencing methods. The next-generation sequencing has become the most remarkable because it is capable of providing information on virus variants too [9]. The early and accurate diagnosis of plant viral diseases is critical for the management of plant viruses to minimize yield loss. So far, no potent cure is known for plant viruses; however, losses can be minimized by practicing precautions like methodological cultural practices, or the use of virus-resistant seeds. Recently developed techniques, CRISPR-based genome editing, and Ribonucleic acid (RNA) silencing are very useful and promise to generate virus-resistant crop plants [5].

This review provides a comprehensive overview of plant-virus-vector interactions and viral diseases, emphasizing their role in understanding plant virus biology. It aims to highlight the development of virus-specific detection methods and effective management strategies. By presenting the chronological progression of plant virology research, this review also explores how advancements in biochemical, molecular, and genetic techniques have shaped our understanding of plant viruses and their interactions with host plants.

2. Milestone of plant virology

We present the milestones of plant virology in order along with developed research tools, and methods of each age such as traces of plant viral diseases, discovery of the causative agents, discerning the biology of new agents, purification and characterization of viruses, unravelment of the molecular nature and mechanisms of viruses, understanding of molecular genetics of viruses, and development of modern diagnostic techniques and viral disease management strategies.

2.1 *The proto-scientific era-earliest traces of plant viral diseases*

Viral diseases in plants had existed for centuries, however, only in the late 19th century, viruses were proposed as causative agents. Later by the 20th century, the nature of viral pathogens was established. The typical yellowing symptoms on leaves of *Eupatorium lindleyanum* were the oldest reference mentioned in a poem dated back to 752 A.D, written by a Japanese Empress named Koken. Later, it was translated into English by T. Inoye [10]. In the 16th century, color-breaking symptoms on tulips flower were reported as an ornamental beauty in western Europe. In 1576, a Flemish botanist, Carlos Clausius became the first scientific person to describe variegations/striping symptoms on the colored tulip petals. The tulip petal break disease a viral disease had been documented in Holland. Thanks to the tulipomania that existed in the 17th century, which led to an exorbitantly high price for striped bulbs and their paintings. This art and documentation preserve later paved the way for history. In 1692, the yellow stripe disease of Jasmine (now known to be caused by Jasminium mottle virus) was reported, as one of the earliest documents on an experimental transmission of a virus. In 1714, Lawrence had described that this stripping could be inherited via the grafting of jasmine plants on sap exchange. A severe potato leaf roll outbreak and peach yellows were seen in Great Britain and the USA in 1770 and 1791, respectively. In 1869, ablution leaf variegation was seen in France and Belgium. In the 19th century, the sereh disease was found in sugarcane in Java. All these mentioned diseases are known to be caused by plant viruses.

2.2 *Discovery of the causative agent for the suspected symptoms (1882-1900)*

The plant virology era began precisely in the late 1870s, with the Tobacco Mosaic Virus (TMV) and its disease being studied in tobacco plants. This disease was prevalent in Holland and was responsible for severe growth retardation and huge yield losses that render the extremely bitter taste the tobacco also. The Dutch farmers approached Adolf Eduard Mayer (1843-1942), a German chemist and director of the Agricultural Experiment Station at Wageningen, Netherlands (since 1879), and requested to find a cure for this disease. He immediately started an investigation whose etiological agent was unclear at that time. Mayer described that leaf curling is an early and characteristic symptom that makes tobacco unsuitable for cigarette manufacturing. Mayer named this disease Mosaikkrankheit/tobacco mosaic disease because of its characteristic appearance like light and dark green pattern on the leaf lamina of tobacco [11].

Mayer prepared juice extract from infected tobacco plants and rubbed it onto healthy plants, which developed similar mosaic symptoms, suggesting the disease was transmissible [11]. He further hypothesized that the symptoms could spread experimentally, observing that the disease moved from the inoculated leaves to other parts of the plant. Despite applying Koch's postulates and using an optical microscope, Mayer could not identify the pathogen. He then heated the sap at 80 °C, which destroyed the infectivity, leading him to conclude that a small bacterium might be the cause [11].

In 1891, Dmitri Ivanovsky, a Russian botanist, used an unglazed porcelain filter (0.1 to 1-micron pore size) to study the unknown agent [12]. By passing juice extract from infected plants through the filter, which excluded bacteria, and inoculating healthy plants, Ivanovsky found the extract remained infectious. This led him to propose that the agent was a soluble toxin [13].

In 1898, Martinus Beijerinck, a Dutch soil microbiologist, repeated Ivanovsky's experiments and confirmed his findings. Beijerinck used a similar filtration method but added agar to check the filterability of the agent. He observed that the infectious juice diffused through the agar, indicating the agent was not particulate but rather a fluid. He concluded that the agent was smaller than bacteria and described it as contagium *vivum fluidum* (infectious living fluid). This experiment introduced the term "virus" and established TMV as the first known plant virus [14].

The debate between Ivanovsky's concept of a soluble toxin and Beijerinck's notion of a living fluid had significant implications for the foundation of virology. While Ivanovsky's toxin hypothesis was rooted in the idea of a non-living chemical agent, Beijerinck's living fluid concept suggested that the causative agent had properties akin to living organisms, such as the ability to replicate and infect. This shift in understanding paved the way for the development of virology as a distinct scientific field, emphasizing the importance of studying entities that straddle the line between living and non-living. Beijerinck's findings marked the beginning of the recognition that viruses were not just chemical toxins but infectious agents with biological properties, thus setting the stage for future research into the biology of viruses and their role in disease. Beijerinck's work led scientists to conclude that this new pathogen was soluble, ultramicroscopic, smaller than bacteria, and could not be cultured on artificial media, thereby disproving Koch's postulates.

2.3 *Discerning the biology of new agent (biological age 1900-1950)*

Scientists were enthusiastic to decipher the identity of new agents employing a variety of methods like the recreation of disease symptoms through sap inoculation, grafting, early characterized ultrafiltration study to exclude the possibility of bacterial or fungal pathogens, and a standard light microscope to identify inclusion bodies. Many studies were performed to reveal the nature of viral diseases with mosaic and mosaic-like symptoms. These studies included as following, examination for altered phenotypic changes, symptom analysis by microscope, and physiological changes on tobacco and on other crops producing similar mosaic symptoms (Figure 1).

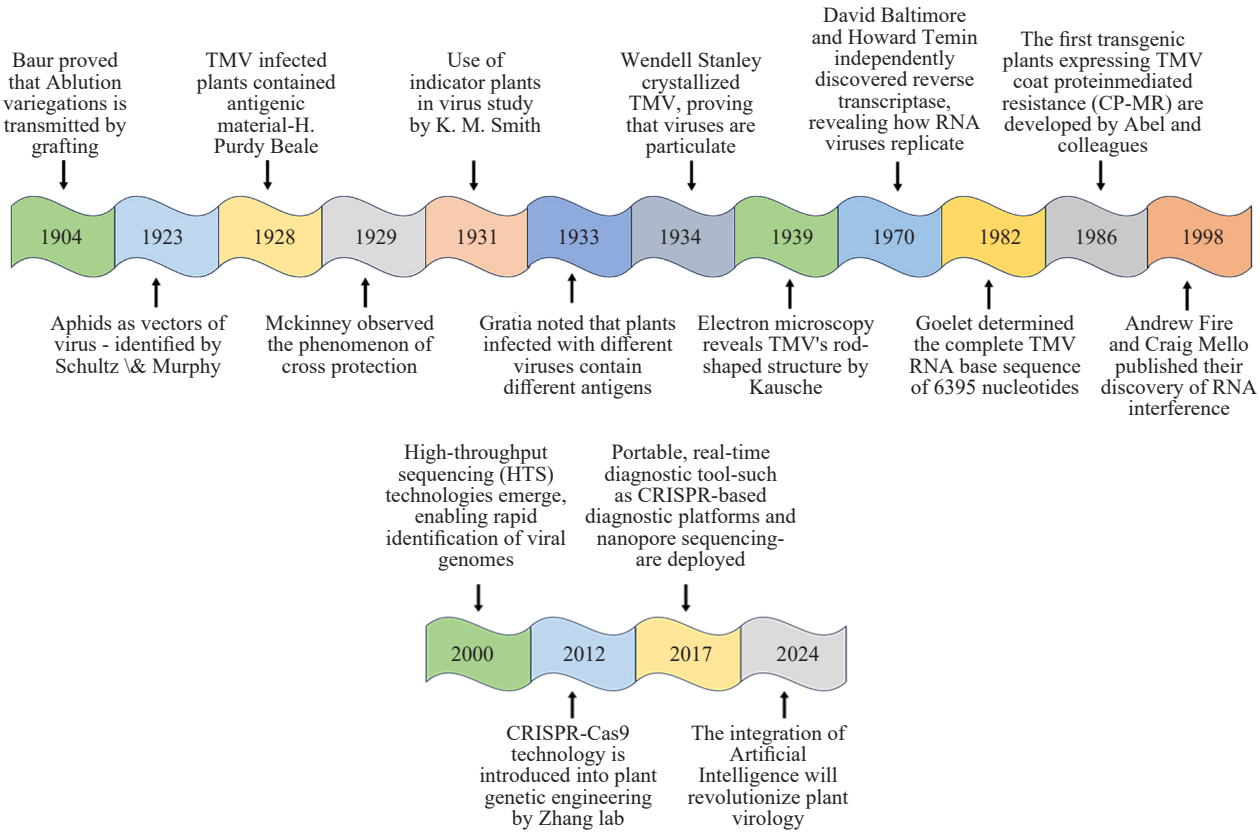


Figure 1. The time segments and technological innovations represented in the timeline, providing context for the progression of plant virology research and the strategies used to manage viral diseases in agriculture

2.3.1 *Historical records of disease transmission*

How the plant viral diseases transmitted from one plant to another was another unanswered mystery. In early 1901, it was revealed that insects play a crucial role in plant virus transmission and epidemiology. Hashimoto from Japan demonstrated the relationship between the plant virus causing rice dwarf disease and an insect leafhopper *Nephotettix apicalis*. It was shown that leafhopper *Eutettix tenella* from an infected plant when fed onto a healthy plant for 5 min. Then the disease would develop [15]. Very soon the leafhoppers [16-17], aphids [18-19], beetles [20], thrips [21], whiteflies [22], and mites [23], were identified which transmit the plant vial disease. Later, the transmission of plant viruses was also reported from seed [24], propagative materials, and pollen [25].

2.3.2 *Discovery of cross-protection*

The principle of cross-protection was introduced by McKinney. He observed that tobacco plants failed to reproduce the characteristic yellow mosaic symptoms upon challenging plant extract sap containing tobacco yellow mosaics [26].

Tobacco plant if initially inoculated with a mild strain of potato virus X then plant observed resistant from subsequent inoculations with severe strains of potato virus X (PVX).

2.3.3 *The development of biological assays for a plant virus*

The more than one virus can infect the same host and can produce the same disease symptoms. In 1925, James Johnson demonstrated that young tobacco leaves showed apparent symptoms when inoculated with the sap prepared from healthy potato plants. Thus, James concluded that a virus can also be carried within its host without producing any symptoms. The researcher, Holmes injected the sap prepared from an infected pathogen into tobacco leaves and observed that lesions were developed as per virus concentration [27]. In 1931, Smith inoculated the potato virus X (PVX) on tobacco which showed the potato mosaic disease symptoms, so he named tobacco crop as an indicator/ differential host. With this study, tobacco became the first and ideal host candidate for PVX [28]. He also demonstrated that infection with two different potato viruses X and Y, in combination could result in severe viral diseases. In another experiment, Smith inoculated the leaves of the Datura (*Datura stramonium*) plant with mixed potato viruses X and Y and observed that only virus Y was identified in young growing leaves. Smith called such crops a filter plant that can filter virus Y from virus X as it travels faster to the growing points. Smith studied the selective multiplication property of vector for separating virus Y from virus X because virus Y is only transmissible through the insect vector aphid *Myzus persicae* vector but not the virus X [28].

2.3.4 *Vector-mediated plant virus transmission*

The concepts of virus-vector interaction for transmission of the virus in a persistent and non-persistent manner were introduced by Watson and Roberts [29]. Four hypothetical mechanisms such as through mechanical, biological, salivary apparatus, and regurgitation are suggested for the transmission of viruses by aphid vectors in a nonpersistent manner [30-31]. The *Myzus persicae* and *Brevicoryne brassicae* vectors take only 1-2 minutes to transfer the virus [32-33]. Nematode as a vector for virus transmission was also discovered which transmits the soil-borne plant virus [34].

The vector-mediated transmission of viruses was studied as below:

I. The retention test: Usually, the stylets of an aphid shed during the molting stage and then replaced by new ones with each advancing instar, therefore, the viruses cannot be transmitted. This test was carried out with the aphid *Myzus persicae* with beet yellow net virus on the turnip plants.

II. Artificial style wetting test: The stylets of aphids are dipped into a concentrated TMV solution and allowed to feed on test plants. The absence of virus symptoms was evidence that viruses were not transmitted via the stylet.

Testing biological transmission of plant viruses: Electron micrograph analysis provided the first logical evidence for the multiplication of rice stunt virus in an arthropod insect vector tissue [35]. Subsequently, Herold published electron micrographs that show the virus-like particles arranged in a regular array of microcrystal size of 242 × 48 microns in length and width concentrated in the cytoplasm in the vicinity of the nucleus of maize leaf cells. The leafhopper *Agallia constricted* species was shown to potentially transmit the two unrelated plant viruses, the wound tumor virus and the potato dwarf virus [35]. The leafhopper *Dalbulus maidis* a vector for corn-stunt virus, exhibited a property of survival for only 4-days on healthy asters but longer periods on infected asters [36]. In the case of nonpersistent, viruses don't pass through the interior of the vector but get associated with the anterior portion of the feeding canal, thus becoming noncirculative or stylet-borne. The mouth part of a vector aphid interacts non-structural protein known as the helper component protein (HC-Pro) of potyvirus, which is required for virus transmission [37-38]. The Hc-Pro P2 and P3 of Caulimovirus bind to aphid mouthparts persist for for several hours and transmit the virus by an attachment mechanism. For transmission [39-40]. The 2b protein Tobraviruses was required to interact with trichodorid nematode. Other stylet-borne viruses such as Cucumoviruses require only the viral coat protein for aphid-mediated nonpersistent transmission [41]. Tospovirus of the *Bunyaviridae* family can replicate in a thrips vector, which propagates in a vector and transmits the virus [42-43]. The luteovirus persists for a long period in aphid vector but without replication, as circulative non-propagative transmission [44-45].

Later, Sylvester recognized a semipersistent form of virus transmission. The persistent virus passes through the insect gut wall and hemocoel to accumulate in the salivary glands of the vector, thus becoming calculative. Circulative viruses are of two types, one that can replicate in the insect vector (termed as propagative) and the other that doesn't

replicate within the insect vector (termed non-propagative).

The plant host, vector, and virus form the complex triad and develop the typical virus disease in different crops. The generalised cycle infection of plant virus starts with the inoculation of the virus into the plant by a suitable vector or mechanical. Then, the virus uncoat to release the nucleic acids which undergoes transcription and translation for replication and multiplication process using the plant host resources. Once the necessary proteins for replication build up, the viral nucleic acid now replicates. The progeny viral nucleic acids can be transported intercellularly to nearby locations or to distant sites by long-distance transport. Once the viral coat proteins form, progeny viruses get assembled and matured. Finally, the vector again acquires the progeny viruses while feeding and the cycle thus continues.

2.4 *Purification and characterization of the virus-biophysical/ biochemical age (1930-1968)*

This age started in the 1930s and marked the virus purification and characterization at the biochemical level. The methods for virus purification became available that helped to study the size and structure of the virus more precisely. TMV was first time precipitated from crude sap of infected tobacco with specific salts, such as lead acetate and safranin with acetone by the Vinson and Peter, at Philadelphia Boyce Thompson Institute. The acetone helped to concentrate the virus and safranin and other salts improved the procedures to obtain a pure form of virus, and amyl alcohol to elute the virus. In 1931, when the virus was purified with acetic acid and acetone, they resulted in some infective crystals that failed to retain the infectivity when crystallized. The precipitate obtained showed the properties of a protein when moved under an electric field. Purdy Beale, from the Philadelphia Boyce Thompson Institute successfully raised antibodies against TMV in rabbits, thus reaffirming the proteinaceous nature.

2.4.1 *Crystallization of TMV*

Stanley's experiments in 1933 and 1934 showed that TMV infectivity was lost in the presence of pepsin, confirming its proteinaceous nature. He also found that the inactivation of infected tobacco juice followed the inactivation pattern of regular proteins across varying pH levels. In 1935, Stanley first crystallized TMV into needle-like structures by adding ammonium sulfate to concentrated infected tobacco juice, revealing crystals approximately 0.03 mm in length [46]. Svedberg, using these crystals, studied the virus protein's molecular weight through ultracentrifugation. Wycoff and Corey, with TMV samples from Bawden and Pirie, confirmed TMV's rod-shaped structure through X-ray diffraction [47]. In 1941, Bernal and Fankuchen improved this analysis, determining the diameter of TMV rods at about 15 nm and their length at ten times their width. Bawden and Pirie suggested that TMV was a nucleoprotein with rod-shaped particles [48]. They also confirmed that Stanley's crystals were para-crystals, regular only in two dimensions. In 1938, Bawden and Pirie, with Bernal and Fankuchen, crystallized the tomato bushy stunt virus and obtained the first 3D image of a spherical virus [49].

In 1950, Watson's X-ray studies indicated that TMV was likely helical, though he couldn't determine the number of subunits per turn. In 1956, Rosalind Franklin, using re-polymerized nucleic acid-free TMV particles, produced high-quality X-ray crystallography images, providing the first true picture of TMV's quaternary structure [50]. Franklin and Caspar confirmed TMV's helical structure and showed that it was hollow, not solid [51]. They also revealed that TMV's single-stranded RNA spirals along the inner surface of the protein capsid, resembling a thread spiraling inside a donut hole [50].

2.4.2 *Blow to the nature of TMV*

Stanley had performed the biuret test and reported positive for protein and Fehling and Molisch tested negative for carbohydrate with TMV. He found approx. 20% nitrogen in the crystals and immediately declared to the world that the precipitate was indeed a protein. He also showed that the crystals if redissolved in the suspension form (up to a billionth dilution) rubbed onto healthy plants and produced the disease symptoms. Thus disproving the earlier notion of the virus being a living soluble liquid. Stanley postulated that viral agents were an autocatalytic protein/enzyme capable of multiplying in living cells [46]. The Stanley studied proteinaceous nature of TMV crystal became vogue very soon. Bawden and Pirie at the UK Rothamsted Institute showed the invariable presence of 2.5% carbohydrate and 0.5% phosphorous besides protein, enation, and aucuba strains of TMV [49]. Both also demonstrated that the carbohydrate

and phosphorus components of TMV could be separated by heat denaturation. Despite several efforts, Bawden and Pirie could never obtain a phosphorous fully active virus preparation. The presence of phosphorous in purified virus precipitate indicated the presence of nucleic acid as an integral component of TMV. The spectrophotometric analysis showed an absorption maximum corresponding to nucleic acid. In 1939, Max Lauffer, along with Stanley, successfully separated the protein and nucleic acid constituents of TMV. Thereafter, HS Loring, a post-doctoral student of Stanley, characterized this nucleic acid to be RNA by treating TMV with ribonuclease enzyme and showing a decrease in TMV infectivity.

2.4.3 *Investigation of TMV by electron microscope*

In 1951, density gradient centrifugation pioneered by K. Brakke became a very critical technique for the isolation of plant viruses. This technique involved layering of crude infectious plant extract on the top of a gradient of varying concentrations prepared of high-density salts like cesium/lithium chloride and centrifuging it at high speed approx. 50,000 rpm [52]. The virus particles were localized in the tube at a position where its density matched with salt, and the same was withdrawn using a needle syringe.

E. Ruska and Von Borris employed by Siemens company in Berlin, designed an electron microscope having a resolution of 10 nm and magnification of up to 1 lakh, that made possible the direct visualization of TMV. In 1939, the first electron microscopic image of TMV came, which supported the rod-like morphology of TMV [54-55]. In 1944, Williams and Wycoff obtained a high-resolution image of TMV under an electron microscope following a specimen preparation technique called shadow casting. The shadow casting technique employs the vaporization of heavy metals like lead, gold, or palladium and their deposition on the particle, thus resulting in a shadow of the particle. These electron microscopic images also confirmed TMV to be of rod shape with a diameter of 15 nm and length of 300 nm [53] (Figure 2).

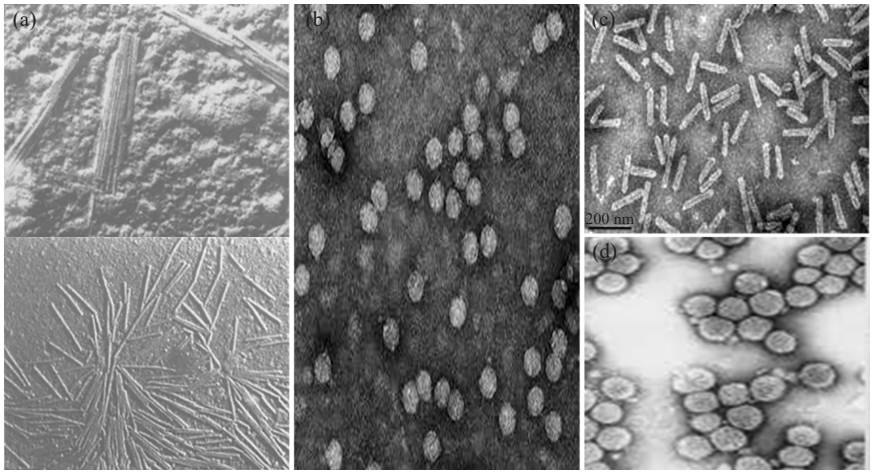


Figure 2. The sketch illustrates the morphology of different plant viruses as observed under an electron microscope. (a) Tobacco Mosaic Virus [56] (b) Luteovirus [57] (c) Banana streak virus [58] (d) Tomato spotted wilt virus [58]

2.4.4 *Morphology of plant viruses*

In 1932, Takahashi and Rawlins at the University of California, Berkeley, carried out the biophysical experiment and provided the earliest information on the shape of TMV by allowing the TMV-rich crude extract juice sample to flow between cross Nicol prisms. Flowing TMV extract bestowed the double refraction pattern that predicted only rod-like particles to be present, as reported earlier by Freundlich [59].

The electron microscope and X-ray diffraction methods enabled to study of the size, shape, and architecture of several plant viruses. The size of plant viruses ranged from 17 nm (alfalfa mosaic virus) to 1,250 X 40 nm (Beet yellow virus) as per characteristics of their species. Under an electron microscope, they were observed as spherical (e.g.,

Tomato Spotted Wilt Virus), Rod-shaped (e.g., Tobacco Mosaic Virus), and Bacilliform (e.g., Banana streak virus). Based on the architecture/symmetry of the capsid, the plant viruses were classified into helical (cylindrical or elongated) and cuboidal (rounded or polyhedral). Helical forms are an isometric, whereas cuboidal forms are isometric in nature. The elongated viruses were further differentiated into rigid Rods (e.g., Tobacco rattle virus), flexuous Rods (e.g., Potato virus X), and Filamentous Rods (e.g., Rice stripe virus). Capsid protein, a protective coat outside the nucleic acid core, was shown to be made up of subunits called capsomeres. Capsomeres were known to be arranged in either helical or geometric forms. Most of the plant viruses were naked in nature (without a viral envelope) with the exception of bunyaviruses and rhabdoviruses (Figure 3).

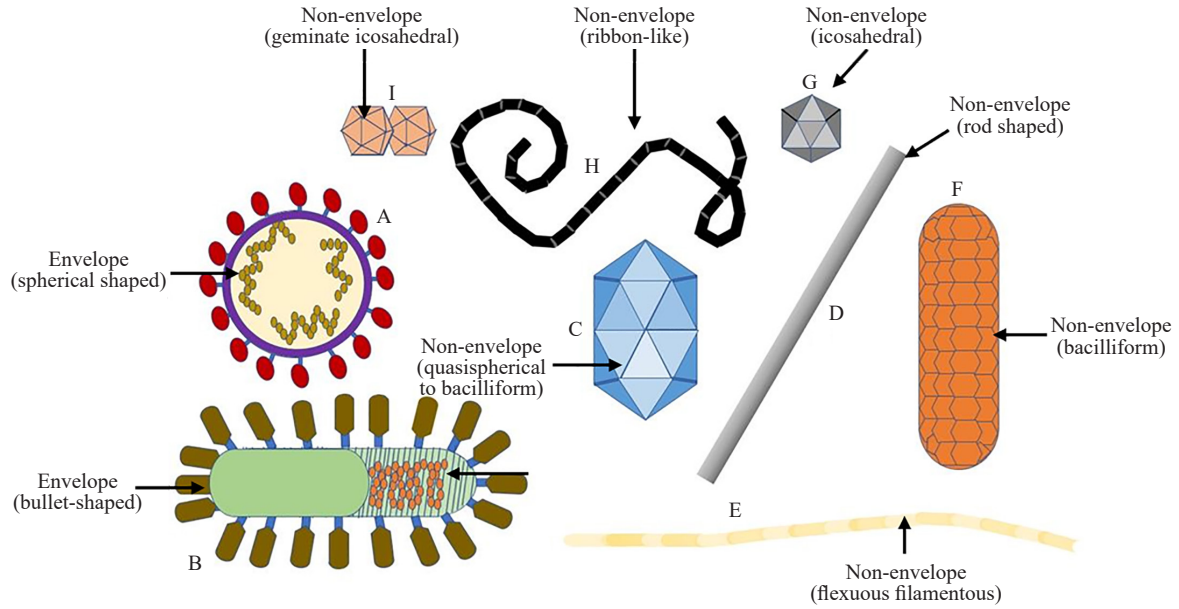


Figure 3. The schematic illustrates the morphology of plant viruses: (A) Tomato spotted wilt virus (spherical), (B) Rhabdoviruses (bullet-shaped), (C) Alfamovirus (quasispherical to bacilliform), (D) Tobacco Mosaic Virus (rod-shaped), (E) Potyvirus (flexuous filamentous), (F) Badnavirus (bacilliform), (G) *Partitiviridae*, *Bromoviridae*, and Caulimovirus (icosahedral), (H) Tenuivirus (ribbon-like), and (I) *Geminiviridae* (geminate icosahedral)

2.5 *Unravelment of the molecular nature and mechanisms (molecular age) (1943-1995)*

The molecular age was the most crucial age in the history of plant virology. After knowing the constituents of TMV, scientists worked on decoding the basis of viral infectivity. It was essential to understand whether it was the nucleic acid that dictated the protein or vice-versa.

2.5.1 *Splitting and reconstitution of two TMV strain*

The biological differences between the different strains of TMV were initially attributed to the differences in their nucleic acid. In 1943, Schramm did a simple experiment to find out which component of TMV was required for reproduction. He first split TMV into pieces with a slightly alkaline solution, and then pH moved back to an acidic condition. He observed that disrupted TMV pieces reassembled back to their original form. Schramm reported that disrupted pieces were incapable of producing new TMV, but the reaggregated forms could produce new TMV rods. Further, Schramm prepared an RNA-free TMV protein by treating it with nucleosidase enzyme from the calf intestine. He further recorded that even though the virus remained crystallizable, it had lost its infectivity. Thus, he asserted RNA is the genetic molecule present within the TMV (Figure 4).

The year 1949 marked the isolation and crystallization of the turnip yellow mosaic virus by Roy Markham and K. Smith. Even though the crystallized virus exhibited homogeneity in migration under regular electrophoresis, the

sedimentation studies using an analytical ultracentrifuge indicated the presence of two components within two-layered fractions. The heavier fraction comprised 70-80% of the total material was thicker containing nucleoproteins that consisted of 37% RNA, while the lighter fraction was solely proteins [60]. Experimenting further, they proved that only the RNA-containing fraction was infectious. Although this was a breakthrough, they weren't very decisive on the fact that RNA alone was sufficient for infection [60].

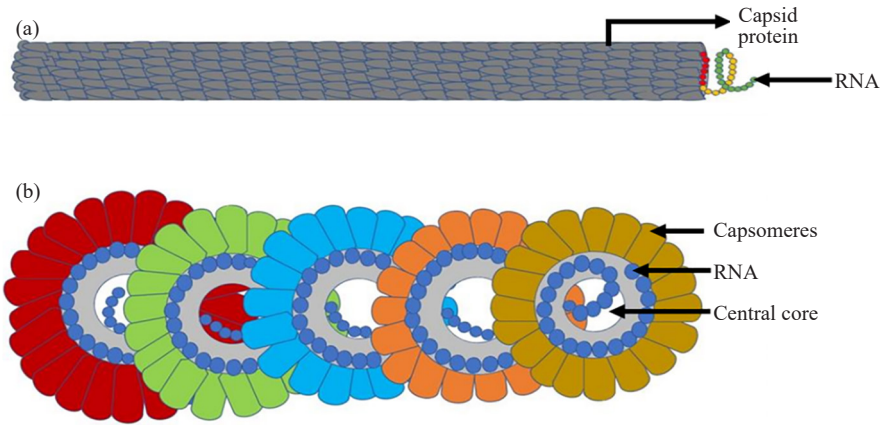


Figure 4. The sketch illustrates the Tobacco Mosaic Virus structure. (a) A detailed representation of the virus, showcasing its helical capsid architecture. (b) A sectional view of the helical capsid, highlighting the internal arrangement of RNA and protein subunits

In 1957, F. Conrad and B. Singer performed reconstitution experiments with 2 different strains of TMV, the first TMV strain produced green mosaics and the second TMV strain produced the ring spot lesions. They first split the two viruses; isolated RNA and protein components by SDS disruption and alkali/acetic acid treatment, respectively, and then reconstituted a hybrid virus using RNA and protein of each other. These chimeric viruses were then used to infect tobacco leaves separately. The progeny analysis revealed that the daughter viruses' phenotype and genotype were identical to the parent strain from which RNAs had been obtained [61]. Thus, it was re-emphasized that the specificity of viral proteins was determined by RNA only, and proteins do not contribute to any of the genetic information [61].

2.5.2 *Experiments with TMV give a breakthrough*

Harris and Knight, in 1952, devised a new experiment wherein they first developed a dethronized TMV by treating TMV with carboxypeptidase (thus removing the C-terminal threonine of TMV coat protein). Experiments showed that these coat protein variant TMV possessed identical infectivity with that of original TMV; therefore, there was no loss of infectivity [62]. Further, dethronized TMV, when inoculated to the plant, produced progeny of the usual type (those with C terminal threonine). This experiment gave the idea that viral RNA controlled the specificity of the viral protein [63].

Pure RNA and pure protein fractions of TMV were obtained by detergent and alkali treatment, respectively. Each fraction, when individually tested for infectivity, was found to be non-infectious. However, by mixing both RNA and protein fractions, an infectious nucleoprotein with the properties of TMV was created. This claimed to be the first artificial creation of the virus by associating two kinds of molecules [64].

Having the doubts still unresolved, Alfred Gierer and Gerhard Schram in 1956 had efficiently separated RNA from protein from TMV using a water-saturated phenol solution where RNA remains in the upper phase, while the protein precipitates with phenol. TMV treated with acid or alkali also yielded the viral protein in its native form. When RNA and protein constituents were inoculated separately into the tobacco leaf, only RNA was found to produce the characteristic lesions but with a lower strength/index of infectivity than that of intact TMV. This result indicated that RNA was labile when alone, and RNase-treated RNA also failed to produce any lesions [65]. Further, no loss of infectivity was observed for RNA even after treatment with antiserum or by undertaking ultracentrifugation (to confirm the exclusion of TMV particles). However, RNase-treated intact TMV particles showed the local lesion upon

inoculation. Thus, Gierer and Schram stated that infectivity was not created de novo by the mixing of 2 different components as reported by Fraenkel and Conrad instead, it was solely the inherent function of the labile RNA, which when associated with protein, protects it from inactivation. Thus, they concluded that the viral genome of TMV must be comprised of RNA [65].

In 1961, Sugiyama and Frankel Conrat developed techniques to understand the base composition of TMV RNA. TMV RNA genome was first digested by the ribonuclease T1 enzyme and then treated with phosphodiesterase generating several unique oligonucleotides [66]. They determined that the 3' end of RNA was made up of unphosphorylated adenosine residues and a 7-methyl guanosine cap at the 5' end [67-68]. In 1966, this observation was later confirmed by Stein Schneider. In another experiment, TMV RNA was treated with phosphodiesterase enzyme which hydrolyse the 5' cap, and this TMV RNA without the cap lost the infectivity [67-68].

In 1972, Jackson purified the polyribosome from TMV-infected tobacco tissue and observed that small TMV RNA was attached to it. This study indicated the participation of TMV RNA in the translation process *in vivo*. This attached TMV RNA was found expressing the coat protein (CP) in an *in vitro* study which indicated the presence of a sub-genomic RNA. This RNA was characterized to have a 5' end capped same as genomic RNA [67-68]. In 1976, wheat germ extract was used as an *in vitro* translation system to study the protein synthesis from the TMV RNA genome. It encoded only two proteins of molecular weights of 130 kDa and 180 kDa, thus suggesting their presence towards the 5' end of TMV RNA. Both proteins were suspected to be involved to play a role in the TMV life cycle [69]. However, despite several efforts, the CP translation was not achieved. It was hence concluded that only full-length TMV RNA was not an efficient template for CP translation. In 1977, Beachy and Zaitlin reported the discovery of RNAs of discrete weights like 0.9-1.6 MDa (termed I-1 RNA/intermediate RNA-1) and 0.68 MDa (termed I-2 RNA /intermediate-length RNA-2). The purified I-2 RNA, when translated *in vitro*, encodes a protein of molecular weight 30 kDa that confirmed that sub-genomic mRNA encodes CP of TMV [70-72].

In 1962, Nirenberg, Conrat, and A. Tsugita introduced the purified genomic RNA of TMV and added it into the cell-free translational system and observed a 75-fold increase in protein production. The supernatant was shown to precipitate with TMV antiserum, thus indicating the synthesis of the TMV coat protein within the cell-free translation system. This experiment also concluded that the single-stranded genome of TMV was (+) sense messenger RNA and revealed the universal nature of codon biology because viral RNA got translated within a bacterial system. The viral genetic code was also verified with the help of TMV coat protein mutants, i.e., by comparing and contrasting the amino acid changes in the mutant and regular TMV [73].

2.5.3 *Development of plant protoplast system to study proteins of TMV*

In 1969, sequencing of the coat protein of TMV was completed by two groups independently, Frankel Conrad and Tsugita Berkeley at the Virus Laboratory, University of California, and H. G Wittmann at the Max Planck Institute of Biology, Tubingen. Wittmann and Braunitzer had first separated the tryptic peptides of coat protein using ion-exchange chromatography and determined the amino acid composition of each peptide, whereas Conrad and Tsugita had used an automatic amino acid analyser machine. Interestingly, both groups concluded that the 158 amino acid sequence was the coat protein of the TMV strain [74-75].

In 1971, Takabe and associates developed tobacco protoplast systems as a live cell model to study the one-step growth curve of plant viruses [76-78]. TMV-infected protoplasts were studied for the first time and showed the proteins of 130 kDa and 180 kDa to be present within them [79]. Upon closer examination, it was found that synthesis of 130 kDa predominates that of 180 kDa. The 180 kDa was expressed because of a read-through skipping of the termination codon of 130 kDa [80]. After the nuclease treatment of whole TMV RNA followed by electrophoresis, the shortest RNA obtained was sequenced to give information on the last 1,000 nucleotides from the 3' end of TMV RNA. This cistron of 1,000 bp coded for the coat protein. Subsequently, the 30 kDa protein was also detected in TMV-infected protoplasts [80]. The proteins of molecular weight 130 kDa and 180 kDa were hypothesized to play a role in TMV replication because they showed significant homology with known RNA-dependent RNA polymerases (RdRp) [81]. In 1986, Ishikawa successfully created mutations at the amber (UAG) stop codon of the gene coding for 130 kDa protein. This mutant produced 130 kDa but not 180 kDa and wasn't infectious, whereas mutants with only 180 kDa retained low levels of infectivity. Thus, a balanced expression of both genes was found to be necessary for the efficient replication of TMV RNA. Finally, Osman and Buck in 1977, had isolated the TMV RNA polymerase complex and showed that 130

kDa and 180 kDa were its constituents [82].

A temperature-sensitive mutant of TMV called Ls1 was used to study the function performed by the 30 kDa protein. Ls1 mutant of TMV replicated and assembled at 32 °C in the protoplast as usual but failed to spread from one cell to another in leaves. The intracellular movement of this mutant virus was possible only at 20 °C and not at higher temperatures. The mutation when analysed was pointed towards a change in the 154th position of the 30 kDa protein from serine to proline. This experiment suggested that regular 30 kDa protein was required for cell-to-cell movement. To further prove the same, transgenic tobacco expressing the wild type 30 kDa protein was used to see if its presence would help/complement the Ls1 mutant to spread cell-to-cell systemically at non-permissive temperatures [83]. The numerous frameshift mutations within the 30 kDa gene were also undertaken and the phenotype of the protein was noticed. Both experiments invariantly proved the 30 kDa protein had a role in virus movement; thus, later, it was known as the movement protein (MP). MP was found to accumulate in large numbers in the infected leaf plasmodesmata. In 1989, Wolf reported that the molecular size exclusion limit for intercellular transport was about ten times higher in transgenic tobacco than in tobacco controls. In 1992, Citovsky deciphered that MP binds to single-stranded nucleic acid and forms an elongated structure. Finally, Heinlein and McLean, in 1995, reported the direct association of MP with the microtubules of TMV-infected protoplasts.

2.5.4 *Nature of plant viral genomes*

The ds RNA genome within plant viruses was first found in wound tumor virus, which resolved into 12 fragments on a 7.5% polyacrylamide gel of 16 mega Daltons [84]. The two sizes described as long and short rods of tobacco rattle virus (a bi/multipartite virus) were discovered with sucrose density fractionation in contrast to TMV, where only one size of RNA molecule was found. Both long and short rods were required to facilitate normal infection and subsequent progeny generation [85]. Other examples include cowpea mosaic virus (bipartite) and alfalfa mosaic virus (tripartite).

Shepherd first reported the DNA genome in the cauliflower mosaic virus while working with the purification of CaMV. He observed that the nucleic acid wasn't hydrolysed by weak acid/alkali treatment (indicating the absence of an RNA genome) and gave a positive diphenylamine test for the DNA. Further, the purified virus, when treated with pancreatic ribonuclease, retained its infectivity, whereas DNase treatment completely abolished the same [86]. Goodman first reported the occurrence of a single-stranded DNA genome in *Geminivirus* through an experiment with exonucleases which showed them to remain insensitive, thus proving that the Geminivirus is composed of a single-stranded DNA with circular (= nonlinear) topology [87-89].

Since the discovery of types of genomes for plant viruses, it was found that plant viruses can have only one nucleic acid, RNA, or DNA but never both. Later, based on the nature of the virus genome, plant viruses were divided into DNA viruses and RNA viruses. The DNA viruses further were split into single-stranded DNA viruses, double-stranded DNA viruses, circular DNA viruses, and linear DNA viruses. Similarly, the RNA viruses were also classified into double-stranded and single-stranded RNA viruses, viruses with a positive sense strand or a negative-sense strand. Within the capsid, the genetic material was shown to be present as a single continuous strand/non-segmented form or as multiple genome segments. In some plant viruses, the genome existes as different segments in more than one virus particle, which is termed as split genome viruses (Figure 5).

Satellite RNA: Basil Kassanis had often observed very small virus particles within the cultures of large Tobacco Necrosis Virus (TNV) 30 nm size. It was almost spherical, with a size of roughly 17nm, and was able to replicate only in the presence of the large TNV virions (which acted as a helper virus) and thus got termed satellite viruses [90]. In 1971, Schneider found another subviral agent, i.e., naked RNAs, in close association with preparations of numerous other viruses and termed them as satellite RNAs [91]. The satellite RNA doesn't code a capsid of its own.

Viroid: Viroid (Potato Spindle Tuber Viroid) was discovered while examining the cause of the famous Potato spindle tuber disease as a free RNA molecule (without a capsid) of smaller genome size and molecular weight between 25-110 KDa [92]. After a year when citrus exocortis disease was studied, its causative agent was found to exhibit similar properties to that of Potato Spindle Tuber Viroid [93].

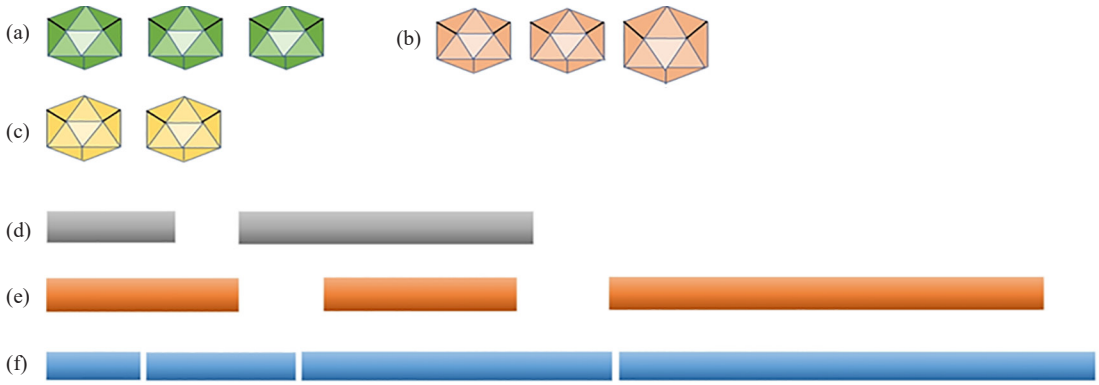
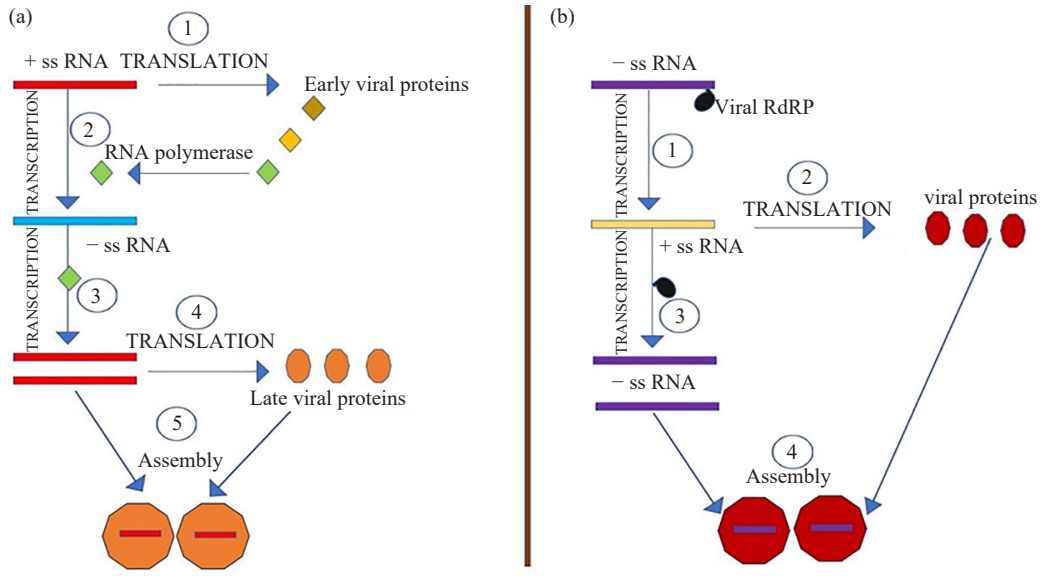


Figure 5. Sketch represents the multipartite viruses. (a) *Cucumovirus* and *Bromovirus* (b) *Ilarvirus* (c) *Comovirus* (d) *Tobravirus* (e) *Pomovirus* (f) *Benyvirus*

2.5.5 *Replication of plant viruses*

In 1963, the first viral RNA-dependent RNA polymerase (RdRp) protein of 34.5 kDa was identified and characterized by the Brome Mosaic Virus, which infects barley seedlings [94-95]. To understand the recruitment of RdRp to the RNA, the DNA templates were generated wherein it was shown that the presence of one additional nucleotide at the 3'end had to be an initiation nucleotide [96]. On the other hand, as reported by Collomer, ds RNAs of Cucumber mosaic virus (CMV) and its satellite had unpaired terminal guanosine, which, together with other proximal downstream elements, were mandatory for its RdRp recognition.

Plant viruses can have either dsDNA or ssDNA genomes. The replication of CaMV requires a reverse transcriptase encoded by ORF-V of the circular dsDNA genome wherein the full-length RNA transcript behaves as an RNA intermediate. On the other hand, the geminivirus ssDNA genome undergoes a rolling circle mechanism for replication with the help of just a single viral encoded replication initiator protein (Rep) [97-99] (Figure 6).



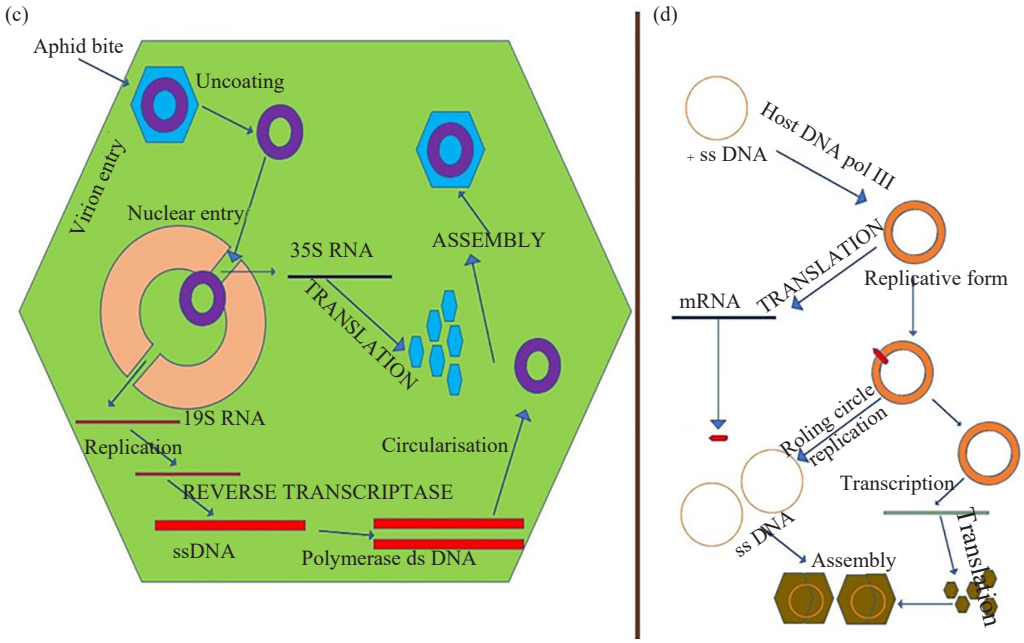


Figure 6. A generalized diagram drawn to show the replication cycle of different plant viruses; (a) replication cycle of positive-sense single-stranded RNA viruses. (b) Replication cycle of negative-sense single-stranded RNA viruses. (c) Replication of double-stranded DNA RT viruses/ *Caulimoviridae* family. (d) Replication cycle of single-stranded DNA viruses

2.6 *Age of viral molecular genetics (1980-2000)*

2.6.1 *Cloning and sequencing of TMV RNA*

In the 1970s, Baltimore, Temin, and Mizutani discovered the reverse transcriptase enzyme and demonstrated that RNA could act as a template to synthesize complementary DNA; thus, it paved the way for cloning. During this period, the restriction endonuclease enzymes were also isolated and characterized, which enabled the desired modifications with the DNA. The discovery of reverse transcriptase and restriction enzymes helped to clone the complete TMV RNA (Vulgare strain) into the M13 bacteriophage vector. Later, the nucleotides of inserted TMV DNA were sequenced by Sanger sequencing. The sequence was analyzed using bioinformatics tools and determined to be of 6,395 nucleotides. The open reading frame (ORF) region coding for protein was also determined on the genome organization of TMV [80].

The TMV genome was shown to consist of a cap structure at the 5’ end and an untranslated leader sequence of 68 nucleotides that follows the first ORF, which codes for 130 kDa protein. The stop codon of ORF coding for 130 kDa protein was leaky and read through nucleotide kDa protein. The stop codon of the second ORF was further found to overlap with that of the third ORF that codes for the 30 kDa protein. The fourth ORF encodes the 17 kDa coat protein and is located towards the 3’ end of the TMV genome. Hence, it was established that the RNA genome of the TMV common strain encodes four gene products. A sequence of 200 nucleotides of a non-coding region was found at the 3’ end, which could fold into a tRNA-like structure [80]. Next, the complete genome sequence of the tomato mosaic virus strain (ToMV) was made available [100]. In 1984, Wilson had initially proposed a co-translational disassembly hypothesis that predicted the release of coat protein first from the 5’ end of the TMV genome. The hypothesis was later proved with fresh TMV virus-infected plant cells, wherein the release of coat protein was followed by immediate ribosome binding to exposed ORF1 to start translation [101].

2.6.2 *Reverse genetics enters the arena*

The complete amino acid sequences of TMV-coded proteins were discerned by bioinformatic tools but the functions of proteins other than the coat protein remained obscure as pure proteins from infected cells could not be obtained in high concentrations. The only solution to this problem was to create a system in which a gene could be manipulated by artificially inducing changes at the genotype level and then analyzing the phenotype. The first time

an RNA manipulation system with brome mosaic virus was established wherein the infectious RNAs of BMV were transcribed in vitro from their cDNA clones [102]. This method was later employed successfully for TMV, and an infectious TMV RNA was synthesized using a full-length genome. Initially, infectious RNA was obtained using the *E. coli* RNA polymerase, which later got substituted with T7 RNA polymerase [103]. Manipulation of genomic RNA can be achieved by deletion/insertion (indels) or substitution of nucleotides of cDNA clones which will then be used to study the function of virus-coded proteins.

2.6.3 *Multifaceted roles of coat protein*

The function of the virus coat protein (CP) was to protect the RNA genome, but the multifunctional role of the CP was also established by reverse genetics. A TMV mutant was created lacking the CP gene and was found to be defective in systemic movement and long-distance movement [104-105]. Powel Abel and co-workers in 1986 showed that viral coat protein genes, when transferred into plant nuclear DNA, could confer virus resistance [106]. Transgenic tobacco expressing CP sense RNA showed a remarkable time lag for symptom development after virus inoculation. Later, the transgene resistance mechanism was explored, and the papaya plant resistant to papaya ringspot virus in Hawaii could be developed and commercialized [107-108].

2.6.4 *Virus-induced changes in a plant*

Table 1. Common disease symptoms induced by virus

External symptoms	
Symptom	Description
1. Stunting	Reduction in growth of different plant parts
2. Mosaic	alternate light and dark green patches on leaves and floral parts
3. Vein banding	dark green lines on either side of leaf vein
4. Stripes (monocots) and streaks (dicots)	long narrow yellow, brown or dark bands on leaf lamina, yellow spots
5. Variegation	A pattern of white patches on leaves
6. Curling	Abnormal shoot and leaf bending
7. Enation	Outgrowth from lower surface of leaf vein
8. Blisters	dark green convex raised spots
9. Shoestrings/filiforms	leaf elongation into needle shape
10. Witche's broom	short internodes and small leaves densely packed together without spreading
11. Fruit abnormalities	concentric rings and mottling
12. Tumerous root outgrowths	Bulged out root (hairy or non-hairy)
Internal symptoms	
1. Hyperplasia, 2. Hypoplasia 3. Internal necrosis 4. lignification, 5. Inclusion bodies 6. Tyloses (xylem tube outgrowths)	Internal symptoms arise because of histological changes and can be diagnosed only by laboratory examination.

Table 1. (cont.)

Physiological symptoms	
Symptom	Description
1. Decreased photosynthetic activity 2. Increased respiration rate 3. Increased phenol oxidase activity 4. Decreased activity of growth hormones 5. Increased accumulation of amides	Physiological changes are those changes that has been found only in virus infected plants but not healthy plants.

Plant viruses-induced disease symptoms within plants can be of external, internal, or physiological nature. It is important to give an idea of the same as they aid in the preliminary diagnosis of viral infections (Table 1).

2.6.5 *Current developments and future perspectives in plant virology: focus on Tobacco Mosaic Virus (TMV)*

Recent years have seen significant advancements in plant virology, fueled by innovative technologies and novel approaches to combating viral pathogens. Among the most extensively studied plant viruses is the Tobacco Mosaic Virus (TMV), a model virus that has greatly contributed to the field. Traditional methods of controlling TMV-such as using resistant cultivars or crop rotation-have had limited success due to the virus's high mutation rate and its ability to overcome resistance. However, recent developments in genetic engineering, particularly through CRISPR-Cas9, have opened new doors for combating TMV. For example, editing the TOM1 gene in tobacco using CRISPR/Cas9 has demonstrated resistance by disrupting the virus's ability to replicate and spread within the plant [109]. This approach shows great promise for developing genetically modified crops with enhanced resistance to TMV and other viral diseases, offering a more sustainable and effective solution to viral management in agriculture.

Another transformative innovation in plant virology is the advent of portable, real-time diagnostic tools. CRISPR-based diagnostic platforms and nanopore sequencing technologies enable rapid, on-site virus identification, allowing early intervention and limiting the spread of infection. These diagnostic innovations are particularly valuable for large-scale agricultural operations, where swift action can prevent extensive crop damage.

In addition to CRISPR-based gene editing, RNA interference (RNAi) has been successfully employed to reduce viral load in infected plants. RNAi works by silencing viral genes, thereby preventing the virus from replicating and spreading within the plant. Several studies have demonstrated the effectiveness of RNAi in controlling TMV in tobacco and other crops, underscoring its potential as a sustainable tool for managing viral diseases in agriculture [110-112].

High-throughput sequencing (HTS) and metagenomics have further revolutionized our ability to study plant viruses like TMV. HTS technologies enable rapid sequencing of viral genomes, which helps researchers identify viral strains, mutations, and even previously unknown variants that may affect resistance. This has facilitated the development of more accurate diagnostic tools and more precise breeding of virus-resistant crops. Metagenomics complements these efforts by allowing a comprehensive analysis of plant viral communities, revealing insights into viral diversity, including the role of insect vectors in spreading TMV and other pathogens, and uncovering novel viral strains that could pose future threats to agriculture.

Biocontrol using engineered bacteriophages is another emerging and promising approach for managing plant viral diseases. Bacteriophage viruses that naturally infect bacteria are being genetically modified to target specific plant viruses such as TMV. These engineered phages are designed to bind to the virus's surface, thereby disrupting its replication and preventing its spread within the plant [113]. This method offers several advantages: high specificity, as phages can target the virus without harming plant tissues or beneficial organisms; and environmental friendliness, as phages are naturally occurring and do not require synthetic chemicals. While still in its early stages, engineered phages hold great potential for sustainable viral disease management, although challenges related to phage delivery, stability in plant tissues, and regulatory approval remain.

Nanotechnology is also revolutionizing the control of plant viruses like TMV. One promising strategy involves the use of nanoparticles such as silver or copper nanoparticles that interact with viral particles at the molecular level. These nanoparticles disrupt the integrity of the viral protein coat, rendering the virus incapable of infecting plant cells [114-116]. Additionally, nano-emulsions and nano-carriers are being developed as delivery systems for antiviral agents. These nano-formulations enhance the stability and absorption of antiviral compounds while reducing the overall chemical load, thus minimizing environmental impact. By combining the precision of nanotechnology with targeted delivery, this approach offers an innovative and eco-friendly method for managing TMV and other plant viral infections.

Despite these advancements, plant virology still faces significant challenges, particularly with the emergence of new viral strains that may overcome existing resistance mechanisms. Climate change and global agricultural trade further exacerbate the risk of viral outbreaks, as rising temperatures influence vector behaviour and host susceptibility, potentially altering the epidemiology of TMV, tomato yellow leaf curl virus (TYLCV), CMV, potato virus Y (PVY), and other plant viruses [117] the progression of virus diseases is strongly affected by changing climate conditions. Among climate-changing variables, temperature increase is viewed as an important factor that affects virus epidemics, which may in turn require more efficient disease management. In this review, we discuss the effect of elevated temperature on virus epidemics at both macro- and micro-climatic levels. This includes the temperature effects on virus spread both within and between host plants. Furthermore, we focus on the involvement of molecular mechanisms associated with temperature effects on plant defence to viruses in both susceptible and resistant plants. Considering various mechanisms proposed in different pathosystems, we also offer a view of the possible opportunities provided by RNA -based technologies for virus control at elevated temperatures. Recently, the potential of these technologies for topical field applications has been strengthened through a combination of genetically modified (GM). Addressing these concerns will require robust monitoring systems and integrated management strategies.

Looking ahead to 2030 and beyond, the integration of Artificial Intelligence (AI) and machine learning into plant virology promises to revolutionize disease management. AI-driven models can predict viral outbreaks based on environmental and epidemiological data, analyze complex omics datasets to identify virus-host interaction patterns and automate resistance design through simulations of genetic edits and breeding outcomes. Future advancements in synthetic biology and gene stacking approaches are expected to further enhance resistance to multiple plant viruses. Moreover, innovative biocontrol methods involving beneficial microbes, endophytes, and natural predators to control vector populations are anticipated to contribute significantly to sustainable agriculture and global food security in the face of evolving plant viral threats.

2.6.6 *Age of virus diagnosis*

In 1927, Dvorak prepared the sap from mosaic virus infected potato plants and injected it into experimental animals, which subsequently developed the antibodies. Purdy Beale also obtained similar results with diseased tobacco plants [118]. He concluded that the sap of infected plants must contain a serologically active component that has antigen-like properties. The two significant breakthroughs in the field of virus diagnostics came with the discovery of serological assays in enzyme-linked immunosorbent assay (ELISA) form and nucleic acid-based assays in polymerase chain reaction (PCR) form. Some of the diagnostic and detection methods developed for plant viruses are as follows:

I. Direct and indirect ELISA have evolved as preferred methods to diagnose plant viral diseases [119]. ELISA can be used to differentiate closely related viruses and their strains through virus/strain-specific antibodies. Double Antibody Sandwich-ELISA format is the most used type of ELISA for virus diagnosis. ELISA is also used for the screening of infections within imported plant materials or germplasm.

II. A lateral flow assay is a simplified form of ELISA for detecting plant viruses was optimized for the first time for CMV and TMV [120-121]. LFA-based diagnostic kits are now used for routine diagnosis of plant viruses.

III. In 1984, Karry Mullis discovered the PCR technique, which was optimized to be used for specific and sensitive detection of plant viruses [122]. Multiplex PCRs were then developed, where few viruses can be diagnosed together in a single reaction. For RNA plant viruses, the reverse transcription-PCR was standardized, which required a preliminary step of reverse transcription required for cDNA synthesis from the template RNA and then PCR. In the same year, Ahmed Hadidi showed that viroid and satellite RNAs from pome fruits or temperate fruits can be used for easy detection by RT-PCR. The real-time quantitative PCR method was also optimized for detecting plant viruses like the citrus tristeza virus and citrus yellow vein clearing virus [123].

IV. In 2000, Loop-mediated isothermal amplification (LAMP) was described by Notomi. This technique required only primers and an enzyme reaction mix to amplify the target sequence at a constant temperature without the use of an expensive thermal cycle machine. LAMP has now been standardized to diagnose many plant viruses infecting apples, citrus, bananas, grapes, and other crops. Reverse transcription-based loop-mediated isothermal amplification was also optimized for plant RNA virus diagnosis [124].

V. In 1995, Fire and Xu originally described the rolling circle amplification (RCA) method to generate single-stranded circular DNA products from circular or linear DNA at a constant temperature. For plant viruses, multi-primed RCA was applied for bipartite Gemini viruses to amplify their DNA B component. RCA followed immediately by RFLP was used to detect small ss circular DNA genomes to diagnose Gemini viruses [125].

VI. In 2004, Vincent developed helicase-dependent amplification (HDA), which is now extensively employed to rapidly detect plant viruses and viroids. HDA method required a DNA helicase, single-stranded binding protein, and endonuclease to create ssDNA as a target template for annealing and extension with two primers at a constant temperature.

VII. In 2006, recombinant polymerase assays (RPA)were a breakthrough for the rapid detection of plant virus members of *Bromoviridae*, *Luteoviridae*, *Potyviridae*, *Reoviridae*, *Virgoviridae*, *Closteroviridae*, *Caulimoviridae*, *Geminiviridae* and *Nanoviridae* [126-127]. In 2016, the first-time detection of viroids by RPA was also reported.

VIII. The microarray technique was used for the first time for a plant RNA virus detection in 2003 [128] and for viroid detection in 2012 [129].

IX. In 2009, Next-generation sequencing (NGS) was first used for plant virus detection. It has accelerated the hypothesis for viral detection at a reduced cost for large-scale surveys. The pepper virome (multiple viral infections from pepper plants) has been reported using the Illumina HiSeq NGS platform. In 2014, Smith used NGS for the first time to sequence the genome of barley stripe mosaic virus isolated from barley grains of approx. 750 years old. Nowadays, metagenomic samples like soil are analyzed by NGS technology to know about the evolutionary history of plant viruses.

X. Deep sequencing on siRNAs isolated from the virus-infected plant samples is done in order to detect many plant viral infections within the fruits and vegetable crops [130].

XI. Digital PCR is an advanced version of traditional PCR that offers unparalleled sensitivity and precision by partitioning the sample into thousands of micro-reactions. Each partition undergoes amplification, allowing absolute quantification of viral genomes. This technique is particularly valuable for detecting low-abundance viral pathogens, including latent infections that might escape conventional methods [131].

XII. CRISPR technology, originally developed for gene editing, has been adapted for rapid virus detection. CRISPR-based diagnostic tools like DETECTR and SHERLOCK utilize Cas enzymes (e.g., Cas12 or Cas13) to recognize specific viral nucleic acid sequences with high specificity. These tools are highly portable, cost-effective, and capable of providing results within minutes, making them ideal for field-based diagnostics [132].

XIII. Artificial Intelligence (AI) and Machine Learning (ML) Integration:

AI and ML are being integrated into diagnostic workflows to analyze complex datasets, such as metagenomic sequences and high-throughput imaging data. These technologies can identify patterns and predict outbreaks based on environmental and epidemiological data, aiding in proactive disease management [133].

XIV. Smartphone-Based Diagnostic Platforms: Smartphone-integrated diagnostic tools now combine advanced imaging and computational power for real-time virus detection. These platforms utilize apps to analyze data from biosensors, lateral flow assays, or microfluidic chips, providing accessible and user-friendly diagnostic solutions for farmers and researchers [134].

3. Methods for viral disease management

Many different conventional, chemical, and molecular methods and integrated methods were developed for the management of plant viral diseases. Some of the practices described below have been used to control viral diseases in crop plants [135].

3.1 *Exclusion and eradication*

Exclusion of plant viruses involves precautionary measures to protect new crops from viral diseases originating in already established crop areas. This is achieved through quarantine or phytosanitary regulations, which are legal requirements designed to manage and control the movement of plant materials. These regulations ensure that plants entering new areas are free from pests and viruses.

Various techniques are employed to eliminate plant viruses, including thermotherapy, cold therapy, meristem tissue culture, chemotherapy, and micrografting, often in combination, to produce virus-free planting material. Phytosanitary certification protocols are strictly followed at national and international borders to verify that plant samples are free from pests and diseases. The European and Mediterranean Plant Protection Organization (EPPO) has developed a certification system that ensures the commercialization of healthy, pathogen and pest-free plants, sterile planting materials, and genetically accurate crops [136].

Eradication measures focus on removing virus-infected plants from cultivated areas, including the removal of weeds that serve as reservoirs for plant viruses. A notable example of exclusion and eradication strategies is the 2024 outbreak of Tomato Brown Rugose Fruit Virus (ToBRFV) in South Australia. In August 2024, Perfection Fresh, a major tomato producer, detected ToBRFV in its glasshouses. In response, the company implemented strict biosecurity measures, including the removal and incineration of infected plants and restrictions on worker movement between glasshouses to prevent the virus's spread.

The South Australian Department of Primary Industries and Regions (PIRSA), in collaboration with a national consultative committee, imposed quarantine measures on 31 of Perfection Fresh's glasshouses. This led to the destruction of over 1.2 million plants, resulting in significant financial losses. While these actions were essential to protect the broader $800 million tomato industry, some growers criticized the response, deeming it excessive and poorly managed. Concerns were raised that such stringent measures might discourage future reporting of similar outbreaks, thus undermining biosecurity efforts.

This case underscores the critical role of exclusion and eradication strategies in managing plant virus outbreaks. It highlights the need for a balanced approach that effectively controls pathogens while minimizing the economic and social impacts on producers. The experience emphasizes the importance of clear communication and collaboration between regulatory bodies and growers to maintain trust and strengthen biosecurity efforts.

3.2 *Vector control*

The insect vectors such as aphids, whiteflies, leafhoppers, mealy bugs, beetles, grasshoppers, thrips, treehoppers, and other vectors were identified which spread the plant viruses within field crops. This must be managed efficiently to control viral diseases. Some of the strategies used to control the vector population are described below.

3.2.1 *Barrier crops and intercropping*

These are agricultural practices used to control insect vectors that transmit plant viruses. Barrier crops are fast-growing, non-host plants planted around the main crop to physically block or reduce the movement of vectors, either through shading or windbreak effects. Intercropping involves planting different crops together, often with one acting as a barrier to insect vectors. Together, these methods create a more complex environment that deters pest proliferation, reducing vector populations and the spread of viral diseases, while potentially enhancing overall crop yield.

A case study investigated the effectiveness of two maize planting patterns-intercropping chilli with maize and surrounding chilli with maize-as barrier crops to control aphid vectors and reduce viral diseases in chilli plants. The results showed a significant reduction in alate aphid populations, with decreases of 65.7% and 59.6% in the first and second trials, respectively, compared to the chilli monocrop control. Additionally, this led to significantly higher fruit yield and fruit number, with a marked reduction in viral disease incidence, likely due to the reduced aphid population. These findings highlight intercropping as an effective, eco-friendly strategy to manage pest populations and enhance crop productivity [137].

3.2.2 *Reflective surfaces*

In the management of insect vectors, especially aphids, the role of colour has been extensively studied. Bright colours such as yellow, orange, and green have been found to attract insect vectors, particularly aphids, while colours like black, grey, and white act as repellents [138]. This principle has been applied in crop management by introducing counter-coloured sheets or surfaces to deter aphids and other insect pests.

For example, counter-coloured sheets, often used in crops like lilies, cucumbers, and lettuce, are strategically placed over or around the crops [139]. These sheets, typically in black or white, serve to repel aphids and reduce their attraction to the plants. By leveraging the natural response of aphids to these colours, growers can effectively minimize aphid populations, thereby reducing the risk of aphid-borne virus diseases and protecting crop health.

3.2.3 *Case study: management of aphids and aphid-borne virus diseases in cantaloupe crops in the San Joaquin Valley, California*

In the inland valleys of California, cucurbitaceous crops, particularly cantaloupes (*Cucumis melo L. var. cantalupensis cv. Primo*), face significant challenges from aphids and several mosaic virus diseases, which can severely reduce yields. Three field experiments were conducted to evaluate the effectiveness of two mulch types-reflective polyethylene and biodegradable synthetic latex spray-for managing aphid populations and aphid-borne virus diseases in late-season cantaloupe crops in the San Joaquin Valley.

The experiments revealed that reflective polyethylene mulches were particularly beneficial under conditions of high aphid populations and substantial virus inoculum potential. Aphid counts on leaves of plants grown over these mulches were consistently lower than those grown on bare soil, highlighting the mulches' role in reducing aphid infestation. Additionally, the onset of symptoms associated with cucumber mosaic cucumovirus, watermelon mosaic potyvirus, and zucchini yellow mosaic potyvirus was delayed by 3-6 weeks in plants grown over reflective mulches, a critical delay that allowed the crops to initiate normal flowering and fruiting.

These findings align with studies on insect vector management, where reflective surfaces play a crucial role in influencing aphid behaviour. Bright colours such as yellow, orange, and green are known to attract aphid vectors, while colours like black, grey, and white have been shown to repel them [138]. This principle was applied in the experiments by introducing reflective polyethylene mulches, which not only reduced aphid populations but also provided an indirect repellent effect on the aphids, likely due to the reflective nature of the material.

Furthermore, counter-coloured sheets are commonly used in crops like lilies, cucumbers, and lettuce to manage insect vectors. By incorporating colours that repel aphids, such as black or white, these sheets can complement the use of reflective mulches to further deter aphid attraction and virus transmission.

The impact of virus diseases on the crops was profound. In control plots where no mulches were used, the virus diseases led to near-total crop loss, with foliar symptoms appearing in 100% of the plants, resulting in almost zero marketable yields. Conversely, mulches significantly improved yields. Full coverage with reflective polyethylene mulch resulted in a 9.5-fold increase in marketable yield, while spray mulch treatment resulted in a 2.5-fold increase. The high incidence of virus disease in control plots made it clear that reflective mulches could be the difference between a saleable crop and total crop failure [140].

Partial coverage with spray mulch or polyethylene film applied in alternate rows was less effective, further emphasizing the importance of complete bed coverage for optimal results. The combination of reflective polyethylene and biodegradable synthetic latex spray mulches, along with the use of counter-coloured sheets, offers a comprehensive strategy for managing aphid vectors and mitigating virus diseases. This approach can significantly improve crop health and yield in high-risk areas like the San Joaquin Valley, ultimately providing a more resilient solution for cantaloupe production.

Insecticides/pesticides: The viruses, unlike other pathogens, cannot be controlled chemically, but their insect vectors can be eliminated or killed by the spraying of organic or chemical insecticides/pesticides to prevent the spread of viruses [141-142]. A study conducted during the 2017 and 2018 cotton seasons evaluated the efficacy of three insecticides-flonicamid, pyriproxyfen, and buprofezin-against the cotton aphid (*Aphis gossypii*) under laboratory and field conditions. The study also examined the combined effects of these insecticides and their impact on natural enemies, including *Coccinella undecimpunctata* (ladybird beetle) and *Chrysoperla carnea* (green lacewing). In laboratory tests,

flonicamid was the most effective, with the lowest LC50 value (0.58 mg/L), followed by pyriproxyfen (3.42 mg/L) and buprofezin (4.26 mg/L). Mixtures of flonicamid with pyriproxyfen or buprofezin at LC25 concentrations showed a synergistic effect, with co-toxicity factors ranging from 23.08 to 37.52. At lower concentrations (LC10 + LC25), these combinations produced an additive effect, with co-toxicity factors of 18.16 (flonicamid + pyriproxyfen) and 10.02 (flonicamid + buprofezin).

Field trials revealed that the highest reduction in aphid populations was achieved with the flonicamid/pyriproxyfen mixture, which reduced populations by 90.45% in 2017 and 87.15% in 2018. This was followed by the flonicamid/buprofezin mixture (87.47% in 2017 and 81.34% in 2018) and flonicamid alone (84.31% in 2017 and 77.89% in 2018). Importantly, all insecticide treatments were classified as harmless or slightly harmful to beneficial insects, including *C. undecimpunctata* and *C. carnea*, in both seasons. These results indicate that flonicamid and its mixtures with pyriproxyfen or buprofezin are highly effective against *A. gossypii*, offering significant population control with minimal impact on non-target organisms. This makes them promising candidates for sustainable pest management in cotton cultivation [143].

3.3 *Generating virus-resistant crops*

A long-term management strategy was to generate a resistant crop cultivar which could be resistant to the virus. The conventional breeding program was an excellent method to develop resistant cultivars; however, it takes longer. After the advent of recombinant DNA technology, the development of virus resistance has become faster, more efficient, and more reliable. In this approach, the desired gene, even from different organisms, can be stably introduced into plants to make them resistant to viruses. Some of the approaches as follows:

i. Pathogen derived resistance: The concept of pathogen-derived resistance (PDR) was developed by Sanford and Johnston in 1985 for the generation of virus-resistant transgenic plants through the expression of virus-derived genes like coat protein (CP) gene of TMV [144], movement protein of potato virus X (PVX) [145-146] CP gene of Alfalfa mosaic virus (AMV) and defective-interfering RNA (or DNA) mediators of Geminivirus [147].

ii. RNA interference: RNAi was first discovered in 1993 as a natural mechanism in host plants to regulate viral replication and gene expression. This discovery highlighted how plants could defend themselves against viral infections through gene silencing. Over time, researchers identified virus-derived RNA molecules that encode suppressor proteins. These proteins play a significant role in interfering with host-mediated RNA silencing, thus allowing viruses to successfully establish infections in plants [148].

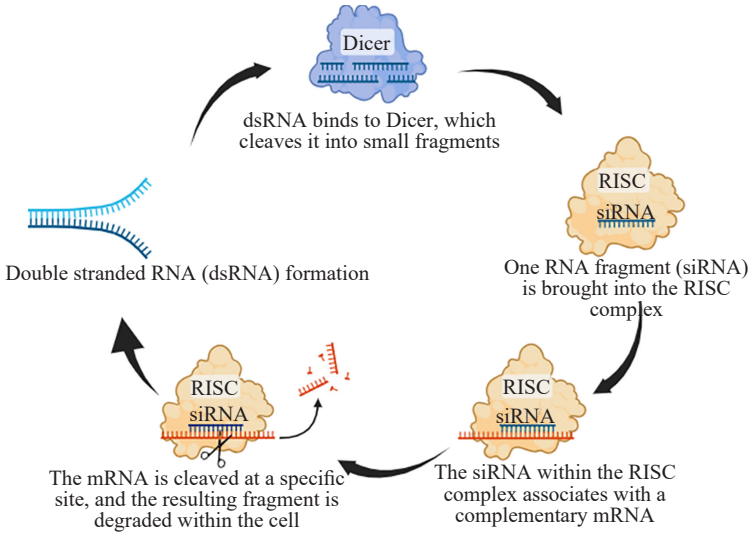


Figure 7. Schematic illustration of the RNAi mechanism: A double-stranded RNA (dsRNA) molecule binds to the Dicer protein, which cleaves it into small interfering RNAs (siRNAs). These siRNAs are then incorporated into the RNA-Induced Silencing Complex (RISC), which directs RISC to complementary RNA targets. Once recognized, the target's expression can be regulated through various mechanisms

RNAi functions as an antiviral defense mechanism by cleaving double-stranded RNA (dsRNA) intermediates of viruses into small RNA duplexes, typically 21-24 nucleotides long. This process is mediated by Dicer, an endonuclease expressed by the host plant. One of the strands of the RNA duplex is incorporated into the RNA-induced silencing complex (RISC), where it guides the complex to recognize and cleave complementary viral RNA sequences. The Argonaute protein within the RISC complex plays a crucial role in enabling the cleavage of target RNAs in a sequence-specific manner, thereby inhibiting the replication and accumulation of viral RNA (Figure 7) [149].

The RNAi mechanism can be artificially induced in plants through genetic engineering approaches. For example, viral genome sequences can be integrated into plant DNA constructs using transformation techniques. These constructs produce viral dsRNA or single-stranded RNA in plants, which activates the RNA silencing pathway and enhances resistance to viral infections. This strategy has been successfully employed to develop plants with increased resistance to various plant viruses, as detailed in Table 2.

Table 2. List of transgenic resistant crops to viruses generated using the RNAi approach

Virus	Resistant crop	Gene modification	Function of yargeted gene	Reference
Tobacco Mosaic Virus (TMV)	Tobacco	RNA interference (RNAi) to block the coat protein (CP) gene of TMV	Function of targeted gene: the coat protein (CP) facilitates virion assembly, stability, and systemic movement within the host plant.	[150]
Potato virus Y (PVY)	Potato	RNA silencing (RNAi) targeting the coat protein (CP) gene of PVY	The coat protein (CP) gene is crucial for PVY as it helps in virion assembly, stability, and movement within the plant.	[151]
Prunus necrotic ringspot virus (PNRV)	Cherry	RNA interference (RNAi) targeting the coat protein (CP) gene of PNRSV	The coat protein (CP) is essential for virion assembly, stability, and systemic movement within the plant.	[152]
Barley yellow dwarf virus (BYDV)	Wheat	RNA interference (RNAi) targeting the P3 and P5 genes of BYDV	P3 encodes a movement protein for viral spread, while P5 is essential for replication and accumulation within the host.	[153]
African cassava mosaic virus(ACMV)	Cassava	RNA interference (RNAi) targeting the AC1 (Rep) and BC1 (MP) genes of ACMV	AC1 (Rep) facilitates viral DNA replication, while BC1 (MP) enables viral spread within the plant.	[154]
Cotton leaf curl virus (CLCuV)	Tobacco	RNA interference (RNAi) targeting the βC1 gene of CLCuV	βC1 is a key pathogenicity factor that enhances viral replication, suppresses host defenses, and facilitates symptom development.	[155]
Mung-bean yellow mosaic India virus (MYMIV)	Black gram plants	RNA interference (RNAi) targeting the AC1 (Rep) and βC1 genes of MYMIV	AC1 (Rep) is essential for viral DNA replication, while βC1 suppresses host defense and enhances symptom development.	[156]
Turnip yellow mosaic virus (TYMV)	Turnip	RNA interference (RNAi) targeting the coat protein (CP) gene of TYMV	The coat protein (CP) is crucial for viral assembly, stability, and movement within the plant.	[157]
Tomato yellow leaf curl Sardinia virus (TYLCSV)	Tomato	RNA interference (RNAi) targeting the V1 gene of TYLCSV	The V1 gene encodes the coat protein (CP), which is essential for virion assembly, stability, and systemic movement of the virus within the plant.	[158]
Mungbean yellow mosaic virus (MYMV)	Soybean	RNA interference (RNAi) targeting the AC1 (Rep) and βC1 genes of MYMIV	AC1 (Rep) is essential for viral DNA replication, while βC1 suppresses host defense and enhances symptom development.	[159]
Watermelon mosaic virus (WMV), Zucchini yellow mosaic virus (ZYMV) and Cucumber mosaic virus (CMV)	Squash	RNA interference (RNAi) targeting the coat protein (CP) gene of WMV, ZYMV and CMV	The coat protein (CP) is essential for the assembly, stability, and movement of the virus within the plant.	[160]
Rice black-streaked dwarf virus (RBSDV)	Rice	RNA interference (RNAi) targeting the P7-1 and P10 genes of RBSDV	P7-1 gene: involved in viral replication and movement; P10 gene: facilitates viral spread within plant tissues.	[161]

However, despite the promising potential of RNAi, there are several challenges that limit its practical application on a larger scale. The cost of developing and implementing RNAi-based solutions remains high, particularly for small-scale farmers in developing regions. Additionally, the technical complexity of generating stable RNAi constructs and ensuring consistent expression levels in plants can pose significant hurdles. Factors such as off-target effects, degradation of RNA constructs, and unintended impacts on plant physiology or beneficial organisms must also be carefully considered.

Another challenge is scalability. While RNAi-based approaches have shown success in controlled laboratory or greenhouse environments, translating these results into field-scale applications can be difficult. Environmental factors such as variability in viral populations, the stability of RNA constructs, and interactions with non-target organisms can influence the effectiveness of RNAi under field conditions. Furthermore, regulatory hurdles and public perception of genetically modified organisms (GMOs) may limit the widespread adoption of RNAi technologies in agriculture.

To overcome these challenges, efforts should focus on reducing costs through improved biotechnological methods, simplifying delivery systems, and enhancing the stability and specificity of RNAi constructs. Additionally, educating stakeholders, including farmers and policymakers, about the benefits and safety of RNAi could help improve acceptance and facilitate its broader implementation in sustainable agriculture.

3.4 *Genome editing*

Table 3. List of gene edited virus resistant crops

Plant(s)	Genome/editing location	Viruses	Outcomes	References
N. benthamiana	Virus DNA, Rep, IR, and CP	Beet curly top virus, Merremia mosaic virus, Tomato yellow leaf curl virus	Indels in viral genome	[162]
N. benthamiana	Virus DNA, Rep A/Rep and LIR	Bean yellow dwarf virus	Resistant	[163]
Arabidopsis *N. benthamiana*	Virus DNA, Rep, IR, and CP	Beet severe curly top virus	Resistant	[164]
N. benthamiana	Virus DNA and satellite sequences	Cotton leaf curl Kokhran virus, Tomato yellow leaf curl virus, Merremia mosaic virus, BCTV-Logan, BCTV-Worland	Indels in viral genome	[165]
Arabidopsis	*eIF4E*	Turnip mosaic virus	Resistant	[166]
Cucumber	*eIF4E*	Cucumber vein yellowing virus (Ipomovirus), Zucchini yellow mosaic virus, and Papaya ring spot mosaic virus-W (Potyviruses)	Resistant	[167]
Arabidopsis	Virus RNA genome	Turnip mosaic virus	Indels in viral genome	[168]
N. benthamiana	Virus RNA genome	Turnip mosaic virus	Indels in viral genome	[169]
Arabidopsis *N. benthamiana*	Virus RNA genome	Cucumber mosaic virus, Tobacco Mosaic Virus	Reduce the virus severity	[170]
Tomato *N. benthamiana*	Virus DNA Rep, IR, and CP	Tomato yellow leaf curl virus	Resistant	[171]
Rice	*Eif4g*	Rice tungro spherical virus	Resistant	[172]
Rice *N. benthamiana*	Virus RNA genome	Southern rice black streaked dwarf virus, Tobacco Mosaic Virus	Reduce the virus severity	[173]
N. benthamiana	Multiplex editing at Rep and IR	Cotton leaf curl Multan virus	Reduce the virus severity	[174]
Cassava	AC2 and AC3	African cassava mosaic virus	Indels in viral genome	[175]
Cassava	nCBP-1, nCBP-2	Cassava brown streak disease	Diseases symptoms are suppressed	[176]
Banana	Virus sequences in the host plantain genome	Endogenous banana streak virus	Most of the infected plants are asymptomatic	[177]
Banana	Eif4e1	Clover yellow vein virus	Virus accumulation rate is very low	[178]
N. benthamiana	Multiplex editing at virus DNA Rep, IR, and CP	Chilly leaf curl virus	Significantly low virus accumulation and decreased disease symptoms	[179]

Genome editing is the most promising techniques which edit the nucleotides within the genome of crops to achieve the desired traits. It involves digestion and ligation of DNA molecules at specific sites for beneficial hereditary changes in the genome. Earlier, the Zinc Finger Nucleases (ZFNs) were the only genome editing tool for the site-specific restriction of DNA. Further, the introduction of unique transcription activator-like effector nucleases (TALENs) and Oligonucleotide-Directed Mutagenesis (ODM) methods enabled more efficient and selective manipulation of target genomic DNA. Recently, CRISPR (Clustered Regularly Interspaced Palindromic Repeats) and its associated protein Cas9 have revolutionized genome editing by enabling precise alterations to plant genomes. This system introduces resistance genes into plants, providing them with the ability to combat viral infections effectively. CRISPR recognizes specific DNA sequences within a pathogen's genome, and the Cas9 protein acts as molecular scissors, cleaving the target DNA at the designated site to disrupt or modify the gene of interest.

This technology has become a cornerstone of genetic engineering, functional genomics, and applied biology due to its wide-ranging DNA-targeting abilities, efficiency, and adaptability (Table 3). It has been successfully applied to generate virus-resistant plants by targeting viral genome sequences or enhancing the expression of plant defense genes.

Despite its potential, CRISPR faces practical challenges. Its implementation requires sophisticated infrastructure and technical expertise, making it costly and less accessible in regions with limited resources. Additionally, the scalability of CRISPR-based strategies for large-scale agricultural use remains a concern. Ethical considerations and regulatory hurdles associated with genetically edited crops also pose significant barriers to widespread adoption. Addressing these limitations will be essential to fully harness CRISPR's potential in plant virology and crop improvement.

3.5 *Strategy used by plants to encounter plant viruses*

Dominant resistance: Dominant R genes within plants trigger a hypersensitive response against viruses through unknown mechanisms. The formation of this kind of pathogenic resistance requires just a single copy of a functional gene [180].

Recessive resistance: Plants can also achieve virus resistance because of a lack of susceptibility factors. This means that due to lack of host factors the virus fails to complete its life cycle within the host [181].

RNA silencing: RNA silencing antiviral mechanisms against both ds RNA and ss RNA viruses is one of the best strategies used by plant systems. This mechanism works by suppressing the translation of viral RNA [182].

4. Conclusion

In conclusion, while the evolution of virology and its impact on plant disease management is well-documented, future research directions require more specificity and focus. Instead of broadly stating that "further research is needed", it is crucial to identify key areas of exploration that will drive advancements in the field. These areas include the discovery of new plant virus species, in-depth investigation of virus-vector-host interactions, the development of advanced diagnostic tools, the creation of genetically resistant crops using cutting-edge technologies like CRISPR, and the exploration of plant viruses for biotechnological applications. Focusing on these targeted research areas will not only enhance our understanding of plant viruses but also contribute to more effective and sustainable management strategies. Ultimately, such focused research will play a vital role in improving food security by increasing the resilience of crops to viral diseases and ensuring higher quality food production in the face of evolving agricultural challenges.

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Author contributions

Aniket Angira and Shobith Suresh Contributed equally to this review.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this review.

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