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REVIEW

Citrus Viroids: A New Frontier in Virus and Virus-Like Pathogens in the Citrus Growing Areas

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ABSTRACT: Citrus viroids are small non-coding RNA pathogens that pose a significant threat to global citrus production by reducing fruit yield, quality, and tree longevity. Several viroids, including Citrus exocortis viroid (CEVd), Hop stunt viroid (HSVd), Citrus bent leaf viroid (CBLVd), and newly identified members such as Citrus Viroid VI (CVd-VI) and Citrus Viroid VII (CVd-VII) have been reported from diverse citrus-growing regions. These pathogens are transmitted mainly through vegetative propagation, contaminated tools, and occasionally via seed or pollen, making their management complex. This review synthesizes current knowledge on the biology, structural diversity, transmission, symptomatology, detection, and economic impact of citrus viroids. In addition to compiling existing findings, it emphasizes critical challenges such as understanding host–pathogen molecular interactions, the implications of viroid infections under climate change, and the limited availability of resistant rootstocks. Recent advances in diagnostic tools, including Reverse Transcription Polymerase Chain Reaction (RT-PCR), Quantitative Polymerase Chain Reaction (qPCR), High-throughput sequencing (HTS), and *in silico* approaches, are evaluated alongside practical constraints in low-resource settings. Furthermore, the review highlights management strategies focused on certified planting material, sanitation, resistant genotypes, and integration into global citrus certification programs. By consolidating existing information while outlining key knowledge gaps and future directions, this work provides a foundation for developing sustainable strategies to mitigate the impact of viroids on the citrus industry.

KEYWORDS: Citrus; viroids; biology; evolution and management; diagnostics; citrus certification programs

1 Introduction

1.1 Citrus Viroids, Isolates, Etiology, and Geographical Distribution

Citrus is one of the most widely cultivated fruit crops globally, but its production is threatened by several viruses and virus-like pathogens, including viroids, which represent a unique group of sub-viral agents. Citrus viroids are tiny pathogens that lack coding ability and consist of circular RNA genomes, but can severely affect citrus crops by affecting the fruit yields and quality [1]. The genome size of viroids ranges from 246 to 401 nucleotides and depends entirely on the cellular machinery of the host for replication [2]. Some of the most significant citrus viroids include Citrus exocortis viroid [CEVd], Citrus bent leaf viroid (CBLVd), Hop stunt viroid (HSVd), Citrus bark cracking viroid (CBCVd), Citrus dwarfing viroid (CDVd), and Citrus viroids V [3] and VI (CVd-V, CVd-VI) [4]. Citrus viroids are categorized into two different families, Pospiviroidae and Avsunviroidae (Table 1). There is a central conserved region (CCR) in Pospiviroidae, while Avsunviroidae lacks a CCR but utilizes hammerhead ribozymes for self-cleavage, respectively [5].

Citrus viroids exhibit different symptoms, like CEVd, which causes bark scaling, stunted growth, and reduced yield [6], while CBLVd causes leaf deformation and poor fruit quality [7]. HSVd infects multiple citrus species and causes symptoms like leaf chlorosis and stunting in trees [8]. Nursery rootstocks get infected by Citrus bark cracking viroid, which causes bark cracking and plant death [9]. CDVd produces symptoms like tree dwarfing, which can be beneficial for high-density planting, but still requires careful management [10]. Viroids often present in mixed infections, and this synergy can intensify disease severity while the diagnosis of the disease becomes more challenging [11].

Transmission mainly occurs through infected plant materials such as budwood and grafts, as well as via contaminated tools [12]. Unlike viruses, viroids lack insect vectors, which makes human-mediated movement the primary mode of spread [13]. They can remain latent for long periods, creating hurdles in early detection. Symptoms include stunting, chlorosis, bark cracking, and poor fruit development, which ultimately reduce economic viability [6]. A study of the distribution and prevalence of citrus viroids is necessary for the evaluation of their impact on citrus production. Reports show that citrus viroids, e.g., CEVd, CVd-III, CVd-IV, HSVd, and CVd-V, are widespread in geographical locations including Greece, Palestine, Japan, and Pakistan. Major citrus viroids have been reported to be found worldwide, but there is less knowledge present for viroids like CVd-VI and CVd-VII [14,15].

Viroids, including CEVd, CBLVd, HSVd, CVd-III, CVd-IV, and CVd-V, have been reported in Africa with cases in Sudan [16] and in the Middle East [17]. These have been found in the Turkey and Palestine region. Reports from Europe confirm the presence of citrus viroids in Italy and in South America; they have been found in Uruguay and Costa Rica areas [18,19]. CEVd has also been identified even in ornamental plants and weeds which are associated with citrus orchards and highlight the broader ecological impact of this viroid [20]. HSVd co-occurring with CEVd and CDVd in mixed viroid infections and found in Greece, etc., is another main cause to intensify disease severity, and this synergy makes management more challenging [21]. HSVd was identified in Japan and reported [22]. CVd-V has been reported in Asia and Punjab, Pakistan, by [15]. CVd-VII has been identified in Japan and North Africa, and recently reported its first case of CVd-V [23] along with CVd-V in China and Australia, which shows the global prevalence of citrus viroids across various geographies [24].

Effective management of viroid disease of citrus depends on accurate diagnostics strategies, and the infection can be avoided to enter into new locations by using viroid-free planting materials and strict budwood certification programs. Quarantine regulations are very important to restrict the movement of infected plant material. Genetic resistance and cross-protection techniques can be a long-term solution for managing these infections. A deeper understanding of viroid diversity and viroid-host interactions is necessary to develop sustainable management strategies for citrus viroid disease management. Although scattered information on different citrus viroids is available, it is limited to molecular detection and characterization. Therefore, this review consolidates current information on the biology, detection, epidemiology, and management of citrus viroids for sustainable fruit production. Critical gaps in knowledge, such as the need for resistant rootstocks and adaptation strategies under climate change, have also been identified. This perspective distinguishes it from earlier information, reviews, and highlights directions for future research.

Table 1: Citrus viroids infecting different citrus varieties.

Viroid	Previous Name	Isolates	Family	Genus	Distribution	Reference
Citrus exocortis viroid (CEVd)	Citrus exocortis viroid (CEVd)	V1, OP925746	Pospiviroidae	Pospiviroid	Mediterranean basin, USA, South America, Central America, Caribbean, Asia, Africa, Middle East	[6,17,25–27]
Hop stunt viroid (HSVd)	Citrus cachexia viroid/Citrus viroid II (CVd-II)	Plum-type, hop-type, and citrus-type	Pospiviroidae	Hostuviroid	Slovenia, China (Xinjiang), Japan, Australia (grapevines), Germany, Finland (cucumbers), Netherlands, Trinidad, Tobago, Venezuela	[28,29]
Citrus bent leaf viroid (CBLVd) Isolates: CVd-Ia, CVd-Ib, CVd-I-LSS	Citrus viroid I (CVd-I)	CVd-Ia, CVd-Ib, CVd-I-LSS. (CVd-I-LSS Pakistan variant)	Pospiviroidae	Apscaviroid	UAE, Italy, Israel, Pakistan, China, Cambodia, Australia, Malaysia, Iran, Japan, Argentina, Crete (Greece). CVd-I-LSS is prominent in Pakistan and China.	[27,30,31]
Citrus viroid-III	Citrus dwarfing viroid (CDVd)	CVd-III-PS-1, CVd-III-PS-2, OP902248, OP902249	Pospiviroidae	Apscaviroid	Greece (Argolida, Chania, Heraklio, Rethimno, Arta)	[9,17,32]
Citrus bark cracking viroid	Citrus viroid-IV (CVd-IV)	CBCVd-LSS, OP902247	Pospiviroidae	Cocadviroid	Argolida, Chania, Heraklio, Rethimno	[17,27,33]
Citrus viroid-V (CVd-V)		CVd-VCA, CVd-VST, CVd-VNE	Pospiviroidae	Apscaviroid	Oman, California (USA), Spain, Nepal, Spain (Valencia)	[34,35]
Citrus viroid-VI (CVd-VI)		CVd-OS	Pospiviroidae	Apscaviroid	Japan (origin), China, Pakistan	[22,31,36,37]

1.2 Economic Impact on Citrus Production and Trade

Citrus viroids have an impact on citrus production and trade. These viroid diseases can reduce yield and increase management costs, ultimately creating market and economic challenges. Infected citrus orchards show lower fruit quality, which leads to decreased yields. Research on CEVd reported that infected citrus produce fewer large fruits than healthy ones and cause financial losses over multiple growing seasons [38,39]. Management of viroid infections requires additional labor and resources to monitor regular symptoms, infected tree removal, and careful measures during pruning or grafting, which ultimately increase the costs [27,40]. Infected fruits often suffer from malformations due to viroid-induced stress, which reduce their marketability and consumer appeal [17,39]. Quarantine measures or restrictions imposed on trade can increase the economic burden on citrus-exporting countries, which reduces their profitability [27]. The presence of viroid infection significantly influences rootstock selection, compelling growers to invest in resistant varieties or less susceptible rootstocks that impose additional costs for obtaining such materials [39,41]. Citrus viroids pose a significant threat to the sustainability of citrus production, and if not correctly managed, this disease can destroy the productivity of citrus globally [27,40].

2 Structural Differences of Citrus Viroids

2.1 Citrus Exocortis Viroid (CEVd)

Citrus Exocortis Viroid (CEVd) is a viroid having circular and single-stranded RNA viroid with a genome size in the range of 368 to 375 nucleotides (nt), while a few variants of CEVd can have a size up to 463–467 nucleotides [20]. CEVd was first identified in 1948 by Fawcett and Klotz, and later it was recognized as the pathogen causing citrus exocortis disease [42]. It has the ability to from a highly base-paired rod-like conformation, which is necessary and helpful for its infectivity [43]. The variability domain of CEVd shows significant genetic diversity due to frequent insertions and deletions in the genome, while its pathogenicity domain is responsible for inducing bark scaling and stunted growth in infected citrus trees [6].

2.2 Citrus Hop Stunt Viroid (HSVd)

Citrus Hop Stunt Viroid (HSVd) was detected for the first time in hops (*Humulus lupulus*) in 1977 [44] and later found associated with citrus species [45]. Genome size of HSVd is approximately 298–303 nt and is present in a stable rod-like RNA conformation [46]. The variability domain of HSVd demonstrates the moderate sequence diversity while its pathogenicity domain causes the severe stunting and fruit deformation in infected citrus cultivars [45].

2.3 Citrus Bent Leaf Viroid (CBLVd)

Citrus Bent Leaf Viroid (CBLVd) was known as Citrus Variable Viroid (CVaV) previously and was first studied in 1977 for the first time [47]. It belongs to the *Apscaviroid* genus with a genome size of 315–330 bp [7]. Its structure is a circular RNA forming a rod-like secondary conformation (Chebli & Afechtal, 2018). The variability domain is present as the three recognized variants of CBLVd, which are CVd-Ia, CVd-Ib, CVd-I-LSS [48], while the pathogenicity domain is responsible for the infections and symptoms formation, like leaf bending and potential yield reductions [49].

2.4 Citrus Viroid III (CVd-III)—Citrus Dwarfing Viroid (CDVd)

Citrus Viroid III (CVd-III) was previously named Citrus Dwarfing Viroid (CDVd), and it is a member of the *Apscaviroid* genus. It has a genome size of approximately 294–300 nt [47], and it was identified in citrus trees first. CDVd has a rod-like RNA structure [9]. The variability domain shows close resemblance to the *Citrus Exocortis Viroid*, while its pathogenicity domain causes dwarfing and reduced fruit production effects in infected plants [50].

2.5 Citrus Viroid IV (CVd-IV)—Citrus Bark Cracking Viroid (CBCVd)

Citrus Viroid IV (CVd-IV) is also known as Citrus Bark Cracking Viroid (CBCVd), and it was first identified in citron, while later its association with severe bark cracking symptoms in trifoliate orange (*Poncirus trifoliata*) was identified [51]. It has a genome size of 284–288 bp and belongs to the *Cocadviroid* genus [52]. The circular RNA structure of CVd-IV forms a rod-like conformation [10], which has the variability domain that helps in strain diversity, while the pathogenicity domain causes the severe bark scaling and cracking symptoms [53,54].

2.6 Citrus Viroid V (CVd-V)

Citrus Viroid V (CVd-V) was first reported in the early 2000s [3], and it has a genome size of 293–294 nt bp, which forms an RNA structure in rod shape with a GC-rich sequence (68.7% paired nucleotides) [34]. The variability domain of CVd-V consists of minimal sequence deviation, and the pathogenicity domain interacts with other viroids and creates a synergistic effect that ultimately influences disease severity [3,55].

2.7 Citrus Viroid VI (CVd-VI)

Citrus Viroid VI (CVd-VI) had the name Citrus Viroid-D (CVd-OS) previously, and it was first reported in Japan from the citrus cultivar *Shiranui* [22]. Its genome size ranges from 326 to 331 nt and has a rod-like circular RNA structure [56,57]. The variability domain has a similar conserved region with other *Apscaviroid* members, but the sequence similarity with other viroids is only 68% [58]. The pathogenicity domain remains unclear and less studied, but is suspected to cause different citrus growth abnormalities [59].

2.8 Citrus Viroid VII (CVd-VII)

Citrus Viroid VII (CVd-VII) is a recently discovered viroid and is categorized in the *Apscaviroid* genus (Table 2). It has been identified in research stations in Dareton, New South Wales, Australia [4], while its Chinese variant has been reported as well, and the genome size is 326 to 331 nt [58]. It has a rod-like RNA conformation [32] while its Pathogenicity (P) Domain is associated with the ability to cause disease symptoms in host plants [60]. Mutations here can alter symptom severity. The Variability domain contributes to the adaptability and host range [10].

Table 2: Comparative citrus viroids.

Viroid	Genome Size (nt)	Structure	Variability Domain	Pathogenicity Domain	References
CEVd	369–394	Circular, rod-like	High genetic variation (insertions/deletions)	Bark scaling, stunted growth	[6,20,42,43]
HSVd	246–401	Circular, rod-like	Moderate sequence diversity	Stunting, fruit deformation	[8,44,46]
CBLVd	330–340	Circular, rod-like	Three variants (CVd-Ia, CVd-Ib, CVd-I-LSS)	Leaf bending, yield reduction	[7]
CVd-III (CDVd)	291–296	Circular, rod-like	Similar to CEVd	Dwarfing, reduced fruit production	[9,47]
CVd-IV (CBCVd)	275	Circular, rod-like	Strain diversity	Severe bark scaling and cracking	[10,54]
CVd-V	293–294	Circular, rod-like	Minimal sequence variation	Interacts with other viroids, modifying severity	[3,35,61]
CVd-VI	328–331	Circular, rod-like	68% sequence similarity with other viroids	Minor citrus abnormalities	[22,56–59]
CVd-VII	369–394	Circular, rod-like	High sequence variability	Mild slight stunting or leaf epinasty and still not studied extensively	[4,24,32]

3 Transmission and Host Range of Citrus Viroids

Citrus viroids exhibit various symptoms, host specificity, and transmission pathways that influence their spread and impact citrus production (Table 3). CEVd [32] affects multiple citrus species and spreads primarily through contaminated tools, grafting, and mechanical means with no known insect vectors [31]. CBLVd is associated with reduced fruit yield and detected in sweet oranges and some hybrid citrus varieties, and is transmitted through grafting and mechanical means, though seed transmission remains uncertain [60]. HSVd infects citrus, hops, and other woody hosts species, which leads to reduced tree vigor, smaller fruit size, and occasional leaf yellowing [62]. It is primarily spread through contaminated tools, grafting material, and possibly by pollen or seeds [63]. CVd-III induces symptoms like gumming, leaf epinasty, and vein necrosis, particularly in sweet oranges, mandarins, and lemon cultivars [17]. This viroid is transmitted mechanically, and through infected propagation material [32]. CVd-IV causes bark cracking and gumming and reduces tree longevity [64]. It primarily infects oranges, mandarins, and grapefruit. At the same time, it spreads through mechanical means, grafting and seed transmission [65] CVd, CVD-VI, and CVd-VII often result in latent infections, but can take part in tree decline when occurring alongside other viroids [4]. These viroids have been infecting various citrus species and are transmitted through mechanical means or grafting [32]. While the possibility of seed transmission has been reported but remains controversial. The presence and transmission of Citrus exocortis viroid (CEVd) through seeds in ornamentals like Impatiens and Verbena, with infection rates as high as 66% in fresh seedlings but declining to 26% after two years of storage [66]. However, conclusive evidence in citrus remains scarce, and the epidemiological significance of this pathway is still debated.

Table 3: Symptoms, host range, and transmission methods of citrus viroids.

Viroid	Symptoms	Host Range	Transmission Modes	References
Citrus Exocortis Viroid (CEVd)	Stunted growth, bark scaling, cracking	Multiple citrus species, especially trifoliate orange	Contaminated tools, grafting, mechanical means, no insect vectors	[6,25]
Hop Stunt Viroid (HSVd)	Reduced tree vigor, smaller fruit, leaf yellowing	Citrus, hops, and other woody hosts	Contaminated tools, grafting, possibly by pollen or seeds	[8]
Citrus Bent Leaf Viroid (CBLVd)	Mild leaf bending, delayed growth, reduced yield	Sweet oranges, hybrid citrus varieties	Grafting, mechanical transmission, uncertain seed transmission	[17,30]
Citrus Viroid III (CVd-III)	Gumming, leaf epinasty, vein necrosis	Sweet oranges, mandarins, lemons	Mechanical transmission, infected propagation material	[9,67]
Citrus Viroid IV (CVd-IV)	Bark cracking, gumming, reduced tree longevity	Oranges, mandarins, grapefruit	Mechanical means, grafting, possibly seed transmission	[17,33]
Citrus Viroid V (CVd-V)	Latent infection contributes to tree decline	Various citrus species	Mechanical transmission, grafting, unclear seed transmission	[34,68]
Citrus Viroid VI (CVd-VI)	Often latent but may weaken trees	Sour orange	Mechanical transmission, grafting	[17,22]

3.1 Detection of Citrus Viroids

Citrus viroids are essential pathogens that affect the health and productivity of citrus plants. Their detection is crucial for effective management and disease control. Various methods, from biological observations to advanced molecular techniques, have been developed for citrus viroid detection (Table 4, Fig. 1). Visual inspection remains the most basic approach, where symptoms such as leaf curling, stunted growth, and bark cracking may indicate viroid infections. However, these symptoms are not definitive since they can be caused by other factors, including environmental stress and other pathogens [68]. Molecular methods such as reverse transcription polymerase chain reaction (RT-PCR) are widely used to achieve accurate diagnosis. RT-PCR enables the amplification of viroid RNA from plant tissues, offering high sensitivity and specificity in detecting viroid infections [69,70]. Multiplex RT-PCR further enhances diagnostic efficiency by allowing the simultaneous detection of multiple viroids in a single reaction, making it particularly useful for large-scale testing [36,71]. However, implementation of these advanced molecular techniques can be challenging in low-resource regions due to high costs, specialized equipment and technical expertise requirements. Another significant technique is real-time RT-PCR (qPCR), which confirms the presence of viroids and quantifies their levels in infected tissues, providing insights into disease severity and progression [72,73].

Table 4: Methods for detecting citrus viroids and their salient features.

Detection Method	Salient Features	References
Visual Inspection	Observes symptoms like leaf curling and bark cracking but lacks specificity	[68]
RT-PCR	Highly sensitive and specific; detects low viroid loads	[70,74]
Multiplex RT-PCR	Simultaneously detects multiple viroids; useful for large-scale screening.	[36,71]
Real-Time RT-PCR (qPCR)	Provides quantitative data on viroid abundance, aiding disease monitoring	[72,73]
Dot-Blot Hybridization	Uses labeled probes for detection; useful as a confirmatory method	[72,74]
High-Throughput Sequencing (HTS)	Identifies known and novel viroids; useful for viroid discovery.	[75,76]
In Silico Detection	Analyzes sequencing data for viroid identification.	[77]
Lateral Flow Devices	Rapid field-based detection using antibodies or nucleic acid probes.	[71]

Besides PCR-based methods, hybridization techniques like dot-blot hybridization are also employed. This method involves transferring RNA onto a membrane and hybridizing it with labeled probes specific to the target viroid [72,74]. Although it is less common than PCR-based approaches, dot-blot hybridization remains useful for confirming viroid presence. High-throughput sequencing (HTS) has emerged as a powerful tool for detecting known and novel viroids. HTS enables direct RNA sequencing from plant tissues, allowing comprehensive analysis of viroid populations and discovery of new variants [75,76]. Additionally, *in silico* detection tools have been developed to analyze sequencing data and identify viroid-specific sequences, improving diagnostic accuracy [77]. Rapid diagnostic tests, such as lateral flow devices, are also being developed for field-based detection. These devices utilize antibodies or nucleic acid probes to deliver fast results, making them highly beneficial for real-time monitoring [72]. Despite advancements, viroid detection faces challenges such as asymptomatic infections, mixed infections, and the need for cost-effective diagnostic solutions. Many infected citrus trees do not exhibit symptoms until significant damage has occurred, complicating early detection efforts [32,78]. Mixed infections involving multiple viroid species further obscure diagnosis, as overlapping symptoms can lead to misidentification [79]. Moreover, molecular techniques require specialized equipment and trained personnel, challenging accessibility in resource-limited regions [80]. Standardization of diagnostic protocols across laboratories is also necessary to ensure consistency in results [81]. Future advancements in citrus viroid detection will likely focus on developing cost-effective, rapid, and field-deployable diagnostic assays, integrating molecular and serological methods, and improving bioinformatics tools to enhance viroid identification and monitoring [82]. While these molecular techniques offer high sensitivity and specificity, their application in low-resource countries presents practical challenges. The high cost of reagents, the need for specialized equipment, and limited technical expertise often restrict widespread use [11]. In many citrus-growing regions, laboratories may lack infrastructure for qPCR or HTS, leading to reliance on conventional RT-PCR or biological indexing.

Capacity building, affordable diagnostic kits, and decentralized testing facilities are critical for ensuring reliable surveillance in such contexts [43].

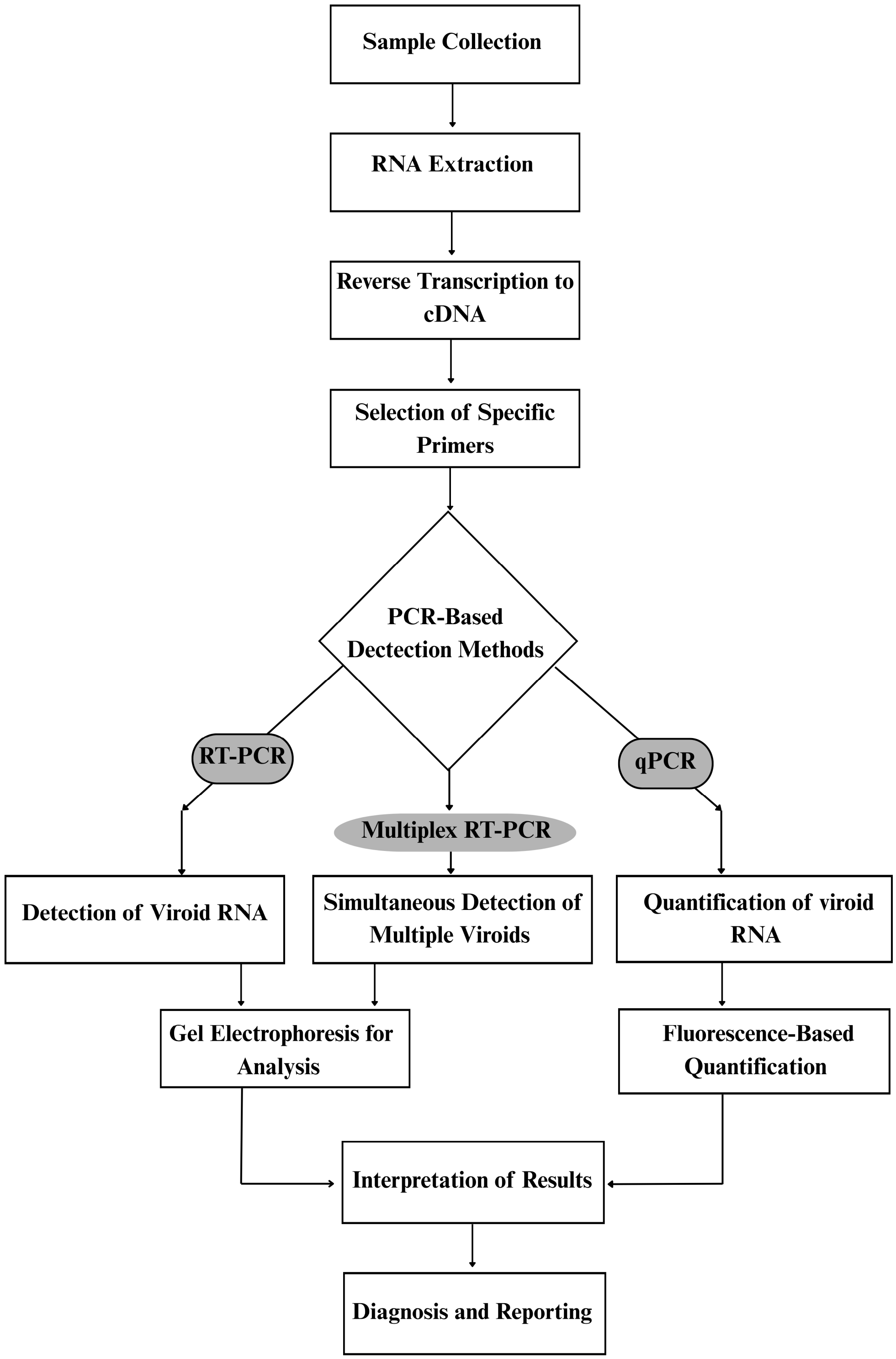

Figure 1: Workflow of molecular characterization techniques for citrus viroids, including RNA extraction, reverse transcription, PCR amplification, and sequencing steps.

3.2 Control and Management Strategies for Citrus Viroids

Currently, there are no direct chemical controls available for citrus viroids. Therefore, management must rely on preventive and integrated approaches. Certified disease-free planting material remains the cornerstone of viroid management. Strict sanitation practices, including disinfecting tools and avoiding contaminated propagation sources are equally essential. The use of resistant or tolerant rootstocks, where available, can mitigate symptom severity and improve orchard productivity. Cross-protection, using mild strains of certain viroids, has been explored in some cases though it requires careful monitoring to prevent unintended spread of more aggressive variants.

3.2.1 Quarantine Measures

Quarantine regulations are fundamental in preventing the introduction and spread of citrus viroids across regions and countries. Import regulations mandate rigorous inspection and testing of citrus plants before entry into a country, ensuring that only disease-free material is transported [83]. The USDA, for instance, enforces strict protocols for testing citrus plants for viroids such as Citrus Exocortis Viroid (CEVd) and Hop Stunt Viroid (HSVd) before import [84]. Regular monitoring programs further aid in the early detection and containment of viroid outbreaks. In Spain, national surveillance programs routinely inspect citrus orchards for viroid infections, enforcing phytosanitary standards [60]. Infected trees are often subjected to eradication protocols, which involve removing diseased plants and replacing them with certified, disease-free stock [85]. Additionally, public awareness campaigns educate growers on the risks of viroid infections and the importance of compliance with quarantine measures [86,87].

3.2.2 Citrus Certification and Registered Nurseries

Sanitary measures play a crucial role in minimizing the spread of citrus viroids. Certification programs ensure that nursery stock is tested and verified to be viroid-free before distribution [85]. For instance, the California Certified Nursery Stock (CCNS) program mandates testing of nursery stock for CEVd and other pathogens before approval for sale [88]. Clean stock programs further reinforce the use of disease-free planting material, regularly testing mother plants for viroid infections to maintain healthy propagation sources [60,83]. Proper sanitation during orchard management practices, such as disinfecting pruning tools with sodium hypochlorite or alcohol, reduces the mechanical transmission of viroids [89]. Training initiatives also educate citrus growers on recognizing viroid symptoms and implementing effective sanitation protocols [87].

3.2.3 Chemical and Biological Methods

While there are no direct chemical treatments for viroids, proper fertilization and pest management can enhance tree resilience against infections [90]. Some studies suggest that biostimulants may improve plant health, indirectly reducing the effects of viroid infections [32]. Biological control strategies are an emerging area of research. Beneficial microorganisms such as endophytic fungi show promise in enhancing plant defenses and outcompeting pathogens, though further studies are needed to establish their efficacy [91,92]. Integrated pest management (IPM) strategies, which focus on controlling aphid populations that may contribute to viroid transmission, are also considered effective [93].

3.2.4 Genetic Resistance and Breeding Strategies

Developing viroid-resistant citrus cultivars remains a long-term solution. Some citrus rootstocks, such as trifoliate orange (*Poncirus trifoliata*), exhibit tolerance to CEVd and are being explored for commercial use [94]. Advances in molecular biology have enabled marker-assisted selection, allowing breeders to identify genetic markers associated with resistance traits [95]. Genetic engineering is also being explored as a potential tool, although commercial application remains in its early stages [96]. Field trials assessing the performance of resistant hybrids in natural conditions provide valuable insights for breeding programs [97].

3.3 Mechanism of Infection by Citrus Viroids

Citrus viroids infect host plants through a non-coding RNA-based mechanism, relying entirely on the host's cellular machinery for replication and systemic movement (Fig. 2). The primary mode of viroid entry into citrus plants is through grafting, mechanical wounds, or vegetative propagation, although seed transmission has been observed in some cases [98]. Once inside the plant, viroids move through plasmodesmata, the microscopic channels connecting plant cells, and are further transported via the phloem network, enabling systemic infection [99]. Unlike viruses, viroids do not encode proteins; instead, they replicate autonomously through a rolling-circle mechanism. This replication process is mediated by host DNA-dependent RNA polymerase II, which the viroid hijacks to transcribe its RNA genome [52]. The replication cycle involves circular or linear RNA intermediates that undergo cleavage and ligation to generate mature viroid RNA molecules capable of further infection [100].

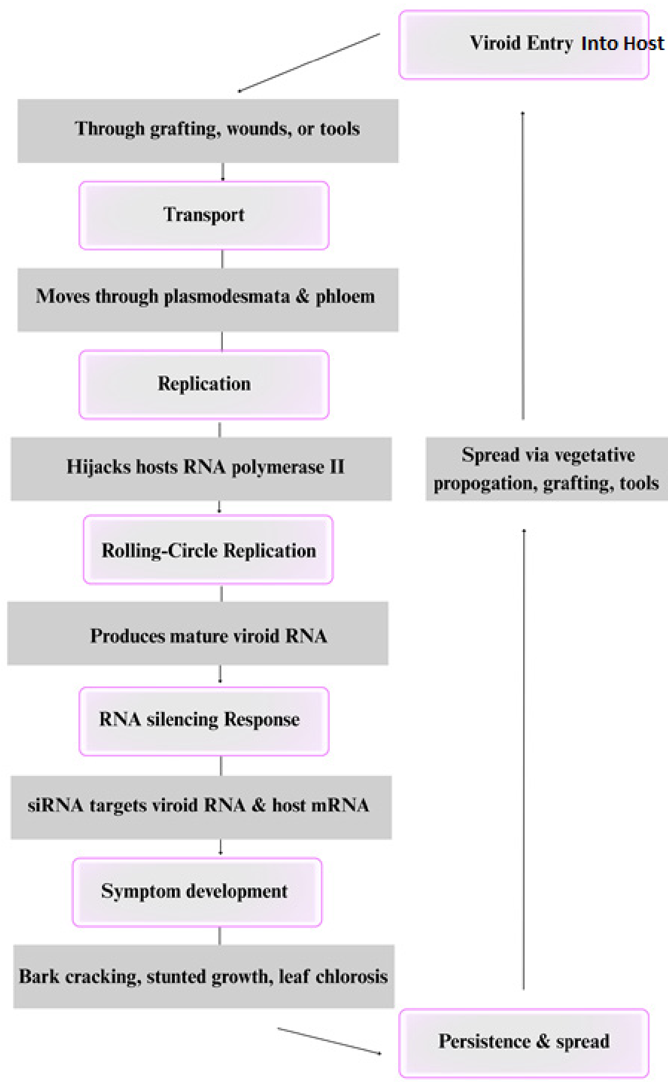

Figure 2: Transmission and infection cycle of viroids in the citrus host.

Once viroids accumulate in host cells, they trigger the plant's RNA silencing defense mechanisms, producing small interfering RNAs (siRNAs) that target both viroid RNA and, in some cases, host mRNA [84]. These siRNAs serve a dual function: on the one hand, they contribute to plant defense by degrading viroid RNA; on the other, they can inadvertently silence host genes, leading to symptoms such as bark cracking, leaf chlorosis, and stunted growth [61]. The severity of these symptoms is influenced by viroid strain, host genotype, and environmental factors [26]. In cases of co-infection, viroids can interact in synergistic or antagonistic ways, altering disease severity. Some mild viroid strains, such as mild CEVd, can offer cross-protection against more severe strains by pre-activating host defenses through siRNA pathways, thereby reducing symptom severity [99,100]. Conversely, certain viroid combinations, such as CBLVd with CVd-IV or CDVd, have been observed to exacerbate symptoms, leading to increased bark cracking and growth reduction [11]. Citrus viroids persist as systemic infections and are efficiently transmitted through vegetative propagation, grafting, and contaminated tools, making them a major concern for citrus production worldwide [101]. Given their significant impact on citrus health, understanding their infection mechanisms is crucial for developing viroid management strategies, including breeding for resistant cultivars and employing cross-protection techniques [102].

3.4 Importance of Continued Research and Collaboration to Address Citrus Viroid Challenges

The ongoing threat posed by citrus viroids necessitates continued research efforts to deepen our understanding of these pathogens and their interactions with host plants. Key areas for future research include:

3.4.1 Viroid-Host Interactions

Investigating the molecular mechanisms underlying viroid-host interactions can provide insights into how these pathogens manipulate host cellular processes [102]. Understanding RNA silencing mechanisms and the role of viroid-derived small interfering RNAs (vd-siRNAs) in symptom expression will be critical for developing targeted management strategies [103].

3.4.2 Development of New Diagnostic Tools

As new strains or variants of citrus viroids emerge, there is a need for the development of advanced diagnostic tools that can rapidly identify these pathogens in diverse environments. Innovations in portable diagnostic devices could facilitate early detection in the field [77].

3.4.3 Novel Management Strategies

Exploring genetic engineering approaches to develop resistant citrus varieties could offer long-term solutions to combat viroid infections. Additionally, integrating biological control methods with traditional management practices may enhance effectiveness in controlling viroid spread [104].

3.4.4 Collaboration across Disciplines

Addressing the challenges of citrus viroids requires collaboration among researchers, extension services, growers, and regulatory agencies. Sharing knowledge about best practices for detection, management strategies, and emerging research findings will be essential for developing comprehensive solutions [21].

3.4.5 Global Monitoring Programs

Establishing international monitoring programs for citrus viroids can help track their spread across regions and inform regulatory measures to prevent outbreaks. Collaboration among countries can facilitate data sharing and improve response strategies to mitigate risks associated with these pathogens [105]. Several international initiatives provide strong examples of such cooperation. The International Organization of Citrus Virologists (IOCV) facilitates global expertise and diagnostic standards exchange. The European and Mediterranean Plant Protection Organization (EPPO) coordinates regional surveillance, sets phytosanitary guidelines and supports certification schemes. Similarly, national programs, such as those led by the USDA Citrus Health Response Program in the United States and the Citrus Certification Program in India provide frameworks for systematic viroid testing and certified propagation material. These initiatives underscore the importance of harmonized surveillance systems in minimizing the spread of citrus viroids across borders. In conclusion, while significant progress has been made in understanding citrus viroids and their impact on citrus production, ongoing research and collaboration are vital for effectively addressing the challenges. By leveraging advances in molecular biology, diagnostics, and integrated pest management strategies, stakeholders in the citrus industry can enhance their ability to combat these persistent pathogens while ensuring sustainable production practices.

4 Future Perspectives

Citrus viroids threaten global citrus production by compromising plant vigour, yield, and overall orchard sustainability. While progress has been made in unravelling viroid–host interactions and advancing diagnostic technologies, substantial gaps remain in management and long-term control. Future research should priorities the development of viroid-resistant citrus cultivars, integrating bioinformatics and molecular surveillance tools for early detection, and further exploring viroid–virus synergism to understand its role in disease severity better. Strengthening these areas will be critical for designing more effective, sustainable, and globally applicable management strategies.

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