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Recent Advances in Immunological and Biosensing Approaches for Sensitive Detection of *Citrus tristeza virus*

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ABSTRACT

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Citrus tristeza virus (CTV) is among the most devastating viruses of citrus crops worldwide that create significant losses by rapid decline, stem pitting, seedling yellows and quality reduction of fruit. Widespread distribution of the virus, its genetic variation, and efficient aphid-mediated transmission have made early and accurate detection of virus crucial for disease management. Biological indexing and serological tests like ELISA, DIBA, DTBIA and immunoelectron microscopy have been very useful in large-scale screening programs due to their simplicity and low cost. There have been some limitations of sensitivity, specificity and strain differentiation, however, that have spurred the advancement of more sophisticated molecular and immunological methods. There have been recent advancements in CTV detection with RT-PCR, real-time PCR, loop-mediated isothermal amplification (LAMP), or droplet digital PCR (ddPCR), which has greatly enhanced the specificity and sensitivity of the detection. Immunodiagnostic systems have been improved by the development of antibody engineering technologies such as monoclonal antibody panels, recombinant antibodies, single-chain variable fragments (scFv), nanobodies, and epitope-guided antibody design during the same period. Advances in emerging biosensing and nanotechnology-based platforms like gold nanoparticle immunosensors, electrochemical and optical biosensors, CRISPR based systems, fluorescent nanoprobe, and microfluidic lab-on-a-chip are also revolutionizing plant virus detection by providing highly sensitive, portable, and rapid detection in the field. This review highlights the genomic structure and the coat protein structure of CTV, its transmission method, conventional and advanced diagnostic techniques, advancement in antibody engineering and nanotechnology based diagnostic platforms. Furthermore, a comparative study of diagnostic systems according to their sensitivity, specificity, cost, feasibility, complexity and applicability in the field is provided to show their practical importance in the context of current citrus disease surveillance and management.

Introduction

Citrus is an economically important genus of *Rutaceae* and is widely cultivated around the world. It probably came from the Himalayan region of China, India and Myanmar and is cultivated in over 140 countries (Liu et al., 2022). The UN Food and Agriculture Organization report that world citrus fruit production has grown rapidly over the past few years, at 144 million metric tons (Benny et al., 2023). The citrus fruits contain many phytochemicals and bioactive compounds which have health promoting properties. They also contain high amounts of vitamins C, folic acid, potassium and pectin. Citrus fruits have acquired great interest because of the high polyphenols mainly flavonoids and antioxidant content. Flavanones like naringin and hesperidin typically occur in the peels and tissues of citrus fruits, and possess many therapeutic benefits because of their anti-oxidative, anti-inflammatory, and anti-carcinogenic properties (Suri et al., 2022). Citrus tristeza disease is caused by *Citrus tristeza virus* (CTV), and is the most widespread citrus viral disease in the world. There are three major types of symptoms for different strains of the virus, namely: rapid decline, stem pitting and seedling yellows. Resistant rootstocks and mild virus strains (cross-protection) are commonly used to control the effect of the virus. However, high incidence of severe strains, especially those causing rapid decline and stem pitting, still cause much yield loss, particularly when sour orange (*Citrus aurantium*) is used as a rootstock and other popular cultivars, e.g. sweet orange (*Citrus sinensis*), and grapefruit (*Citrus paradisi*) are grown on a large scale. The virus is transmitted primarily in a semi-persistent, non-circulative manner by aphids, particularly *Toxoptera citricida*, *Aphis gossypii* and *Aphis citricola*. The aphid can become infected within a few hours of ingesting infected tissue and will be infectious for some time, typically 1 to 2 days, and more efficiently infects the longer the feeding time. Of these species, the brown citrus aphid, *Toxoptera citricida* is the most effective vector species, and *Aphis gossypii* is also a good vector species, while other aphid species play a smaller role (Chen et al., 2023). CTV is reported from many citrus-growing areas worldwide, with two types of CTV, stem pitting and quick decline, being the more serious. Biological indexing on indicator plants, molecular methods (ELISA and PCR), are common methods used to identify the virus. For areas where CTV is not present, control is primarily dependent on preventative measures, including quarantine and good regulatory practice. Other management techniques involve cross protection with milder strains of the virus and resistant rootstocks, especially for reducing losses due to stem pitting and quick decline strains. While resistance or tolerance will be most sustainable, breeding is considered difficult because few sources of resistance are available and the need to combine resistance with desired agronomic traits. Signs can be confused with nutrient deficiencies or other phloem-restricted pathogens and severe infections can cause major yield loss, with up to 30% or total crop loss from premature fruit drop (Villar-Luna et al., 2024). Citrus fruit quality can be severely affected by CTV and may reduce market value and result in high losses to growers. No cure is currently known for CTV and infected trees are removed or died. Mild infections with CTV also result in leaf yellowing of citrus trees in some cases, but have a relatively small impact on crop yields. Strategies to reduce losses caused by CTV include seedling production of disease-free plants, regular scouting for CTV in citrus groves and timely control of vectors (Shang et al., 2024). This review focuses on the advanced detection techniques of the *Citrus tristseza virus* (CTV) coat protein (CP), aiming to compile previously identified epitopes along with their amino acid positions, corresponding antibodies, and strain-dependent recognition patterns, while also summarizing the main approaches used for their characterization, including recombinant protein expression, mutational analysis, peptide libraries, and structure-based prediction methods.

Economical significance

Citrus fruits are among the most widely grown and important fruit crops in the world. In 2021, they ranked as the second most produced fruit group, with about 161.8 million tons harvested from over 10.2 million hectares, second only to bananas and plantains. Oranges make up the largest share of citrus production, contributing nearly half of the total with around 75.6 million tons grown on 9.93 million hectares. Tangerines come next, with about 42 million tons produced on 3.11 million hectares, followed by lemons and limes, which account for roughly 20.8 million tons from 1.34 million hectares. Other citrus fruits, including pomelos, grapefruits, and less common types like citron, bergamot, and kumquat, also add to global production, contributing about 9.6 million tons and 13.9 million tons, respectively (Pereira Gonzatto and Scherer Santos, 2023). *Citrus tristeza virus* (CTV) is considered one of the most damaging viral diseases of citrus worldwide. Over the years, it has become more destructive than many other previously known citrus viruses and virus-like diseases. The rapid spread and severe decline caused by CTV have had a major impact on the citrus industry, resulting in the death of more than 100 million citrus trees across the world (Shafique et al., 2024).

Genomic Organization

Citrus tristeza virus (CTV) is one of the most important viruses transmitted by aphids and responsible for devastating citrus crops for over 100 years in numerous countries around the world and remains a serious threat today. It is in the genus *Closterovirus* of the family *Closteroviridae*, which are viruses with large single-stranded RNA genomes. CTV has one of the largest genomes among this group (approx. 19.3 kb) and resides in a long flexible thread-like particle. The results of the virus

are dependent upon the variety of citrus plant especially the scion/rootstock combination and the strain of the virus. It is mainly linked with two important diseases: quick decline and stem pitting. Rapid tree death is caused by quick decline, particularly if the trees are grafted onto sour orange (*Citrus aurantium*) rootstock. In contrast, stem pitting is independent of the rootstock in crops like sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*) and lime (*Citrus aurantifolia*). The disease causes the formation of pits and grooves in the stems and trunks, weakens the plant, decreases growth, decreases yield and causes small fruit of poor quality. It has to be noted that, in some citrus hosts, the action of some strains of CTV may be either mild or be completely unnoticed (Folimonova and Sun, 2022). *Citrus tristeza virus* (CTV) has 12 ORFs which code for proteins that are required for replication, assembly, movement in plant, interactions with host plants and insect vectors. In infected citrus plants, the virus is mainly restricted to the phloem tissues. Transmitted from plant to plant in a semi-persistent manner by aphids which acquire the virus by sucking sap from infected trees and pass it on to healthy trees within a few days or hours. The brown citrus aphid (*Aphis citricidus*) is the most efficient vector, although other species like the cotton aphid (*Aphis gossypii*) and the green citrus aphid (*Aphis spiraecola*) can also spread the virus, but less effectively. CTV is mostly spread over longer distances by infected planting material, but over shorter distances by aphid movement (Aknadibossian et al., 2026).

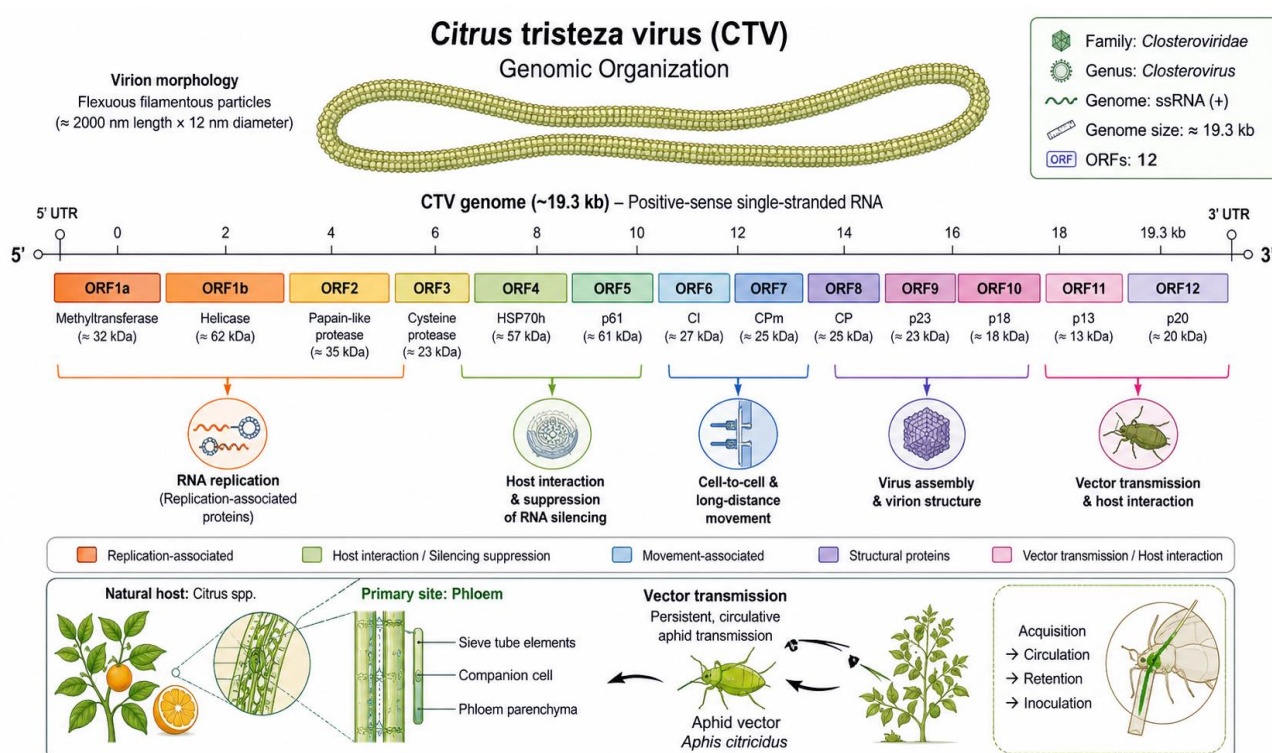


Figure 1. The structure and biology of *Citrus tristeza virus* (CTV).

The *Closterovirus* genus CTV is represented by the approximately 19.3-kb single stranded plus sense RNA genome of CTV. The genome has 12 open reading frames (ORFs) coding for proteins that are associated with RNA replication, host interaction, movement between cells and long-distance, virion assembly and vector transmission. The replication associated proteins are encoded by ORF1a and ORF1b, while the proteins encoded by the downstream ORFs contain a papain-like protease, HSP70h, p61, coat-associated proteins, coat protein, p23, p18, p13 and p20. Characteristic flexuous filamentous virion, association with phloem tissues of Citrus spp. and aphid-mediated transmission, especially *Aphis citricidus*, is also demonstrated.

Coat protein structure

The virus has a single-stranded, positive-sense RNA genome of almost 19.3 KB with 12 open reading frames (ORFs) that specify at least 19 proteins. ORFs 1a and 1b encode proteins involved in the replication component, while ORFs 2-11 encode proteins that are synthesized with coterial 3' subgenomic RNAs. The role of several proteins, such as p61 (HSP70h), p61, p27 (minor coat protein, CPm) and p25 (coat protein, CP) in virion assembly and movement within the host plant is important. Besides, p25, p20 and p23 are RNA silencing suppressors that allow the virus to evade host defense mechanisms. Of these, p23 is a protein which is not shared by other members of the family, and which has a zinc finger domain with RNA binding properties. It is a component of the machinery that regulates the production of positive- and negative-strand RNAs in viral replication and resides in the nucleolus, Cajal bodies and plasmodesmata of infected cells. The p23 protein is also

associated with symptom development and may play a role in the long distance spread of the virus via intercellular connection. Moreover, the coat protein p25 resides at the host cell cytoplasm and nucleus, compromising the transmission of silencing signals, and playing a role in virus-host interactions (Yang et al., 2021). Pathogenicity of CTV isolates was assessed using a series of indicator plants to assign different isolates to biogroups, which has been more and more complemented, and in many cases, replaced, with molecular techniques that enable faster and more precise classification of CTV isolates. The variability of the isolates has been studied by means of the coat protein analyses with the monoclonal and polyclonal antibodies, peptide mapping and, especially, the MCA13 monoclonal antibody, which can help discriminate severe isolates from mild ones by recognizing specific epitopes. Various nucleic acid-based techniques have also been used, such as dsRNA profiling, probe hybridization, restriction fragment length polymorphism (RFLP) analysis of the CP gene, RT-PCR with genotype-specific primers and single-strand conformation polymorphism (SSCP) analysis, which have helped to elucidate the genetic diversity of CTV populations. These strategies have helped improve our understanding of the diversity of isolates and pathogenicity of the virus, demonstrating that there is as much diversity as with other plant viruses, but more than in many members of the *Closteroviridae* family, and that studying these phloem-limited pathogens that cannot be cultured, and which are subject to complex plant-vector interactions, is difficult (Bendix and Lewis, 2018).

Transmission of CTV

Citrus tristeza virus (CTV) is transmitted mainly by long-distance spread through infected propagation materials, while the short-distance spread is by the transmission of the virus by vectors such as *Toxoptera citricidus* and *Aphis gossypii* in a semipersistent manner. The virulence and appearance of symptoms associated with CTV is affected by a combination of factors that are acting synergistically such as the viral strain composition, host species, scion-rootstock compatibility, and environmental conditions. The mild isolates cause either asymptomatic or very mild infections while the severe ones are responsible for three major disease syndromes, namely quick decline (tristeza), stem pitting, and seedling yellows. It usually causes rapid or progressive tree decline in trees grafted on susceptible rootstocks, such as a sour orange. Stem pitting occurs as deep, longitudinal grooves in the wood of trunks and branches of grapefruit and sweet orange, which have no relationship to the rootstock. Seedling yellows are a disease affecting young citrus plants, causing chlorosis and growth retardation in hosts including lemon, sour orange and grapefruit. These syndromes include not only the more problematic ones, such as quick decline and stem pitting, but also various others that are much less significant in causing a decline in tree vigor and yield (Zindović et al., 2024). The transmission of CTV occurs via several aphid species, such as *Aphis gossypii*, *Aphis spiraecola*, *Toxoptera aurantii* and *Toxoptera citricidus*, with the brown citrus aphid (*T. citricidus*) being the most efficient and widespread vector in citrus-producing areas. It is a narrow host range species that mainly feeds on Rutaceae, and is a more efficient vector of CTV strain isolates than other aphids, and it has a semipersistent vectorial capacity, with the acquisition of the isolates occurring within seconds to 30 minutes and they can be transmitted within a few hours, with retention in the vector lasting about 24-48 hours. With the advent of serological diagnosis, the detection of CTV has greatly improved, with antibody-based methods being a key part of research, quarantine and extension programmes. Quality mono and polyclonal antibodies have been developed, such as hybridoma technology against severe isolates, which has improved the precision for diagnosis. A variety of serological methods are now available including ELISA (double antibody sandwich ELISA), immunoblotting, dot immuno-binding assay (DIBA), tissue blot immunoassay (DTBIA), Western blotting, serologically specific electron microscopy, lateral flow assays and IP-PCR, of which DASELISA and DTBIA are the most commonly used due to their reliability and suitability for large-scale screening programmes (ASHOKRAO, 2019).

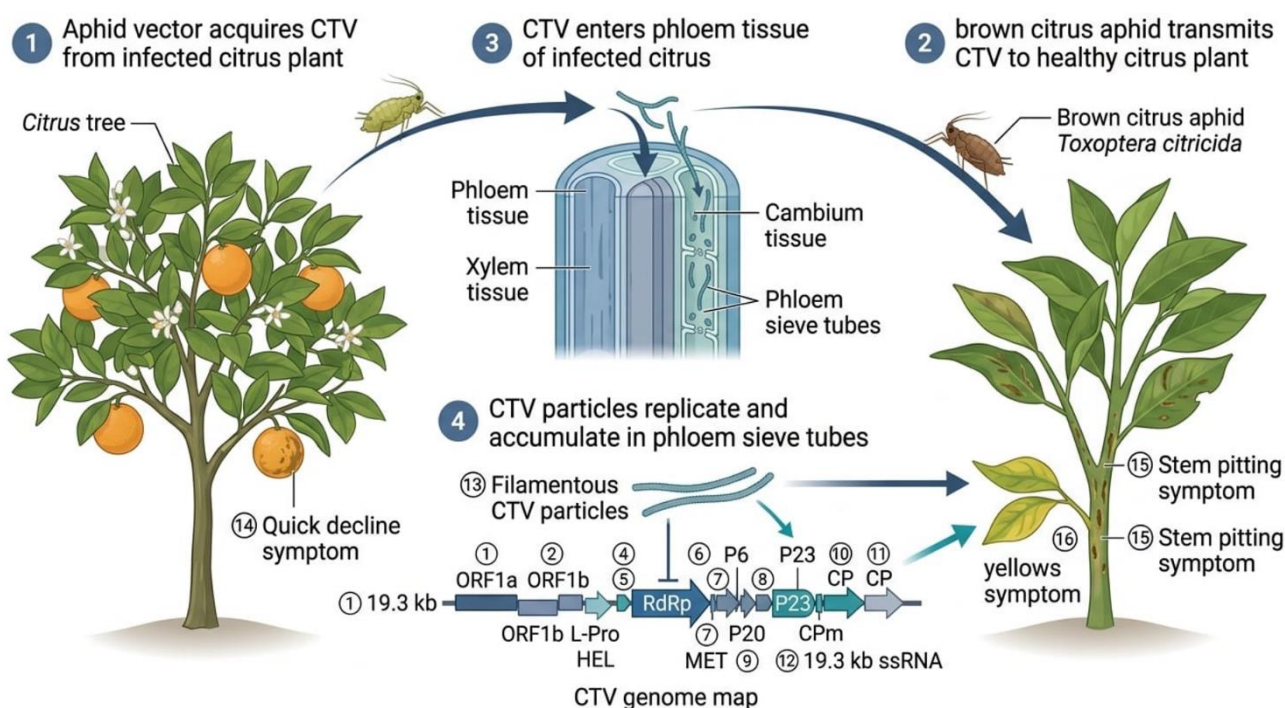


Figure 2. Transmission cycle, vascular tropism, genome architecture and symptom development of Citrus tristeza virus (CTV).

Schematic representation indicating how CTV is acquired and transmitted by the brown citrus aphid (*Toxoptera citricida*) vector from host plant to host plant. The virus infects and accumulates in the phloem sieve tubes of the host vascular tissue. The ~19.3 kb single-stranded positive-sense RNA genome organization shows some important open reading frames (ORFs) that code for the polyprotein, replication modules (L-Pro, HEL, RdRp) and structural/accessory proteins (P6, P20, P23, CPm, CP). When infected by a virus, it produces different clinical symptoms such as rapid decline, a yellowing of the foliage and pitting of the trunks and stems.

Conventional Immunological Detection Methods

Detection methods for *Citrus tristeza virus* (CTV) have been extensively studied and developed. Biological indexing is one of the most common methods for CTV detection. In this technique, the suspected scion is graft-inoculated onto an indicator plant *Citrus aurantifolia*, and characteristic vein clearing symptoms appear within a few weeks. Serological methods, such as enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIBA), electron microscopy (EM) and immunoelectron microscopy (IEM) using polyclonal or monoclonal antibodies have been widely used for CTV detection. Molecular techniques have increasingly been used for CTV identification. The reverse transcription PCR (RT-PCR) and real-time RT-PCR are the most reliable, sensitive and convenient methods for CTV detection. Variations of the RT-PCR technique such as one-step, two-step, multiplex and immunocapture (IC) RT-PCR have been developed to increase the sensitivity of the detection. In addition, the one-tube loop-mediated isothermal amplification (LAMP) technique was recently proposed as a rapid detection method for CTV (Warghane et al., 2020). Serological methods such as ELISA have been widely used in plant virus diagnostics due to their economy and adaptability to large-scale field screening, using especially highly specific monoclonal antibodies (mAbs). *Citrus tristeza virus* (CTV), a phloem-limited insect-transmitted bug-borne cloverleaf-shaped plant virus, is one of the most relevant plant pathogens affecting citrus industries worldwide. Over the past forty years both polyclonal (pAbs) and monoclonal (mAbs) antibodies have been used in ELISA for CTV detection. Some studies have reported the use of both pAbs and mAbs in combination in ELISA assays for CTV. Production of efficient and reliable antibodies for diagnostics remains one of the main limitations faced by the scientific community. Phage display technology is a powerful tool for making monoclonal antibodies using recombinant DNA technology. Phage display allows selection of antibodies to be produced using recombinant DNA technology. Selected antibodies can be produced as Fab fragments or single-chain variable fragments (scFvs) and expressed in prokaryotic or eukaryotic hosts. The epitope recognized by an scFv consists of the six CDRs (L1-L3 and H1-H3). Several important crop pathogens have been detected using recombinant antibodies made by phage display, including *Citrus tristeza virus*, Tomato spotted wilt virus, *Candidatus Phytoplasma aurantifolia*, *Potato leafroll virus*, *Fusarium verticillioides*, *Xylella fastidiosa* subsp. pauca, *Xanthomonas citri* subsp. citri and Chickpea chlorotic dwarf virus.

(Raeisi et al., 2022). Enzyme-linked immunosorbent assay (ELISA) is widely used for the assessment of both the quality and quantity of various antibodies and antigens, including plant pathogens. ELISA is known for its accuracy and sensitivity for disease diagnosis, especially for plant viruses, and can easily process large numbers of samples. ELISA is an immunoassay technique using enzyme-coupled antibodies and a support matrix. A variety of serological and molecular techniques for citrus virus particle identification, the Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method is commonly used for its rapid viral detection. This method is based on the specific interaction of the viral particles with antibodies adsorbed onto the surface of microtiter plates, followed by enzymatic amplification using conjugated secondary antibodies. Results obtained from ELISA assays were cross-verified with Reverse Transcription Polymerase Chain Reaction (RT-PCR) results to increase the reliability of the results from ELISA assays. This cross-verification method can be very helpful for strategic planning and decision-making related to the conservation of citrus species (Baral, 2024). Direct tissue blot immunoassay (DTBIA) and dot immunobinding assay (DIBA) methods have been successfully used for virus detection by employing crude plant extracts or freshly cut tissues directly immobilized on membranes, making them particularly useful for rapid field diagnostics and for large-scale epidemiological studies (Choudhary et al., 2013). The performance of these assays depends on the antibodies employed. The use of polyclonal antibodies (pAbs) in serological methods allows the detection of multiple viral isolates because pAbs react with several epitopes (regions of antigenic determinants on viruses). However, cross-reactions with related pathogens can limit the use of pAbs (Iracheta-Cardenas et al., 2009). Monoclonal antibodies (mAbs) are more specific and react with single epitopes, which makes them ideal for the discrimination of distinct CTV strains. For example, the MCA13 antibody can recognize severe CTV isolates. Although conventional serological methods are flexible, easily scalable, cost-effective and perform diagnostics directly from plant extracts, they can show reduced sensitivity when the virus titer is low. Furthermore, they are unable to detect genetically distinct CTV strains whose coat proteins exhibit distinct antigens (BR et al., 2015). Diagnostic approaches for *Citrus tristeza virus* (CTV) include biological indexing, serological assays and nucleic acid-based methods. Serological techniques such as ELISA, Direct Tissue Blot Immunoassay, and dot-immunobinding assays are widely used for large-scale screening due to their cost-effectiveness and suitability for high-throughput analysis. Molecular methods, including RT-PCR, real-time TaqMan RT-PCR, and loop-mediated isothermal amplification, provide enhanced sensitivity and accurate detection of the virus. ELISA-based systems using polyclonal and monoclonal antibodies are commonly employed for detection of diverse CTV isolates, they are associated with limitations such as cross-reactivity, variable sensitivity, and batch-to-batch inconsistency. Double-monoclonal antibody sandwich ELISA systems offer improved specificity, stability, and reliability, making them more suitable for accurate detection and management of CTV (Shang et al., 2026). Serological differences have been detected among various *Citrus tristeza virus* (CTV) isolates, reflecting diversity in their coat protein antigenic regions. Three linear epitopes located at amino acid positions 48-63, 97-104, and 114-125 were identified using bacterially expressed truncated coat proteins along with a set of monoclonal antibodies raised against native CTV-S4 virions. Site-directed mutagenesis revealed that a single amino acid change (D98G) within the 97DDDSTGIT104 epitope completely disrupted recognition by monoclonal antibodies 1, 4, and 10, highlighting its key role in antibody binding. A similar loss of recognition was observed when glycine was naturally present at position 98 in the HB1 isolate, confirming the importance of this residue in antigen-antibody interaction. Furthermore, structural variations within the epitope region spanning amino acids 48-63 between isolates S4 and HB1 were

associated with differing reactivity to monoclonal antibodies 5 and 6, suggesting conformational changes can significantly influence serological behavior among CTV strains (Wu et al., 2014).

Table 1: serological detection of *Citrus tristeza virus* (CTV)

Type	Advantages	Limitations	Sample Type	Antibodies	Characteristics	Reference
ELISA	Commercial Availability, highly specific and sensitive, minimum cost Detection of large volume	Requires highly specialized instrumentation and labour	Freshly prepared sap of leaf	MCA13 MCAs, 3DF1 + 3CA5 MCAs	antibody coating and monoclonal antibody are used for detection	(Shojaei et al., 2016)
DTBIA	Easy storage and transport Field-based testing Cost-effective	Requires visual interpretation Limited sensitivity	Pieces of membranes	3DF1 + 3CA5 MCAs linked to AP or 3DF1 scFv-AP/S + 3CA5 scFvAP/S proteins	No antibody coating, pAbs, monoclonal or single chain antibodies	(Saponari et al., 2008)
LFIA	Easy to handle	pretreated	Crude	goat anti-CTV	Capture antibody	(Maheshwa

	short detection time Low cost	sample, limited Sensitivity	leaf sap	antibody	for detection	ri et al., 2017)
Immuno- electron microscop y (EM)	The virion morphology can be observed Lesions can be observed	Comparatively higher cost Difficult to detect high throughput	Purified parts of tissues	873, 894 and 879 pAbs	Polyclonal antibody used detection	(Gulati et al., 2019)

Advances techniques for detection of *Citrus tristeza virus* (CTV)

Advances in the development of new tests for *Citrus tristeza virus* (CTV) have a general pattern that is primarily linked to recent innovations in antibody engineering and modern biosensing technologies: namely, a clear tendency for more sensitive, faster and field-deployable detection systems. Monoclonal antibody panels targeting conserved and variable regions of the CTV coat protein have been improved to generate a more strain-differentiating CTV diagnostic, while epitope-guided antibody design driven by mapping of the coat protein facilitated strong antigen-antibody interactions and increased sensitivity when diagnosing viral infection (Singh et al., 2025). Meanwhile, unique recombinant antibody formats are emerging such as single-chain variable fragments (scFv) and nanobodies due to their small size, stability, and applicability towards portable diagnostic platforms. Furthermore, the development of more specific and less cross-reactive double antibody sandwich ELISA systems has made them particularly suitable for large scale screening. In addition, biosensing technologies are revolutionizing plant virus detection through the integration of biological recognition elements with highly sensitive signal transduction systems (Asaadi et al., 2021). Electrochemical biosensors have the advantages of fast response time and low cost, especially nanomaterial-enhanced electrochemical biosensors; Optical biosensors based on surface plasmon resonance and fluorescence technology can achieve real-time analysis without labeling but require high sensitivity. This reduction in the distances translates into lower limits of detection and increased performance of an assay on nanoparticle-based signal amplification. In general, these novel methods provide faster, more sensitive and portable assay platforms as compared to standard immunoassays render them as excellent candidates for the effective monitoring of citrus diseases (Xu et al., 2025).

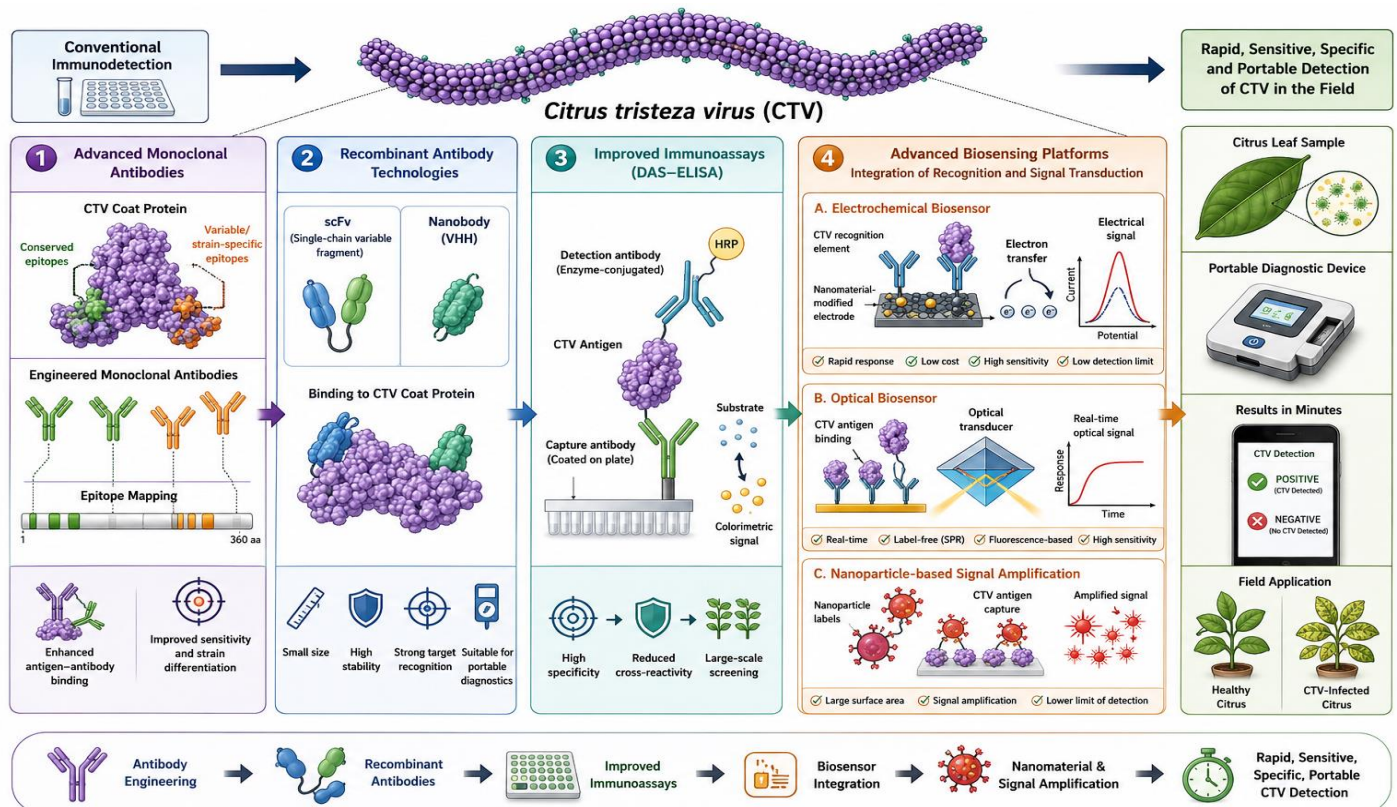


Figure 3. Adopted advanced immunological approach and biosensing platforms for detecting *Citrus tristeza virus* (CTV) in the field.

The engineering of monoclonal antibodies against conserved and variable coat protein epitopes, recombinant formats such as scFv, V_HH nanobodies, enhance binding affinity, stability and strain differentiation compared to conventional approaches.

These recognition elements are used to achieve low detection limits in advanced electrochemical biosensing platforms, label-free optical platforms (SPR, fluorescence), and nanomaterial-modified electrochemical sensors and in advanced setups of double-antibody sandwich ELISA (DAS-ELISA). The entire pipeline is integrated into a portable diagnostic device and interfaces to smartphones to allow for rapid CTV detection in minutes in the field, without the need to transport samples to a lab.

Molecular Biological detection

In recent years, the capacity to detect CTV strains has been greatly enhanced, which has helped in better managing this pathogen. Advanced molecular techniques, such as reverse transcription polymerase chain reaction (RT-PCR), real-time PCR (RT-qPCR) and loop-mediated isothermal amplification (LAMP) have been developed to supplement the traditional methods like biological indexing and serological assays. These approaches are more specific and sensitive, and enable rapid and accurate detection of CTV, which is key to minimizing the effects of CTV on citrus production (Warghane et al., 2017). One of the most discussed issues in CTV detection is the issue of the genetic variation of the virus, and the current diagnostic methods are not very effective. Although molecular methods have come a long way, some limitations remain, including RNA instability and the requirement for highly qualified personnel, limiting the detection certainty in a range of agricultural settings. A fast and specific RT-LAMP method to detect CTV was developed. The RT-LAMP assay had a 100-fold higher sensitivity compared to a traditional RT-PCR method. The Acc I digest of the RT-LAMP products were used to confirm the products. The products produced by the RT-LAMP could be detected using add SYBR Green I, suggesting that it is possible to use the RT-LAMP directly as a method for detecting CTV. Traditional methods have been supplanted by molecular detection methods that have improved in terms of specificity and sensitivity of the detection of *Citrus tristeza virus* (CTV) (Ooi et al., 2022). The main molecular methods are the reverse transcription polymerase chain reaction (RT-PCR) variants, enzyme-linked immunosorbent assays (ELISA), and newer amplification technologies. Polymerase Chain Reaction (PCR) and its variants are now crucial to the proper identification of CTV. These methods allow the amplification of particular nucleic acid sequences with high sensitivity and specificity (Shang et al., 2024). Recent developments include the introduction of droplet digital PCR (ddPCR) which is more precise and not affected by the need for standard curves, improving the quantification capabilities. However, traditional RT-PCR has a small percentage of samples that lack detection of the low-titer pathogens, and the development of real-time RT-PCR methods that enable quantitative analysis and enhanced detection limits is needed (Kokane et al., 2021).

Nanotechnology and Next-Generation Diagnostic Tools

The development of nanotechnology has greatly revolutionized the detection of Citrus tristeza virus (CTV) and other plant viral pathogens in terms of sensitivity, speed, portability and field applicability. Among the most widely investigated nanodiagnostic devices are gold nanoparticle-based immunosensors, which exhibit a strong enhancement of the detection signal due to their optical properties, biocompatibility, and the ability to amplify detection signals. The use of such nanoparticle-assisted systems has improved immunoassays and lateral flow assays, enabling the detection of viral particles within just minutes and easily seen by the naked eye, even at low concentrations (Mallick et al., 2025). Likewise, quantum dots and fluorescent nanoprobe have received a great deal of attention because of their high fluorescence stability, wide range of excitation spectrum, and high intensity of fluorescence, allowing ultrasensitive and multiplex pathogen detection. In addition, the development of nano sensor-based diagnostics further shows that, nanomaterial-assisted electrochemical and optical biosensors can enhance the real-time monitoring and decrease the false-positive reaction during detection of plant virus (Aatreyi et al., 2026). This is particularly relevant in the field of plant virology, where emerging detection systems based on the CRISPR technology are increasingly relevant, since they are highly specific and able to recognise nucleic acid molecules rapidly, and are therefore suitable for low-cost, portable diagnostic platforms. Furthermore, the microfluidic technologies used for lab-on-a-chip systems combine sample preparation, amplification and detection into a single small device with low reagent and technical skill requirements. They are miniaturized systems that provide fast processing, low contamination and high throughput screening of citrus pathogens. The recent development of duplex PCR-lateral flow immunoassay (d-PCR-LFIA) platforms for simultaneous detection of *Candidatus Liberibacter asiaticus* and CTV further emphasizes the significance of point-of-care diagnostics for citrus orchards, where fast and early disease monitoring in the field is critical for prompt disease management and citrus fruit certification programs (Bharsakale et al., 2025). Nanotechnology-based diagnostics can revolutionize plant disease surveillance by offering highly sensitive, portable and easy-to-use alternatives to traditional laboratory assays (Marwal, 2026).

Comparative Performance of Diagnostic Platforms

There is an increasing number of platforms available for detection of *Citrus tristeza virus* (CTV) and comparative trials have been used to evaluate the platforms for performance in terms of sensitivity, specificity, ease of operation, scalability, cost, and practicality in the field. ELISA is still widely employed due to its relative simplicity, low cost and suitability for large-scale testing, but may lack sensitivity and require laboratory equipment and more processing time. Depending on expensive equipment, skilled personnel and controlled laboratory conditions, molecular techniques including RT-PCR and qPCR are more sensitive and specific than other techniques (Aydin et al., 2025). Nanotechnology-enabled biosensors and CRISPR-based detection systems, on the other hand, are very potential for real-time monitoring of diseases, providing nearly immediate results, requiring little sample preparation and having high analytical sensitivity (Choi and Yoon, 2023). Immunosensors and fluorescence nanoprobe based on gold nanoparticles have shown to have good signal amplification and detection limits, and electrochemical biosensors offer a more cost-effective and portable option for orchard level diagnostics. Similarly, microfluidic lab-on-a-chip systems decrease the complexity of operation by combining several diagnostic procedures in a small, automatic device. These next generation systems provide substantial time-to-result savings and a greater useability on location, as compared to traditional laboratory assays, especially when dealing with the early stages of disease detection and quick management decisions in citrus groves. Despite these benefits, there are still difficulties with standardisation, stability and commercialisation of these materials at larger scales for widespread adoption (Kudłacik-Kramarczyk et al., 2025; Hemdan et al., 2025).

Table 2: Comparison of detection techniques of *Citrus tristeza virus* (CTV)

Diagnostic Platform	Principle	Characteristics	Time to Result	Cost	Field Applicability	Major Advantages	Limitations	References
ELISA	Antigen-antibody interaction	Moderate sensitivity and specificity, Low complexity	3–6 h	Low	Limited to semi-field/lab use	Cost-effective, suitable for large-scale screening	Lower sensitivity, possible cross-reactivity	(Jeon et al., 2025)
RT-PCR / qPCR	Amplification of viral nucleic acids	High sensitivity and specificity, High complexity	2–5 h	High	Mostly laboratory-based	Highly accurate and reliable	Requires skilled personnel and expensive equipment	(Papaneri et al., 2025)
Gold Nanoparticle-Based Immunosensors	Nanoparticle-assisted signal amplification	Very High sensitivity and high specificity, Moderate complexity	15–60 min	Moderate	Excellent for field use	Rapid detection, visual signal enhancement, portable	Stability and standardization issues	(Basso et al., 2025)
Electrochemical Biosensors	Electrical signal generated after target binding	Very High sensitivity and high specificity, Moderate complexity	Minutes to 1 h	Moderate	High	Rapid, sensitive, low sample requirement	Sensor fabrication may be complex	(Wu et al., 2025)
Quantum Dot / Fluorescent Nanoprobes	Fluorescent nanomaterial-based detection	Very High sensitivity and high specificity, Moderate complexity	30–90 min	Moderate to High	Moderate	Strong fluorescence stability	Requires specialized imaging systems	(Cedrún-Morales et al., 2026)

CRISPR- Based Detection Systems	CRISPR- mediated nucleic acid recognition	Extremely High sensitivity and specificity, Moderate complexity	30–60 min	Mode rate	Excellent potential for field deploy- ment	Highly precise, rapid, and adaptable	Still under optimization for large- scale use	(Ghounei my et al., 2023)
Duplex PCR- Lateral Flow Immunoassay (d-PCR-LFIA)	Combined PCR amplification and lateral flow detection	High sensitivity and high specificity, Moderate complexity	1–2 h	Mode rate	High	Simultaneous detection of multiple pathogens	Requires optimization for routine commercial use	(Papini et al., 2025)

Conclusion

The widespread host range, genetic variation, high speed of spread facilitated by aphid vectors and the devastating effects of citrus tristeza virus (CTV) on tree health and fruit production continue to make this virus a significant threat to citrus production worldwide. Timely and correct identification is still one of the best ways to manage the disease and minimize economic losses. The principle of routine screening and disease monitoring has been well established by conventional diagnostic methods like biological indexing and serological assays but their limitations in terms of sensitivity, strain discrimination and field applicability have inspired the development of more sophisticated technologies. With molecular methods such as RT-PCR, qPCR, RT-LAMP and ddPCR, the accuracy and reliability of detection are significantly enhanced, especially in low-titer infections and mixed strain populations. In addition, in recent years, antibody engineering has progressed to a level that has greatly improved the specificity and precision of the immunological assays, particularly the development of monoclonal antibodies, recombinant antibodies, nanobodies, and epitope- based diagnostic systems. New, cutting-edge diagnostic technologies, such as nanotechnology-based biosensors, systems based on CRISPR, and microfluidic lab-on-a-chip systems, are becoming increasingly promising new tools for rapid response, portability, high sensitivity, and on-site orchard monitoring. These innovative technologies hold great promise to transform citrus disease diagnostics, but challenges with standardization, commercialization, and scaling up still exist. The integration of molecular biology, antibody engineering, biosensing, and nanotechnology will be crucial to the creation of rapid, inexpensive and field deployable diagnostic systems that will facilitate sustainable citrus production and effective global citrus disease management programs.

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