

Advances in Viral Pathogenesis and Control in Plants: A review

Abstract

Plant viral diseases pose a significant threat to global agriculture, leading to substantial yield losses and affecting food security. Advances in viral pathogenesis and control have been driven by a combination of traditional and modern approaches. This review highlights key areas of progress, including virus-host interactions, molecular mechanisms of viral replication, and host immune responses. Traditional control methods, such as crop rotation, sanitation, and vector management, remain foundational but face limitations due to the evolving nature of plant viruses. Breeding for genetic resistance, while effective, is challenged by the rapid adaptation of viral pathogens. The emergence of biotechnological strategies, such as RNA interference (RNAi), CRISPR/Cas systems, and the development of transgenic plants, has provided novel tools for enhancing resistance. Furthermore, molecular diagnostics, including PCR and next-generation sequencing (NGS), have revolutionized virus detection, enabling precise and early diagnosis. The integration of omics approaches—genomics, transcriptomics, proteomics, and metabolomics—has facilitated a plant-virus interactions, while synthetic biology and systems biology are opening new frontiers in engineering virus-resistant crops. Climate change exacerbates the challenge by altering virus spread, vector dynamics, and host susceptibility, necessitating adaptive strategies. Emerging and re-emerging plant viruses underscore the need for robust surveillance and biosecurity measures, emphasizing the role of international collaboration in controlling these threats.

Keywords: *Plant viruses, viral pathogenesis, genetic resistance, RNA interference, CRISPR/Cas*

I. Introduction

A. Plant Viruses

1. Plant Health in Global Agriculture

Plant health is fundamental to global food security, ecosystem stability, and economic development. Agriculture, which forms the backbone of many economies worldwide, relies heavily on healthy crops to ensure food production, livelihoods, and raw materials for various industries. Over 80% of the human diet comes from plants, with staple crops such as rice, wheat, maize, and potatoes being essential for nutritional needs [1]. Plant diseases caused by various pathogens, including viruses, pose significant challenges to maintaining crop productivity. Viruses are among the most damaging plant pathogens, often resulting in severe yield losses and reductions in crop quality. The impact of plant viruses can be particularly devastating in regions that depend heavily on agriculture, especially where resources for disease management are limited. Furthermore, emerging and re-emerging viral pathogens are increasingly being reported due to climate change, global trade, and agricultural practices, emphasizing the need for robust control measures and research into plant viral pathogenesis.

2. Economic and Ecological Impact of Plant Viruses

The economic impact of plant viruses is significant, causing billions of dollars in annual losses globally [2]. These losses arise not only from reduced yields but also from costs associated with managing viral diseases, such as the use of pesticides for vector control, the development and deployment of resistant varieties, and labor for implementing cultural practices. The Tomato Yellow Leaf Curl Virus (TYLCV) alone is responsible for severe economic losses in tomato crops worldwide, with yield losses ranging from 20% to 100% depending on the timing of infection. Similarly, the Cucumber Mosaic Virus (CMV) affects a wide range of crops, leading to considerable economic damage. Ecologically, plant viruses can alter the dynamics of plant communities by influencing the fitness and competitive interactions of infected plants. Viruses may also affect non-cultivated plant species, leading to changes in biodiversity and ecosystem function [3]. In some cases, viruses can spread to wild relatives of crop plants, posing a risk to natural ecosystems. The use of chemical control measures to manage virus vectors can lead to environmental concerns, such as pesticide resistance and effects on non-target organisms.

3. General Characteristics of Plant Viruses (Structure, Replication, and Transmission)

Plant viruses are obligate intracellular pathogens that depend on host cellular machinery for replication and spread. They exhibit diverse morphologies and genetic organizations but are typically classified based on their nucleic acid type (RNA or DNA), shape (icosahedral, rod-shaped, or filamentous), and the presence or absence of an envelope.

a. Structure

Most plant viruses are either RNA or DNA viruses, with the majority being RNA viruses [4]. Their genomes can be single-stranded (ssRNA) or double-stranded (dsRNA) in RNA viruses, or single-stranded (ssDNA) or double-stranded (dsDNA) in DNA viruses. For example, the Tobacco Mosaic Virus (TMV), one of the first viruses to be discovered, is a single-stranded RNA virus with a rod-like structure. The viral genome encodes structural proteins (such as coat proteins) and non-structural proteins (such as replicases, movement proteins, and suppressors of host defenses).

b. Replication

Viral replication begins with the virus entering the plant cell, often through wounds or vector transmission. Upon entry, the viral genome is uncoated and hijacks the host's transcriptional and translational machinery to replicate and produce viral proteins. RNA viruses replicate in the cytoplasm, while DNA viruses typically replicate in the nucleus. For example, Geminiviruses, which are DNA viruses, replicate through a rolling circle mechanism and utilize host DNA replication machinery [5].

c. Transmission

Plant viruses are transmitted through various mechanisms, including:

- **Vector-mediated transmission:** Most commonly by insect vectors such as aphids, whiteflies, and thrips, which transmit viruses like Begomoviruses and Potyviruses.
- **Mechanical transmission:** Occurs through physical contact or abrasion, allowing viruses like TMV to spread from one plant to another.

- **Seed and pollen transmission:** Some viruses, such as the Barley Stripe Mosaic Virus, can be transmitted through infected seeds or pollen.
- **Soil-borne transmission:** Fungi and nematodes can also transmit certain viruses, such as Tobacco Rattle Virus, by acting as vectors in the soil environment [6].

B. Objectives of the Review

1. Summarize Recent Advancements in Plant Viral Pathogenesis

The study of plant viral pathogenesis has advanced significantly in recent years due to the advent of modern molecular biology techniques, including next-generation sequencing (NGS), proteomics, and functional genomics. These tools have shed light on the intricate virus-host interactions, revealing novel insights into how viruses manipulate host cellular machinery to establish infection, evade plant immune responses, and spread within the host. The molecular underpinnings of these processes is critical for the development of effective control strategies [7]. This review aims to consolidate recent findings in the field, emphasizing breakthroughs in our viral pathogenesis.

2. Current Strategies and Innovations in Virus Control

Given the economic and ecological burden of plant viral diseases, various control strategies have been developed, ranging from traditional cultural practices and vector management to cutting-edge genetic engineering techniques. Recent innovations, such as CRISPR/Cas systems, RNA interference (RNAi), and synthetic biology approaches, hold promise for developing virus-resistant crops. This review will discuss these strategies, including their mechanisms of action, efficacy, and practical applications in agricultural systems.

3. Identify Gaps and Future Research Directions

Despite significant progress, several gaps remain in our plant viral pathogenesis and control [8]. The mechanisms underlying virus adaptation and evolution, the role of non-coding RNAs and epigenetic modifications in virus-host interactions, and the environmental factors influencing virus outbreaks are not fully understood. There is a need for more effective and sustainable control methods that can withstand the challenges posed by climate change and globalization.

II. Advances in Viral Pathogenesis

A. Virus-Host Interactions

1. Mechanisms of Virus Entry and Movement within Host Plants

Unlike animal viruses that often rely on endocytosis for entry, plant viruses must overcome the rigid plant cell wall, typically gaining access through mechanical wounds or with the help of vectors such as insects, nematodes, or fungi. Once inside the host cell, viruses initiate cell-to-cell movement through plasmodesmata, the channels connecting plant cells [9]. This process is mediated by viral movement proteins (MPs), which interact with and modify plasmodesmata to facilitate the movement of viral nucleic acids between cells. The Tobacco Mosaic Virus (TMV) MP binds to viral RNA and modifies the plasmodesmata to enable passage. After successful cell-to-cell movement, the virus reaches the phloem, where it spreads systemically throughout the plant, ensuring widespread infection. This systemic

spread relies on interactions between viral proteins and phloem-specific factors, as seen with Cucumber Mosaic Virus (CMV).

2. Host Factors Exploited by Viruses for Replication and Spread

Plant viruses are entirely dependent on host cellular machinery for replication, translation, and movement [10]. They co-opt host transcription and translation machinery, including ribosomes and translation initiation factors such as eIF4E, which are critical for viral protein synthesis. For example, potyviruses exploit eIF4E to enhance viral RNA translation. Heat shock proteins (HSPs), particularly Hsp70 and Hsp90, play roles in the proper folding and assembly of viral replication complexes. Viruses manipulate the host's endoplasmic reticulum (ER) and other membrane systems to form specialized replication factories. For example, Turnip Mosaic Virus (TuMV) induces ER-derived membrane structures to house replication complexes. Furthermore, the cytoskeleton, comprising actin and microtubules, is also utilized by viruses such as TMV for intracellular trafficking of viral components, aiding in their movement within and between cells.

3. Host Signaling Pathways Influenced by Viral Infection

Viral infections significantly alter host signaling pathways to create an environment conducive to viral replication and spread [11]. Viruses often manipulate plant hormonal pathways such as salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA). These hormones are integral to plant immune responses, with SA typically associated with defense against biotrophic pathogens, including viruses. CMV suppresses SA-dependent defense pathways, thereby enhancing susceptibility. Viral suppressors of RNA silencing (VSRs) are critical for neutralizing the RNA silencing pathway, a primary antiviral defense mechanism in plants. The CMV 2b protein binds to small RNAs, preventing their incorporation into the RNA-induced silencing complex (RISC). Viruses modulate reactive oxygen species (ROS) production, which is part of the plant's defense mechanism, to maintain conditions that favor viral replication while avoiding cell death that could limit virus spread [12].

B. Molecular and Genetic Basis of Pathogenicity

1. Roles of Viral Proteins in Pathogenicity

a. Suppression of Host Defense Mechanisms

To establish infection successfully, viruses must overcome host immune responses. One of the key mechanisms by which plant viruses suppress host defenses is through the production of viral suppressors of RNA silencing (VSRs). For example, the HC-Pro protein of potyviruses binds to small interfering RNAs (siRNAs) and blocks the RNA silencing pathway, a critical antiviral defense. Viral proteins can interfere with host hormone signaling to dampen immune responses. The P6 protein of Cauliflower Mosaic Virus (CaMV) disrupts salicylic acid-mediated defenses, thereby facilitating viral infection [13]. This ability to suppress multiple layers of host defense mechanisms highlights the complex interactions between plant viruses and their hosts.

b. Modulation of Host Gene Expression

In addition to suppressing defenses, plant viruses modulate host gene expression to optimize the cellular environment for viral replication. The β C1 protein of Tomato Yellow Leaf Curl

China Virus (TYLCCV) acts as a transcriptional regulator, altering the expression of host genes involved in growth, development, and defense. Geminivirus replication-associated protein (Rep) interacts with host retinoblastoma-related protein (RBR) to manipulate the cell cycle, providing the conditions necessary for viral replication. This targeted modulation of host gene expression is an important aspect of viral pathogenicity and a focus of ongoing research [14].

2. Functional Genomics Studies in Plant-Virus Interactions

Functional genomics has significantly advanced our plant-virus interactions by providing insights into changes in host gene expression and protein interactions during infection. Transcriptomic studies using RNA sequencing (RNA-seq) have revealed how viral infections affect the expression of thousands of host genes. For example, infection of Arabidopsis with Turnip Mosaic Virus (TuMV) led to the differential expression of genes involved in defense signaling, hormone pathways, and RNA processing. Proteomic studies have identified host proteins that interact with viral components, offering insights into how viruses manipulate host machinery. Interactomics approaches, such as yeast two-hybrid screens, have helped identify critical host-virus protein interactions, contributing to our molecular mechanisms underlying pathogenicity [15].

C. Emerging Concepts in Viral Pathogenesis

1. Role of Non-Coding RNAs in Viral Infections

Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play emerging roles in the regulation of plant-virus interactions. Viruses can manipulate host miRNAs to suppress immune responses and enhance infection. For example, CMV targets miR168, which regulates the key RNA silencing protein Argonaute1 (AGO1), thereby suppressing antiviral RNA silencing. Long non-coding RNAs (lncRNAs) are also gaining attention for their role in modulating gene expression and acting as decoys or scaffolds in regulatory networks. Some lncRNAs may act to sequester miRNAs or interact with proteins involved in defense, although the full extent of their roles in plant-virus interactions is still being elucidated [16].

2. Epigenetic Changes Induced by Viruses

Plant viruses are increasingly recognized for their ability to induce epigenetic changes in the host, affecting gene expression patterns and enabling persistent infections. DNA viruses like Geminiviruses can alter host DNA methylation, leading to the suppression of defense-related genes. In addition, viruses can influence histone modifications, altering chromatin structure and regulating gene expression to benefit the virus. For example, changes in histone methylation and acetylation can suppress the activation of defense genes, highlighting an advanced strategy by which viruses reprogram host cellular environments.

3. Quorum Sensing-Like Mechanisms in Viral Populations

An emerging concept in plant virology is that viruses may exhibit quorum sensing-like behaviors, coordinating their actions based on population density [17]. Recent research suggests that viral populations within a host may sense and respond to the density of viral genomes, adjusting replication, movement, or pathogenicity in response. This form of communication may involve feedback mechanisms that regulate viral replication machinery,

optimizing infection dynamics within the host. Such mechanisms parallel bacterial quorum sensing and represent an exciting new avenue of research in viral pathogenesis, offering potential targets for novel control strategies.

III. Host Plant Responses to Viral Infections

Plants, unlike animals, rely solely on innate immunity to defend themselves against pathogens, including viruses [18]. These immune responses are highly sophisticated, involving both local and systemic defense mechanisms (Table 1). Plant immune responses to viral infections include the recognition of viral components, activation of signaling pathways like RNA silencing, and systemic responses such as systemic acquired resistance (SAR). Genetic and environmental factors influence the host's susceptibility or resistance to viral infections.

A. Innate Immune Responses

1. Recognition of Viral Components by Host Receptors

The first step in plant defense against viruses involves the recognition of viral components by the plant's innate immune system [19]. Unlike animal systems, plants do not have specialized immune cells; instead, each plant cell can recognize and respond to pathogens. This recognition often involves pattern recognition receptors (PRRs) and nucleotide-binding leucine-rich repeat receptors (NLRs). PRRs detect conserved pathogen-associated molecular patterns (PAMPs), triggering a basal defense known as PAMP-triggered immunity (PTI). Although PAMP recognition is well-established for bacteria and fungi, plant viruses often evade this pathway. Instead, viruses are typically detected by intracellular receptors, primarily NLRs, which recognize specific viral effectors or their activities, triggering effector-triggered immunity (ETI). One example of this mechanism is the recognition of the TMV replication protein by the N receptor in tobacco, leading to a hypersensitive response (HR). This HR involves localized cell death to limit viral spread. NLRs can detect viral proteins indirectly by sensing perturbations in host cellular processes caused by viral effectors [20]. This indirect detection, known as the "guard hypothesis," exemplifies how plants monitor the integrity of host proteins targeted by viruses to activate immune responses.

2. Activation of Immune Signaling Pathways (e.g., RNA Silencing, Hypersensitive Response)

Upon recognition of viral components, several immune signaling pathways are activated. One of the primary defense mechanisms against viruses is RNA silencing, a sequence-specific process that degrades viral RNAs. RNA silencing is initiated when double-stranded RNA (dsRNA), a replication intermediate of RNA viruses, is processed by Dicer-like (DCL) proteins into small interfering RNAs (siRNAs) [21]. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), where they guide the cleavage of complementary viral RNAs. This mechanism not only restricts viral replication but also generates systemic silencing signals that confer resistance throughout the plant. Another key immune response is the hypersensitive response (HR), a form of programmed cell death at the site of infection, which limits virus spread by sacrificing infected cells. This response is commonly associated with ETI, as seen in the interaction between TMV and tobacco. HR is accompanied by the generation of reactive oxygen species (ROS), salicylic acid (SA) accumulation, and the expression of defense-related genes. These immune responses are highly coordinated and

regulated to balance defense and growth, ensuring that the plant can survive the infection while minimizing resource allocation to immunity [22].

B. Adaptive-like Responses in Plants

1. Memory-like Defense Mechanisms (Priming)

Although plants lack an adaptive immune system like that of animals, they exhibit a form of immune memory known as priming. Priming enhances the plant's ability to respond more robustly upon subsequent infections by the same or different pathogens. This heightened state of alert does not involve changes in the genetic makeup but is rather a result of biochemical and epigenetic modifications that prepare the plant for future attacks. Priming can be triggered by pathogen infection, chemical treatments, or environmental stress. A virus-infected plant might respond more quickly and effectively to subsequent viral or even bacterial infections. Priming can involve the accumulation of inactive signaling proteins or transcription factors that become rapidly activated upon secondary infection. One example is the priming of RNA silencing pathways, where prior infection leads to faster and more robust siRNA production upon subsequent viral challenges [23].

2. Systemic Acquired Resistance (SAR)

Systemic acquired resistance (SAR) is another adaptive-like defense mechanism in plants. SAR is a long-lasting, broad-spectrum resistance response that is activated in uninfected parts of the plant following a localized infection. SAR is often associated with the accumulation of SA and the expression of pathogenesis-related (PR) genes. The signaling molecule methyl salicylate (MeSA) is synthesized at the site of infection and transported to distant tissues, where it is converted back to SA, triggering defense responses in those areas. SAR confers resistance not only to the initial pathogen but also to a wide range of other pathogens, including viruses, bacteria, and fungi. For example, SAR induced by TMV infection in tobacco provides resistance to other viral infections and even bacterial and fungal pathogens. This systemic response enhances the plant's ability to withstand future infections by creating a pre-activated state of defense throughout the plant [24].

C. Host Susceptibility Factors

1. Genetic Factors Influencing Susceptibility/Resistance

The susceptibility or resistance of a plant to viral infection is largely determined by its genetic makeup. Resistance genes (R genes) play a central role in recognizing specific viral components and triggering defense responses. For example, the N gene in tobacco provides resistance to TMV by recognizing the virus's replicase protein, leading to a strong immune response. Similarly, the R gene Ty-1 in tomato confers resistance to Tomato yellow leaf curl virus (TYLCV) by enhancing RNA silencing [25]. In R genes, quantitative resistance loci (QRLs) also contribute to partial resistance by affecting various aspects of the immune response, such as RNA silencing efficiency, hormonal regulation, and cell wall reinforcement. Plants with these loci may not completely prevent viral infection but can significantly reduce viral replication and spread, minimizing the impact of the disease. Certain mutations or natural variations in host factors can either enhance susceptibility or provide resistance. A mutation in the eIF4E gene, which viruses commonly exploit for translation, can render

plants resistant to several potyviruses by disrupting the virus-host interaction necessary for replication [26].

2. Environmental Influences on Host Resistance

Environmental conditions such as temperature, light, and nutrient availability can significantly influence the outcome of plant-virus interactions. High temperatures often suppress plant immune responses, making them more susceptible to viral infections. This phenomenon is evident in the temperature-sensitive response of Arabidopsis to Turnip Crinkle Virus (TCV), where higher temperatures compromise the RNA silencing defense, leading to enhanced viral replication [27]. Certain environmental stresses can prime plants for enhanced resistance. For example, drought stress has been shown to activate ABA signaling pathways, which can cross-communicate with immune signaling pathways to modulate resistance. Nutrient availability, particularly nitrogen and phosphorus, affects the plant's metabolic state, which can influence its susceptibility or resistance to viruses. Nutrient-rich conditions often favor rapid growth, which may come at the expense of immune investment, potentially increasing susceptibility.

Table:1 Host Plant Responses to Viral Infections (Source- [20], [24], [26])

Host Plant Response	Mechanism	Examples/Applications	Significance
Hypersensitive Response (HR)	Localized cell death at infection site to prevent virus spread.	Tobacco mosaic virus (TMV) in tobacco plants	Limits virus spread to neighboring cells.
Systemic Acquired Resistance (SAR)	Activation of defense genes throughout the plant upon initial infection.	Cucumber mosaic virus (CMV) in cucumbers	Provides broad-spectrum resistance against diverse pathogens.
RNA Silencing	Degradation of viral RNA by small interfering RNAs (siRNAs).	Tobacco plants against Tobacco etch virus (TEV)	Reduces viral load and confers resistance.
Production of Pathogenesis-Related Proteins (PR Proteins)	Induction of proteins that degrade viral particles or inhibit replication.	PR-1 proteins in Arabidopsis during virus attack	Strengthens plant defenses and limits infection.
Alteration in Hormone Signaling	Modulation of hormones like salicylic acid, jasmonic acid, and ethylene.	Salicylic acid response in tomatoes against Tomato yellow leaf curl virus (TYLCV)	Enhances resistance or susceptibility depending on the virus.

Antioxidant Enzyme Activation	Upregulation of enzymes like superoxide dismutase (SOD) to counter oxidative stress.	Increased SOD in rice infected with Rice tungro virus	Protects cells from damage due to reactive oxygen species (ROS).
Lignification and Cell Wall Fortification	Strengthening of cell walls to prevent viral entry and movement.	Lignin deposition in potato against Potato virus X (PVX)	Reduces virus movement within plant tissues.
Callose Deposition	Formation of callose around plasmodesmata to restrict virus movement.	Callose in Arabidopsis infected with Cucumber mosaic virus (CMV)	Limits systemic spread of the virus.
Programmed Cell Death (PCD)	Controlled cell death in infected areas to contain the virus.	Programmed cell death in barley infected with Barley yellow dwarf virus (BYDV)	Prevents virus from reaching uninfected tissues.
Metabolic Reprogramming	Alteration of primary and secondary metabolism to reduce virus replication.	Increased phenolic compounds in grapevines infected with Grapevine fanleaf virus (GFLV)	Reduces virus replication and enhances defense.

IV. Technological Advances in Detection and Diagnosis of Plant Viruses

The accurate and timely detection of plant viruses is critical for effective disease management and mitigation of crop losses. Over the past few decades, technological advancements have significantly improved the sensitivity, specificity, and speed of diagnostic methods (Table 2)& (Table 3)[28]. These advancements range from molecular diagnostics, which target the genetic material of viruses, to immunological techniques that detect viral proteins, and the application of biosensors and nanotechnology for on-site, rapid diagnostics.

A. Molecular Diagnostics

1. Polymerase Chain Reaction (PCR) and Its Variants

Polymerase Chain Reaction (PCR) has revolutionized the detection of plant viruses by allowing the amplification of minute quantities of viral nucleic acids. Traditional PCR targets specific DNA sequences, enabling the detection of DNA viruses or cDNA synthesized from RNA viruses. Due to its high sensitivity and specificity, PCR is a cornerstone in plant virology diagnostics [29].

- **Reverse Transcription PCR (RT-PCR):** This variant is particularly valuable for RNA viruses, which constitute the majority of plant viruses. RT-PCR converts viral RNA into complementary DNA (cDNA) using reverse transcriptase, followed by amplification. This method has been widely used for detecting RNA viruses such as Tobacco Mosaic Virus (TMV) and Cucumber Mosaic Virus (CMV).
- **Real-Time PCR (qPCR):** Also known as quantitative PCR, this technique allows for the quantification of viral load in real-time. It uses fluorescent dyes or probes to detect DNA during the amplification process, offering greater sensitivity and enabling quantification. qPCR is essential for assessing viral titers, studying virus dynamics in host plants, and evaluating resistance in genetically modified crops [30].
- **Loop-Mediated Isothermal Amplification (LAMP):** LAMP is a faster alternative to PCR that operates at a constant temperature, eliminating the need for thermal cycling. It has gained popularity due to its simplicity, rapid turnaround, and potential for field application. LAMP has been successfully used for detecting viruses such as Banana bunchy top virus (BBTV) and Plum pox virus (PPV).

2. Next-Generation Sequencing (NGS)

Next-Generation Sequencing (NGS) has transformed the detection and diagnosis of plant viruses by allowing the comprehensive and unbiased identification of viral genomes. Unlike PCR, which requires prior knowledge of the target sequence, NGS can detect both known and novel viruses by sequencing the entire nucleic acid content of a plant sample [31]. NGS technologies, such as Illumina, PacBio, and Oxford Nanopore, generate massive amounts of data, providing insights into viral diversity, genome organization, and evolutionary relationships. NGS has been instrumental in identifying new plant viruses and studying complex viral communities (viromes). For example, it has been used to uncover mixed infections and virus-associated satellite RNAs in crops such as tomatoes and cucumbers. Metagenomics, an NGS-based approach, allows for the simultaneous detection of multiple pathogens in a single sample without prior knowledge of the pathogens present. This capability is particularly valuable for diagnosing complex syndromes caused by mixed infections or novel viruses in crops like wheat, rice, and grapevine [32].

B. Immunological Approaches

1. Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) is one of the most widely used immunological techniques for detecting plant viruses. ELISA is based on the specific interaction between viral antigens and antibodies. In the case of plant viruses, antibodies are raised against viral coat proteins, allowing the detection of viral particles in plant tissues.

- **Direct and Indirect ELISA:** In direct ELISA, the antigen is directly immobilized on the plate, and the detection is carried out using an enzyme-linked antibody that binds to the antigen. Indirect ELISA involves an additional antibody layer, providing increased sensitivity. ELISA is highly sensitive and specific, capable of detecting low concentrations of viral antigens [33]. ELISA has been extensively used for detecting viruses like TMV, CMV, and Potato virus Y (PVY). Its advantages include cost-effectiveness, ease of use, and high throughput, making it suitable for large-scale

screening in breeding programs and diagnostic labs. ELISA is less effective in detecting viruses during early stages of infection when antigen levels are low.

2. Lateral Flow Assays

Lateral flow assays (LFAs) are rapid, user-friendly diagnostic tools similar to home pregnancy tests. They detect viral antigens or antibodies in plant sap and provide visual results within minutes. LFAs are based on the principle of immunochromatography, where antibodies specific to the target virus are immobilized on a nitrocellulose membrane [34]. LFAs are highly portable and do not require specialized equipment or trained personnel, making them ideal for on-site field diagnostics. They have been successfully developed for detecting viruses such as PVY, TMV, and Tomato yellow leaf curl virus (TYLCV). Although LFAs are less sensitive than ELISA, their speed and convenience make them valuable for rapid decision-making in disease management.

C. Biosensors and Nanotechnology in Virus Detection

1. Nanoparticle-Based Detection Systems

Nanotechnology has introduced innovative approaches for virus detection, leveraging the unique properties of nanoparticles to enhance sensitivity and specificity. Nanoparticles, such as gold nanoparticles (AuNPs) and quantum dots, are used as labels or signal amplifiers in biosensing platforms [35].

- **Gold Nanoparticles (AuNPs):** AuNPs are widely used in colorimetric assays due to their unique optical properties. In the presence of a target virus, AuNPs aggregate, causing a visible color change. This principle has been employed for the detection of TMV and other plant viruses.
- **Quantum Dots:** Quantum dots are semiconductor nanocrystals that exhibit size-dependent fluorescence properties. They are used in fluorescence-based biosensors for detecting viral proteins or nucleic acids with high sensitivity. Quantum dot-based assays have shown potential in detecting plant viruses such as PPV and CMV, providing quantitative data and multiplexing capabilities [36]. Nanoparticle-based detection systems are not only sensitive and specific but also allow for miniaturization and integration into portable devices, making them suitable for on-site diagnostics.

2. Field-Deployable Biosensors for Rapid Diagnosis

Field-deployable biosensors represent a significant advancement in plant virus diagnostics, enabling real-time monitoring and rapid decision-making. These biosensors integrate biological recognition elements (e.g., antibodies, nucleic acids) with transducers that convert the recognition event into a measurable signal, such as electrical, optical, or electrochemical signals [37].

- **Electrochemical Biosensors:** These sensors detect viral nucleic acids or proteins based on changes in electrical signals. They are highly sensitive and can detect low concentrations of viral targets in complex plant tissues. Electrochemical biosensors have been developed for the detection of viruses like PVY and BBTV.
- **Optical Biosensors:** Optical biosensors utilize changes in light properties (e.g., fluorescence, absorbance) upon binding of the viral target to the recognition element.

These sensors are capable of providing rapid and quantitative results and have been used to detect a variety of plant viruses [38].

Table:2 Technological Advances in Detection and Diagnosis of Plant Viruses (Sources: [28], [29], [34], [36])

Detection Technology	Principle	Advantages	Examples/Applications
Enzyme-Linked Immunosorbent Assay (ELISA)	Detection of virus-specific antigens using antibodies.	High sensitivity, cost-effective, easy to perform.	Tomato spotted wilt virus (TSWV) detection in solanaceous crops
Western Blotting	Separation of viral proteins followed by detection with specific antibodies.	High specificity, suitable for protein confirmation.	Tobacco mosaic virus (TMV) protein analysis
Polymerase Chain Reaction (PCR)	Amplification of specific DNA sequences for virus identification.	High sensitivity, allows for early detection.	Detection of Banana bunchy top virus (BBTV)
Reverse Transcription PCR (RT-PCR)	Conversion of viral RNA into cDNA followed by PCR amplification.	Effective for RNA viruses, highly sensitive and specific.	Papaya ringspot virus (PRSV) detection in papaya
Real-Time PCR (qPCR)	Quantitative detection of viral DNA/RNA using fluorescence.	Real-time monitoring, quantifies viral load.	Quantification of Cucumber mosaic virus (CMV) in cucurbits
Loop-Mediated Isothermal Amplification (LAMP)	Amplifies viral DNA/RNA at a constant temperature.	Rapid, cost-effective, no need for thermal cyclers.	Tomato yellow leaf curl virus (TYLCV) in tomato fields
Next-Generation Sequencing (NGS)	High-throughput sequencing of viral genomes for comprehensive analysis.	Detects novel viruses, high resolution.	Identification of unknown viruses in mixed infections
CRISPR-Based Diagnostics (CRISPR-Cas)	Uses CRISPR-Cas systems to detect viral nucleic acids.	Ultra-sensitive, rapid, and portable.	Detection of Potato virus Y (PVY) in potato
Biosensors	Utilizes biological molecules to detect virus presence electrically or optically.	Real-time, on-site diagnostics, highly sensitive.	Detection of Tomato leaf curl virus (ToLCV)
Digital PCR (dPCR)	Partitioning of samples for precise quantification of viral load.	Extremely accurate, detects low viral concentrations.	Quantification of Grapevine fanleaf virus (GFLV)
Microarray-Based	Detection of	High throughput,	Detection of mixed viral

Detection	multiple viral pathogens simultaneously using hybridization.	useful for complex samples.	infections in ornamental plants
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Table:3 Comparison of Plant Pathogen Detection Techniques (Sources- [35], [36])

Method	Detected Pathogen	Minimum Detectable Quantity	Time Required
Droplet Digital PCR (ddPCR)	<i>Botrytis cinerea</i>	2.67 copies/ μ L of DNA	~minutes
LAMP	<i>Phytophthora agathidicida</i>	1 fg DNA	60 min
Multiplex RPA with SERS	<i>Botrytis cinerea, P. syringae</i>	2 genomic copies	40 min
Quantum Dot Biosensor	<i>Citrus tristeza virus</i>	220 ng/mL	–
Lateral Flow Immunoassay	<i>Potato virus Y</i>	330–5.4 ng/mL	15 min

V. Current Strategies for Controlling Plant Viral Diseases

Plant viral diseases are among the most challenging issues in agricultural production, as viruses lack effective curative treatments once the infection is established (Table 4). Therefore, managing plant viral diseases primarily focuses on preventative strategies, including traditional agricultural practices, breeding for resistance, biotechnological interventions, and chemical and biological controls. Each approach contributes uniquely to disease management, often requiring integration for optimal results.

A. Traditional Control Methods

1. Cultural Practices (Crop Rotation, Sanitation)

Cultural practices form the cornerstone of traditional disease management by minimizing the exposure of crops to viral inoculums [39]. Crop rotation is a time-tested strategy that involves alternating susceptible and non-susceptible crops in the same field across growing seasons. This practice disrupts the life cycles of viruses and their vectors by depriving them of their preferred hosts. For example, rotating cereal crops with non-host plants can reduce the incidence of Barley yellow dwarf virus, as its aphid vectors do not survive on alternative hosts. Sanitation is equally critical in controlling plant viruses. It involves the removal and destruction of infected plants and plant debris that can harbor viruses or their vectors. Ensuring clean tools, seeds, and planting materials is essential to prevent the mechanical transmission of viruses like Tobacco Mosaic Virus (TMV), which can persist in plant debris and on contaminated tools for extended periods. Eliminating volunteer plants and weeds, which often serve as reservoirs for viruses and vectors, helps reduce the viral inoculum in the field [40].

2. Vector Control Strategies

Since many plant viruses rely on insect vectors for transmission, controlling these vectors is an essential component of traditional virus management. Insecticides have been widely used

to manage vector populations such as aphids, whiteflies, and thrips, which are responsible for transmitting viruses like Tomato yellow leaf curl virus (TYLCV) and Cucumber mosaic virus (CMV). The indiscriminate use of insecticides can lead to resistance development in vector populations, environmental contamination, and harm to beneficial insects. Integrated Pest Management (IPM), which combines chemical, biological, and cultural practices, offers a more sustainable approach. IPM includes the use of natural predators, insect traps, and reflective mulches to deter vectors. Reflective mulches, for example, repel aphids and whiteflies by disorienting their visual navigation, reducing the incidence of viral diseases they spread [41]. Planting barrier crops that serve as physical barriers or decoys to attract vectors away from the main crop can also reduce virus transmission.

Table:4 Current Strategies for Controlling Plant Viral Diseases (Sources: [38], [40], [41])

Strategy Type	Mode of Action	Examples
Genetic Resistance	Use of resistant plant varieties that contain specific resistance genes.	Tomato mosaic virus (ToMV) resistant tomatoes, Potato virus Y (PVY) resistant potatoes
Cross Protection	Pre-inoculation with a mild strain to protect against severe strains.	Citrus tristeza virus (CTV) management in citrus crops
RNA Interference (RNAi)	Gene silencing through small interfering RNA molecules to inhibit viral replication.	Papaya ringspot virus (PRSV) resistance in transgenic papaya
CRISPR/Cas9 Genome Editing	Targeted editing of plant genomes to disrupt viral susceptibility genes.	Beet curly top virus (BCTV) resistance in sugar beet
Chemical Control	Application of antiviral chemicals to inhibit virus replication or vector transmission.	Imidacloprid against aphid-transmitted viruses
Biological Control	Use of beneficial organisms to suppress virus-carrying vectors.	Release of parasitoids like <i>Encarsia formosa</i> to control whitefly-transmitted viruses
Cultural Practices	Implementing crop rotation, sanitation, and vector control to reduce virus spread.	Use of reflective mulches to deter whiteflies
Quarantine and Certification	Restricting movement of infected plant materials and ensuring pathogen-free seeds.	Virus-free certification for potato tubers
Vaccination and Biocontrol Agents	Application of plant vaccines or beneficial microbes to enhance resistance.	Plant Growth-Promoting Rhizobacteria (PGPR) against Tobacco mosaic virus (TMV)
Heat Therapy and Meristem Culture	Use of high temperatures or tissue culture techniques to eliminate viruses from infected plants.	Elimination of viruses from banana and potato tissue cultures

B. Genetic Resistance in Plants

1. Breeding for Resistance (Conventional Breeding Techniques)

Breeding for genetic resistance is one of the most effective and sustainable approaches to managing plant viral diseases. Conventional breeding involves selecting and crossing plants with naturally occurring resistance genes to develop resistant varieties. These resistance genes, often referred to as R genes, provide plants with the ability to recognize and combat specific viruses. For example, resistance to Potato virus Y (PVY) in potatoes has been

introduced through conventional breeding programs that incorporate the Rysto gene from wild potato species *Solanum stoloniferum*. The N gene from wild tobacco (*Nicotiana glutinosa*) has been bred into cultivated tobacco to confer resistance to TMV [42]. Conventional breeding is a time-consuming process, as it involves multiple generations of crossing, selection, and evaluation. It remains a cornerstone of plant virus management due to its cost-effectiveness and the long-lasting resistance it provides.

2. Use of Resistant Varieties and Hybrids

The deployment of virus-resistant varieties and hybrids has significantly reduced the impact of plant viral diseases. These varieties are developed through either traditional breeding or genetic engineering and are widely adopted by farmers to prevent disease outbreaks. Resistant tomato varieties have been developed to combat TYLCV, a devastating disease in tomato production. The resistance gene Ty-1, which encodes an RNA-dependent RNA polymerase (RdRp), provides broad-spectrum resistance by enhancing the plant's ability to degrade viral RNA. Similarly, hybrids of maize resistant to Maize streak virus (MSV) have been developed and successfully deployed in Africa, significantly reducing yield losses [43].

C. Biotechnological Approaches

1. RNA Interference (RNAi)-Based Technologies

RNA interference (RNAi) is a powerful gene-silencing mechanism that has been harnessed to develop virus-resistant plants. RNAi-based technologies involve the introduction of double-stranded RNA (dsRNA) corresponding to viral genes into the plant, which triggers the plant's RNA silencing machinery to degrade the viral RNA, thereby preventing replication and spread. RNAi has been successfully used to engineer resistance to a variety of plant viruses. For example, transgenic papaya expressing a coat protein gene of Papaya ringspot virus (PRSV) effectively silences the virus and confers high levels of resistance [44]. Similarly, RNAi has been employed to confer resistance to CMV in transgenic tobacco and tomato plants. This approach is particularly promising because it can provide resistance to multiple viruses simultaneously if the dsRNA targets conserved viral sequences.

2. CRISPR/Cas Systems for Virus Resistance

The advent of CRISPR/Cas technology has opened new avenues for engineering virus-resistant crops. CRISPR/Cas systems, particularly Cas9 and Cas12a, can be programmed to target and cleave viral DNA or RNA, thereby disrupting the replication cycle of DNA and RNA viruses. For example, CRISPR/Cas9 has been used to target the genome of Tomato yellow leaf curl virus (TYLCV), resulting in reduced viral accumulation and symptom severity in tomato plants [45]. Similarly, CRISPR/Cas13, which targets RNA, has been used to confer resistance to Turnip Mosaic Virus (TuMV) in Arabidopsis, demonstrating the versatility of this technology in combating RNA viruses. CRISPR/Cas systems offer the advantage of high specificity and the ability to target multiple viral strains by designing guide RNAs against conserved viral regions.

3. Development of Virus-Resistant Transgenic Plants

Transgenic approaches involve the introduction of foreign genes into plants to confer virus resistance. One of the most notable successes is the development of transgenic papaya

resistant to PRSV, which saved the Hawaiian papaya industry from collapse. The transgenic papaya expresses the coat protein gene of PRSV, which confers resistance through a mechanism similar to RNAi [46]. Another example is transgenic potatoes expressing the PVY coat protein gene, which exhibit resistance to multiple PVY strains. The development of virus-resistant transgenic plants has been particularly beneficial for crops where conventional breeding is difficult or time-consuming. The adoption of transgenic crops faces regulatory hurdles and public acceptance issues, particularly in regions where genetically modified organisms (GMOs) are contentious.

D. Chemical and Biological Control

1. Use of Antiviral Chemicals

The use of antiviral chemicals in plant virus management is limited due to the difficulty in targeting viruses without affecting the host plant. Some chemicals can inhibit viral replication or interfere with virus-vector interactions. For example, salicylic acid (SA) and its derivatives can enhance plant defense mechanisms, including the RNA silencing pathway, leading to reduced viral replication [47]. Other chemical agents, such as plant growth regulators and induced resistance compounds, can prime plants to better resist viral infections. Although antiviral chemicals are not widely used due to efficacy and environmental concerns, ongoing research aims to identify new compounds with improved specificity and safety profiles.

2. Biocontrol Agents (e.g., Beneficial Microorganisms, Natural Plant Compounds)

Biological control agents, including beneficial microorganisms and natural plant compounds, are emerging as sustainable alternatives to chemical treatments. Certain rhizobacteria and endophytic fungi can induce systemic resistance in plants, enhancing their ability to withstand viral infections. For example, *Bacillus subtilis* has been shown to induce systemic resistance in plants against Cucumber Mosaic Virus (CMV) by activating the plant's immune system [48]. Natural plant compounds, such as flavonoids, alkaloids, and essential oils, also exhibit antiviral activity. These compounds can directly inhibit viral replication or enhance plant defense responses. Extracts from neem (*Azadirachta indica*) and garlic (*Allium sativum*) have shown efficacy against a range of plant viruses in laboratory settings.

Conclusion

Managing plant viral diseases remains a significant challenge due to the complexity of virus-host interactions, the rapid evolution of viruses, and the influence of environmental factors such as climate change. While traditional control methods, genetic resistance, and biotechnological advances have made considerable progress, limitations persist in achieving broad-spectrum, durable resistance and adapting strategies to emerging threats. Innovative approaches such as systems biology, synthetic biology, and precision agriculture offer promising avenues for improving our control of plant viruses. International collaboration and enhanced biosecurity measures are crucial for tackling the transboundary nature of viral diseases.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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