J.Mycopathol.Res. 63(3) : 477-490, 2025, ISSN : 0971-3719 (Print), 2583-6315 (Online)
10.57023/JMycR.63.3.2025.477

REVIEW

Innovations in molecular diagnostics, characterization and novel management of graft transmissible pathogens in horticulture crops

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Received : 16.06.2025 Accepted : 25.07.2025 Published : 29.09.2025

Horticultural crops such as citrus, apple, banana, grapes form the backbone of India's fruit industry, contributing significantly to food security, rural livelihoods, and economic development. However, the productivity and quality of these crops are severely compromised by a diverse group of graft-transmissible pathogens, including viruses, viroids, and phloem-limited bacteria. These pathogens are often systemic in nature and perpetuated through vegetative propagation, resulting in long-term persistence and widespread dissemination. Major diseases such as Citrus tristeza virus (CTV), Citrus greening disease (*Candidatus*Liberibacter asiaticus), Apple mosaic and stem pitting diseases, Banana bunchy top disease (BBTD), and Grapevine leafroll and fanleaf complexes continue to cause substantial economic losses due to yield reduction, quality deterioration, and restricted germplasm exchange. Recent innovations in molecular diagnostics have transformed our ability to detect and manage these pathogens. Techniques such as RT-PCR, real-time PCR, multiplex PCR, and isothermal amplification methods like RT-RPA-LFIA and RT-LAMP offer high sensitivity and specificity. Additionally, the advent of high-throughput sequencing (HTS) has facilitated the discovery of novel viruses and viroids and provided insights into pathogen diversity and co-infections. These tools support early detection, epidemiological studies, and the development of certified virus-free planting material. Despite these advancements, challenges remain in translating laboratory-based methods into affordable, field-level diagnostics. This review summarizes the current knowledge on the incidence, symptomatology, molecular characterization, transmission, and epidemiology of graft-transmissible pathogens in major Indian horticultural crops. It highlights the development and application of advanced diagnostic technologies and discusses integrated management strategies including clean plant programs, phytosanitary measures, and resistant cultivar development. A coordinated approach combining diagnostics, clean planting material, vector control, and regulatory support is essential to mitigate the threats posed by these pathogens and ensure the sustainability and profitability of the horticulture sector.

Keywords : Graft-transmissible pathogens, molecular diagnostics, horticultural crops, viruses, viroids, citrus, apple, banana, grapes, disease management, clean plant programs (CPP)

INTRODUCTION

India's horticultural sector holds a pivotal position in the country's agricultural framework, contributing significantly to both nutritional security and economic growth. According to the Third Advance Estimates for the agricultural year 2023–24 released by the Department of Agriculture and Farmers' Welfare, the total horticulture production in India is estimated at 353.19 million tonnes, cultivated over an expansive area of approximately 28.98 million hectares. Within the fruit sector, which accounts for 112.73 million tonnes of the total horticultural production, vegetatively propagated crops like citrus, grapes, banana, apple etc emerge as major crops of significant economic importance. Banana, in particular, holds a dominant position in terms of both cultivated area and production volume. In 2023–24, banana was grown over 994,000 hectares, yielding 37.807 million tonnes with a remarkable average productivity of 38.02 tonnes per hectare. Its high productivity and adaptability across various agro-climatic zones underscore banana's strategic importance in Indian horticulture. Moreover, it serves as a dietary staple for a large segment of the population and holds considerable export potential, especially from key producing states such as Maharashtra, Tamil Nadu, and Gujarat

4th Professor R.P.Purkayastha Memorial Award Lecture delivered on 6th December 2024 during National conference held at Ramakrishna Mission Cultural Centre, Golpark, Kolkata

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(FAO, 2024; NHB, 2024).Citrus fruits, includes lime, lemon, mandarin, and sweet orange, also hold a 3rd prominent place in terms of production in India. During the same period, the total area under citrus cultivation reached nearly 1.1 million hectares, yielding 14.576 million tonnes of produce. The average productivity for citrus fruits stood at 13.25 tonnes per hectare. These fruits are valued not only for their high vitamin C content but also for their wide range of culinary and nutritional uses. Major citrus-producing states include Maharashtra, Andhra Pradesh, and Punjab. However, the citrus industry faces increasing threats from emerging viral and vector-borne diseases, particularly those associated with graft-transmissible pathogens. Among the most serious are Citrus Tristeza Virus (CTV) and *Candidatus*Liberibacter asiaticus, the causal agent of citrus greening disease (Huanglongbing). These pathogens substantially reduce both yield and fruit quality, highlighting the urgent need for resistant cultivars and strengthened diagnostic capabilities (Ghosh *et al*. 2018,2020).

Apple cultivation is predominantly confined to the temperate regions of Jammu & Kashmir, Himachal Pradesh, and Uttarakhand. In 2023–24, apples were grown on 304,000 hectares, with a total production of 2.699 million tonnes. However, productivity remains relatively low at 8.88 tonnes per hectare. This is largely due to the crop's sensitivity to climatic variability, a limited availability of high-yielding cultivars, and vulnerability to virus and viroid infections (Shah *et al*. 2022).Despite the fruit's high market value and nutritional appeal, production has been increasingly challenged by climate change and biotic stresses. The adoption of improved horticultural practices and the implementation of clean plant programs emphasizing the use of virus-free planting material are key strategies for enhancing apple productivity and resilience. Although grape cultivation occupies a smaller area compared to other major fruit crops, it boasts significantly higher productivity. In 2023–24, grapes were cultivated over 169,000 hectares, producing 3.911 million tonnes with an average productivity of 23.13 tonnes per hectare (FAO, 2024). Grapes are among the highest-value horticultural commodities, with uses ranging from fresh table consumption to raisin production, juice extraction, and winemaking. Maharashtra leads in grape production, followed by Karnataka and Tamil Nadu. However, grapevines, being perennial in nature, are highly susceptible to a range of viral and phytoplasmal diseases that adversely affect vine health and fruit quality. The application of molecular diagnostic techniques, particularly high-throughput sequencing (HTS), has become instrumental in the early detection and management of these pathogens, offering a pathway toward more sustainable grape production.

The productivity, quality, and longevity of key horticultural fruit crops in India such as citrus, apple, banana, grape are severely affected by a range of viral and viroid infections. Citrus is particularly vulnerable to graft-transmissible pathogens like Citrus tristeza virus (CTV, *Closterovirus tristezae*), Huanglongbing (HLB, *Candidatus* Liberibacter), Citrus yellow mosaic virus (CYMV, *Badnavirus tessellocitri*), and several viroids, which lead to fruit drop, reduced size, and tree decline. In apples, viruses such as Apple mosaic virus (ApMV, *Ilarvirus ApMV*) and Apple stem pitting virus (ASPV, *Foveavirus mali*), along with viroids, can reduce yields by 15–25% and compromise fruit quality. Banana production is constrained by Banana bunchy top virus (BBTV, *Babuvirus musae*), Banana bract mosaic virus (BBrMV, *Potyvirus nusae*), and Banana streak virus (BSV,*Badnavirus alphavirgamusae*), causing stunted growth and malformed fruits. Grapevines face threats from Grapevine leafroll-associated viruses (GLRaVs, *Ampelovirus tetravitis*), and Grapevine fanleaf virus (GFLV, *Nepovirus foliumflabelli*), all of which affect berry quality and vine longevity (Bharsakale *et al*. 2023; Rana *et al*. 2011; Selvarajan *et al*. 2010; Holkar *et al*. 2024).

Vegetative propagation in these crops facilitates the systemic spread of pathogens, emphasizing the critical need for virus-free planting materials and reliable molecular diagnostics. To address this, the Clean Plant Program (CPP) promotes the use of rigorously screened mother blocks maintained in protected environments for multiplication of disease-free planting materials. This approach significantly reduces the risk of using infected propagules and mitigates the

spread of graft-transmissible pathogens, thereby enhancing the overall health and productivity of horticultural crops.

CITRUS

Disease incidence and economic importance

Citrus is the third-largest fruit industry in India after banana and mango, contributing 142.62 lakh tonnes of fruit annually from 10.86 lakh hectares (NHB, 2022). However, productivity remains lower than in developed countries, largely due to systemic diseases caused by viruses, viroids, and Virus like Pathogens (VLPs). Citrus hosts over 50 graft-transmissible pathogens, most of which are spread through infected budwood used in propagation **(**Prabha and Baranwal, 2011; Ghosh *et al*. 2018; Warghane*et al.*2020).

Among the most damaging pathogens is Citrus tristeza virus (CTV) has long, flexuous filamentous particles (2000 × 11 nm) and a 19.3 kb single-stranded RNA genome encoding at least 19 proteins (Dawson *et al*. 2015; Warghane*et al*. 2017). CTV-infected trees show symptoms such as vein clearing, stunting, stem pitting, chlorosis, epinasty, seedling yellows, and abnormal fruit development, all of which significantly reduce yield (Dawson *et al*. 2013; Ghosh *et al.* 2009). Another major pathogen, Indian citrus ringspot virus (ICRSV) has rod-shaped virions (650 × 13 nm) and a 7.6 kb genome encoding six ORFs, and is known to infect kinnow mandarin in India, causing chlorotic rings and mottling (Thind *et al*. 2000; Kokane *et al*. 2021b). Citrus yellow mosaic virus (CYMV), a pararetrovirus causes severe yellow mosaic disease across various citrus cultivars. It has non-enveloped bacilliform particles (30 × 120–150 nm) and a double-stranded DNA genome of 7.5–8 kb (Ghosh *et al.* 2014; Kumar *et al*. 2018) (Fig.1).

Identification of Phytoplasma one of the destructive pathogens caused by candidatus phytoplasma cynodontis (16SrXIV group) organism of Witches' Broom Disease of acid lime (WBDL) in India. Furthermore, first time reported the hop stunt viroid infection in citrus in India (Ghosh *et al.*2013, 2017, 2019; Ramachandran *et al*. 2005).Perhaps the most devastating disease affecting citrus is Huanglongbing (HLB), or citrus greening, caused by the phloem-limited bacterium *Candidatus*Liberibacter asiaticus. This fastidious, gram-negative á-proteobacterium has spread to over 64 countries, posing a global threat to citrus production (Ghosh *et al*. 2023). In the United States, HLB has caused annual financial losses exceeding $3.6 billion (Molki*et al*. 2020).

Symptomatology

These pathogens lead to a range of symptoms including chlorosis, mottling, vein clearing, stem pitting, stunted growth, premature fruit drop, and fruit deformation (Nageswara-Rao *etal*. 2013; Ghosh *et al.* 2023). These infections not only reduce yield by causing premature fruit drop and tree decline but also compromise fruit quality. For instance, CTV can cause phloem death, resulting in stunted trees that bear small, poor-quality fruits (Zhou *et al*. 2020).HLB has been shown to decrease fruit sugar content, increase acidity, and lead to the accumulation of bitter and astringent compounds such as limonoids and flavonoids, which severely impact marketability (Singerman *et al*. 2017; Dala-Paula *et al*. 2018; Zhang *et al*. 2021).

Host Range, transmission and epidemiology

Most of these pathogens infect a wide range of citrus species and hybrids. For instance, CTV and CYMV affect several sweet orange, mandarin, and lime varieties, while ICRSV is more specific to kinnow mandarin. Graft transmission via vegetative propagation is the predominant mode of spread. Insects such as aphids and psyllids also contribute to vector-mediated transmission, particularly for viruses like CTV and HLB.

ICRSV and CYVCV is primarily spread through vegetative propagation, especially via infected buds and grafts. Although not transmitted by insects, nematodes, seed, or soil, its presence in the pollen of infected Kinnow mandarin plants suggests a possible but unconfirmed route through pollination (Pant and Ahlawat, 1998; Singh *et al*., 2006).The virus is also mechanically transmissible, though it is not seed-transmitted despite being detectable in seed coats. Its

widespread occurrence in Indian citrus orchards is largely attributed to the use of contaminated scion material during nursery propagation. The disease is particularly severe in high-density Kinnow cultivation regions such as northern India, where infection rates can reach 100%, and yield losses range from 66% to over 91%. Recent genomic studies have led to the proposal of two distinct ICRSV species ICRSV-A and ICRSV-B which has significant implications for improving diagnostics and refining management strategies in affected citrus-growing areas (Bharsakale*et al.* 2025).

Molecular Characterization and Pathogen Diversity

CTV isolates from central India revealed significant genetic diversity. A decline-inducing isolate, CTV-DKG1, was collected from mosambi (*Citrus sinensis*) and maintained on indicator plants. RT-PCR amplification using coat protein gene (CPG) specific primers yielded a 672 bp product. Sequence analysis showed 97% nucleotide identity with Chinese isolates (S4, CTV-0002, HN-K) and 96% similarity with isolates from Israel and Jordan. The deduced coat protein consisted of 223 amino acids and exhibited 95–97% homology with closely related isolates, with minor variations at specific positions. Phylogenetic analysis placed CTV-DKG1 in a clade with Chinese, Israeli, and Jordanian isolates, distinct from other Indian strains (Ghosh *et al*. 2009, 2020, 2022). Recent studies have used coat protein (CP), p23, and p18 gene sequences as genetic markers to characterize CTV isolates from Bhutan and India. Bhutanese isolates showed close genetic relatedness to the virulent VT strain and clustered into groups II and III based on specific genomic loci, similar to northeast Indian isolates. In India, four regional CTV isolates—Kat1, D1, B5, and G28—were sequenced over the 32 half of the genome (~8.4 kb), covering ORFs 2–11 and the 32 UTR. Sequence identity among these and previously reported isolates ranged from 92–99%. Phylogenetic analysis grouped Indian isolates into four of ten total genogroups, with Kat1, D1, and Kpg3 forming the “Kpg3Gr” cluster, a subtype of the Indian VT genotype. Distinct lineages were identified for B5 and G28, highlighting novel CTV variants. Recombination analysis also confirmed multiple recombinant events, emphasizing the extensive genetic diversity of CTV in Indian citrus orchards (Ghosh *et al*. 2021; Biswas *et al*. 2018).

Whole-genome sequencing of *Candidatus* Liberibacter asiaticus (*C*Las) has revealed a compact genome (~1.23 Mb) marked by the absence of genes for key metabolic processes. To sustain itself, *C*Las relies on a suite of ABC transporters that facilitate the import of essential metabolites. The bacterium also produces virulence-associated proteins such as Sec-dependent effectors (e.g., SDE1), peroxidases, and serralysin proteases, which suppress plant immune responses and enhance colonization. Additionally, prophages like SC1 and SC2 contribute effectors that disrupt host signaling and oxidative stress responses. CLas is transmitted by the psyllid vector *Diaphorina citri*, colonizing both the insect’s gut and salivary glands, with vector competence varying by temperature- *C*Las thrives at 30–35°C, while *Candidatus* Liberibacter africanus (*C*Laf) prefers cooler climates (Ghosh *et al.* 2013, 2015, 2023; Thakuria and Singh, 2023). Also characterized a bacterioferritin co migratory protein family 1-Cys peroxiredoxin from *C*las(Singh *et al*. 2017; Kumar *et al*. 2019a,b). Citrus ringspot disease is caused by Indian Citrus Ringspot Virus (ICRSV) has flexuous, filamentous virions (~650 × 13 nm) and a positive-sense ssRNA genome (~7.56 kb) containing six ORFs. These encode a replicase, a coat protein, a nucleic acid-binding protein, and a triple gene block (TGB) involved in cell-to-cell movement (Rustici *et al.,* 2000; Adams *et al*. 2004; Prabha and Baranwal, 2012).

Impact on yield and fruit quality

Pathogen infections in citrus crops have a profound impact on both yield and fruit quality. *Citrus tristeza virus* (CTV) causes phloem necrosis, which gradually leads to tree decline. Infected trees exhibit reduced vigor and produce small, misshapen fruits, ultimately lowering market value and overall productivity (Zhou et al. 2020). Similarly, Huanglongbing (HLB), caused by *CandidatusLiberibacter asiaticus*, severely affects fruit quality and quantity. It has been

associated with up to a 223.4% increase in orange prices due to diminished supply and quality. Fruits from HLB-infected trees typically exhibit lower sugar content, elevated acidity, and the accumulation of bitter compounds such as limonoids and astringent flavonoids, making them less palatable and commercially viable **(**Singerman *et al.* 2017; Dala-Paula *et al*. 2018; Zhang *et al*. 2021).

Diagnostics

Effective disease detection remains a cornerstone of management. Traditional methods such as biological indexing, while historically important, are slow and require specific indicator hosts. ELISA offers a high-throughput alternative but is constrained by the availability of high-quality antisera and limited specificity when dealing with closely related strains (Meena and Baranwal, 2016; Steel *et al*. 2010). Molecular techniques offer more precision: SYBR Green-RT-qPCR and PCR are widely used for their sensitivity and speed, although they require costly and sophisticated infrastructure (Kokane *et al*. 2021; Kokane *et al*. 2021). Loop mediated isothermal amplification (LAMP) assays gives rapid and sensitive detection of CTV, ICRSV and HLB in plant as well as in vectors like *Diaphorinacit-rikuwayama* (Ghosh *et al*. 2016, Warghane*et al*. 2017; Kokane *et al*. 2021). Recombinase Polymerase Amplification (RPA), a point-of-care technology, has recently gained attention, but its adoption in India is limited due to the high cost of reagents and lack of local manufacturing (Ghosh *et al.* 2020, 2018; Ponnusany *et al*. 2018). Multiplex PCR (mPCR) is emerging as a valuable tool for simultaneous detection of multiple pathogens. Assays have been developed for identifying up to seven citrus viruses and viroids, including *Candidatus* Liberibacter *asiaticus*, in a single reaction (Ito *et al*. 2002; Baranwal *et al.* 2005; Meena and Baranwal, 2016).

Management Strategies

Integrated management of citrus diseases, particularly Huanglongbing (HLB), increasingly relies on a combination of chemical, genetic, and molecular approaches. At the pathogen level, foliar sprays of broad-spectrum antibiotics such as penicillin, streptomycin, and oxytetracycline have demonstrated efficacy in suppressing *C*Las populations and mitigating symptom expression under both greenhouse and field conditions. Research has also identified several therapeutic candidates, including Clidinium bromide, pimozide, sulfasalazine, and folic acid, which exhibit potential in targeting key bacterial pathways. Innovations in targeted delivery such as nanoparticle-based systems and alternative strategies like thermotherapy, as well as metal-binding and amino acid-binding proteins, further expand the toolkit for pathogen suppression (Lonare *et al*. 2023; Gupta *et al*. 2023).

On other side antimicrobial Nano-Zinc Oxide- 2S Albumin protein formulation has been developed that significantly inhibits growth of CLas by reducing titer in plant (Ghosh *et al*.2018; Saini *et al*. 2018). The biotechnological advances have enabled the development of genetically engineered citrus varieties with enhanced resistance. For instance, the introduction of the 2S albumin gene into *Citrus sinensis* and overexpression of the *PRpnp* gene (*Putranjiva roxburghii* purine nucleoside phosphorylase) in *Citrus aurantifolia* have conferred improved tolerance to abiotic stress and resistance to insect pests. These traits are mediated by upregulated defense-related phytohormones such as abscisic acid (ABA) and jasmonic acid (JA). At the vector level, efforts are ongoing to control *Diaphorina citri*, the psyllid vector of CLas, using both chemical and biological methods. Collectively, these multi-tiered approaches reflect a growing emphasis on sustainable, scalable, and scientifically informed solutions to manage HLB and ensure the long-term viability of citrus cultivation (Singh *et al*. 2023; Lonare *et al*. 2024).

APPLE

Disease incidence and economic importance

Apple (*Malus* × *domestica* Borkh.), a temperate fruit from the family *Rosaceae*, is cultivated extensively across temperate and subtropical regions worldwide and holds immense commercial importance (Hanke *et al*. 2020). However, apple productivity is severely impacted by a wide range of graft-transmissible viral and

viroid diseases, with over 19 distinct pathogens reported globally. These include Apple mosaic virus (ApMV), Apple necrotic mosaic virus (ApNMV), Apple chlorotic leaf spot virus (ACLSV), Apple stem grooving virus (ASGV), Apple stem pitting virus (ASPV), Prunus necrotic ringspot virus (PNRSV), Cucumber mosaic virus (CMV), and viroids such as Apple scar skin viroid (ASSVd) and Apple hammerhead viroid (AHVd), all of which contribute to significant yield and quality losses (Németh, 1986; Nabi *et al*. 2020).

Symptomatology

Although many apple viruses result in latent infections, symptomatic manifestations can be severe in specific cultivars or under particular graft combinations. Mosaic disease, caused by ApMV and more recently ApNMV, is characterized by pale to bright cream-colored spots or bands on spring leaves, yellow line patterns, vein clearing, and blotching. ApNMV has been identified as the primary agent of mosaic in several Asian countries, including India, with reported yield losses of up to 46% in 'Golden Delicious' apples (Cembali *et al.* 2003; Noda *et al*. 2017; Nabi *et al*. 2020). ASPV and ASGV, though often asymptomatic, can cause stem grooving, pitting, and brown line symptoms, especially when grafted onto sensitive rootstocks such as *Malus pumila* (Cho *et al*. 2010). The Apple rubbery wood virus (ARWV1/2) causes abnormal flexibility in shoots due to reduced lignification, leading to lower yields. Symptoms of Apple luteovirus (AaLV) include leaf discoloration, trunk cracking, and rapid tree decline, while *Tomato ringspot virus* (ToRSV) causes union necrosis, delayed budbreak, and premature leaf drop (Rott *et al*. 2018; Liu *et al*. 2018).

Host Range, transmission and epidemiology

Apples are the primary hosts for these pathogens, but many—such as ACLSV, ASGV, and ASPV—can also infect other members of the *Rosaceae* family, including pear and quince. In mixed orchards or where alternate hosts are present, these viruses may spread more rapidly due to cross-infection potential. Moreover, orchard weeds have been identified as alternate hosts for some viruses, such as *Cherry rasp leaf virus* (CRLV), which causes flat apple disease (James *et al*., 2001). The primary mode of transmission for apple viruses and viroids is through vegetative propagation, including grafting and budding. Some viruses, such as CRLV, can also be transmitted by nematodes (*Xiphinemaamericanum*) or mechanically, while seed transmission occurs in a limited number of cases (10–20%) (James *et al*. 2001).International trade and movement of infected propagating material have accelerated the global spread of these pathogens. Viral infections are systemic and often persist undetected for years, making early detection and certification of planting material crucial.

Molecular Characterization

Molecular studies have revealed significant genetic diversity among apple viruses. For instance, ApMV and ApNMV belong to the genus *Ilarvirus* (family *Bromoviridae*) and possess tripartite RNA genomes with icosahedral symmetry. ARWV1/2 are classified under the genus *Rubodvirus* in the family *Phenuiviridae*, while ACLSV is a member of the genus *Trichovirus* (family *Betaflexiviridae*). ASGV and ASPV also belong to *Betaflexiviridae*, the former under the genus *Capillovirus* and the latter under *Foveavirus*. Newer viruses like Apple luteovirus are associated with the genus *Luteovirus* (family *Tombusviridae*), while others such as *AGCaV* (Apple green crinkle-associated virus) have been placed within *Foveavirus* as well (Liu *et al*. 2020; Li *et al.* 2022).Recent studies using high-throughput sequencing (HTS) have confirmed the co-existence of multiple viruses, often in mixed infections, in Indian apple orchards (Nabi *et al*. 2020).

Pathogen Diversity

The apple virome is exceptionally diverse, involving RNA and DNA viruses, as well as viroids, often with overlapping symptom profiles or latent infections. Notably, Temperate fruit decay-associated virus (TFDaV) has been found in systemically infected trees without apparent symptoms, possibly due to its long latent phase or interactions with other co-infecting pathogens

(Al Rwahnih *et al*. 2009). The diversity also extends to vector relationships, with some viruses lacking known natural vectors while others, such as CRLV, are transmitted by nematodes or mechanically.

GRAPES

Disease Incidence

Grapevine (*Vitis vinifera*) is India's fifth-largest fruit crop in terms of productivity and is of significant economic importance due to its dual use in table consumption and winemaking. Globally, grapevines are affected by more than 67 viruses and 6 viroids, contributing to reduced yield, quality deterioration, and increased production costs (Basso *et al*. 2017). In India, five of these six known viroids have been documented: Australian grapevine viroid (AGVd), Grapevine yellow speckle viroid-1 and -2 (GYSVd-1, GYSVd-2), Hop stunt viroid (HSVd), and Grapevine latent viroid (GLVd) **(**Adkar-Purushothama *et al.* 2014; Sahana *et al*. 2013; Singhal *et al.* 2019; Sidharthan*et al*. 2020).

Symptomatology

While many viroid infections are asymptomatic, some can produce noticeable effects under specific environmental conditions or in co-infection scenarios. For example, GYSVd-1 and GYSVd-2 are associated with yellow speckle and vein banding at elevated temperatures (Taylor and Woodham, 1972). Coinfections, such as GYSVd-1 with *Grapevine fanleaf virus* (GFLV), can result in severe vein banding disease and vine decline (Di Seria*et al*. 2017).HSVd, while mild in grapevines, has caused substantial economic losses in hop cultivation (Sano, 2012).

Host Range, transmission and epidemiology

Grapevines are the primary hosts for these viroids, though HSVd has a broader host range including hop and other plant species. The widespread cultivation of grapevine cultivars in varied agro-climatic zones increases the risk of pathogen spread and symptom expression in mixed infections. Viroids are primarily transmitted through infected propagating material used in grafting and budding (Di Seria*et al*. 2017). Additional transmission routes include mechanical means such as contaminated pruning tools and, to a lesser extent, seeds (Wah and Symons, 1999). However, field studies suggest that routine vineyard operations like pruning are unlikely to be major transmission pathways (Staub *et al*. 1995). These modes of spread, combined with the vegetative propagation of grapevines, have facilitated the global dissemination of viroid pathogens.

Molecular Characterization

Grapevine-infecting viroids are small, circular, non-coding RNAs localized primarily in the nucleolus. Their lack of a protein coat and low abundance complicate isolation and detection (Singhal *et al*. 2021). Traditional techniques such as return-polyacrylamide gel electrophoresis (R-PAGE) and dot blot hybridization were initially employed but later replaced by more sensitive molecular methods. Reverse transcriptase-polymerase chain reaction (RT-PCR), introduced for viroid detection in the early 1990s, remains a standard method but requires purified nucleic acids, thermal cycling equipment, and skilled personnel (Hadidi and Yang, 1990). In recent years, reverse transcriptase-recombinase polymerase amplification (RT-RPA) has emerged as a rapid, sensitive alternative. RT-RPA operates under isothermal conditions (37–42/ °C), completing amplification within 10–20 minutes, and is capable of detecting viroids from crude plant extracts without nucleic acid purification. This technique has been successfully applied to detect Little cherry virus 2, Tomato chlorotic dwarf viroid (TCDVd), and HSVd in hops (Mekuria *et al*. 2014; Kappagantu *et al*. 2017).

Pathogen Diversity

The grapevine virome is remarkably diverse and includes numerous latent pathogens that often go undetected in routine field surveys. Diagnostic challenges are further compounded by the frequent occurrence of mixed infections involving both viruses and viroids, which can exacerbate symptom severity and complicate management decisions (Singhal *et al*. 2021). Given the high incidence of asymptomatic infections and the

Table 1: Summary of hosts, associated viruses and viroids, diagnostic tools, and references for major horticultural crops in India

Host Crop	Viruses and Viroids	Diagnostic Tools	References
Citrus	*Citrus tristeza virus* (CTV), *Citrus yellow mosaic virus* (CYMV), *Indian citrus ringspot virus* (ICRSV), Citrus viroids (e.g., CEVd), *CandidatusLiberibacter asiaticus* (HLB)	RT-PCR, qPCR (TaqMan, SYBR Green), RT-LAMP, RT-RPA-LFICA, Duplex PCR, Serological assays (ELISA), NCM-based DNA extraction	Ghosh *et al.,* 2013; 2018; 2020; Kokane *et al.,* 2021 Motghare*et al*., 2017 Bharsakale*et al*., 2023, 2025
Apple	*Apple mosaic virus* (ApMV), *Apple necrotic mosaic virus* (ApNMV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Apple chlorotic leaf spot virus* (ACLSV), Viroids (e.g., AHVd)	RT-PCR, Multiplex RT-PCR, Real-time PCR, High-Throughput Sequencing (HTS/NGS)	Hu *et al*., 2018; Cho *et al.,* 2017
Banana	*Banana bunchy top virus* (BBTV), *Banana bract mosaic virus* (BBrMV), *Banana streak virus* (BSV), *Cucumber mosaic virus* (CMV)	ELISA, RT-PCR, Rolling Circle Amplification, DNA hybridization, qPCR, LFIA	Kumar *et al*., 2015; Selvarajan et al., 2020
Grapes	*Grapevine leafroll-associated viruses* (GLRaV-1, 3, 4), *Grapevine fanleaf virus* (GFLV), *Grapevine rugose wood-associated virus* (GRSPaV), *Grapevine fleck virus* (GFkV), *Grapevine red blotch virus* (GRBV), *Grapevine geminivirus A* (GGVA), Viroids	RT-PCR, ELISA (limited use), High-Throughput Sequencing (HTS/NGS), qPCR, Duplex PCR	Saha *et al.,* 2021; Singhal *et al*., 2021

increasing detection of new or previously unreported viroids in Indian vineyards, comprehensive molecular surveillance and the use of sensitive multiplex diagnostic tools are essential for effective disease management and certification of planting material.

BANANA

Disease Incidence

Banana and plantain (*Musa* spp.) rank among the world's top ten food crops, cultivated across approximately 10.3 million hectares in tropical regions. These crops, primarily propagated vegetatively through suckers or tissue-culture plantlets, are highly susceptible to systemic viral infections. Due to their perennial growth and clonal propagation, banana plantations facilitate the accumulation and perpetuation of viruses, significantly reducing yields and posing substantial challenges to the international exchange of germplasm. Globally, around 20 virus species from five viral families have been identified as infecting banana, of which Banana bunchy top virus (BBTV), Banana bract mosaic virus (BBrMV), and a complex of Banana streak viruses (BSVs) are the most economically damaging. BBTV, in particular, is responsible for up to 100% yield loss in affected plantations and has caused considerable contraction in banana cropping areas, particularly in the "Old World" regions (Tripathi *et al*. 2016; Singh *et al.* 2018).

Symptomatology

Banana bunchy top disease (BBTD), caused by BBTV, is the most devastating viral disease in banana cultivation. Infected plants exhibit distinct symptoms such as marginal chlorosis of young leaves, upright leaf orientation, stunted growth, and the formation of a characteristic "bunchy" top. Plants often fail to produce fruit or yield only malformed bunches. BBrMV causes reddish-purple streaks on pseudostems and bracts, mosaic patterns on leaves, and fruit deformation, whereas BSV infections are often latent but may express as chlorotic or necrotic streaks on leaves and pseudostems under stress or in certain hybrids (Elayabalan *et al*. 2015).

Host Range

The primary hosts for BBTV, BBrMV, and BSVs are banana and plantain. Some viruses, such as Abaca bunchy top virus (ABTV) and a distinct strain of Sugarcane mosaic virus (SCMV-Ab), extend their host range to related species like

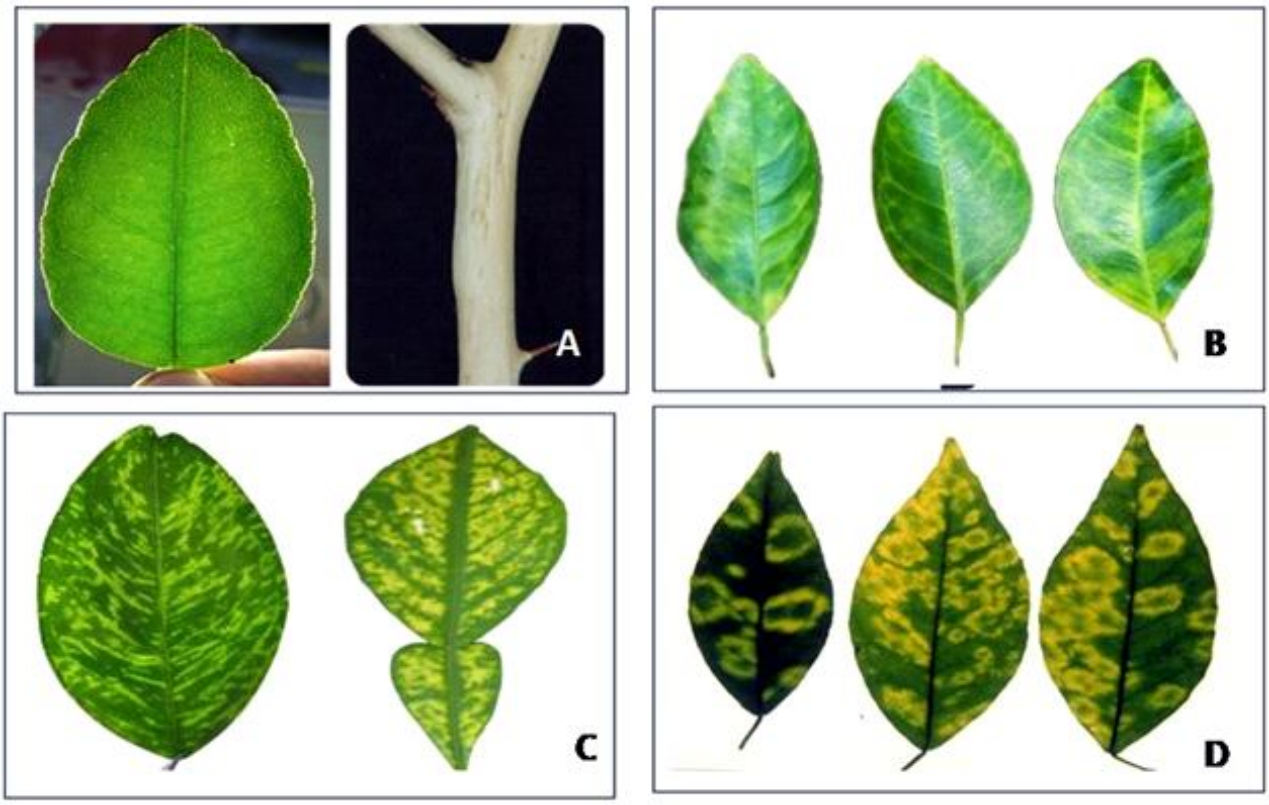

Fig 1: Symptomatology of Major Viral and Virus-like Diseases Affecting Citrus. This figure illustrates characteristic symptoms associated with four major citrus pathogens: **A:**Citrus tristeza virus (CTV), **B**: Huanglongbing (HLB),**C:**Citrus yellow mosaic virus (CYMV), and **D:**Indian citrus ringspot virus (ICRSV). The observed leaf and stem symptoms are important diagnostic indicators for field-level identification and initial disease surveillance

abaca (*Musa textilis*), indicating potential cross-host adaptability. Additional viruses with limited host specificity include Banana mild mosaic virus, Banana virus X (BVX), and Cucumber mosaic virus (CMV), which can infect multiple monocotyledonous crops (Kumar *et al*. 2015; Singh, 2018).

Transmission and Epidemiology

BBTV is transmitted by the banana aphid (*Pentalonianigronervosa*) in a persistent circulative manner and spreads rapidly through infected planting material. BBrMV is also vectored by aphids but does not integrate into the host genome. In contrast, BSVs exist in both episomal and endogenous forms; the latter are integrated into the *Musa* genome and can become activated under biotic or abiotic stress. These transmission modes, coupled with vegetative propagation, greatly complicate virus containment (Jebakumar *et al*. 2018). The epidemiology of banana viruses is also influenced by vector populations, planting practices, and environmental conditions. Global movement of infected propagules further exacerbates the risk of introducing these pathogens into new production zones, as evidenced by recent detections of BBrMV in Colombia and Costa Rica.

Molecular Characterization

BBTV, a member of the genus *Babuvirus* (family *Nanoviridae*), consists of a multipartite circular single-stranded DNA genome of six components. BBrMV belongs to the genus *Potyvirus* (family *Potyviridae*) and contains a positive-sense single-stranded RNA genome. BSVs are members of the genus *Badnavirus* (family *Caulimoviridae*) and are known for their pararetrovirus characteristics, with double-stranded DNA genomes and the ability to integrate into host genomes. Other minor viruses, including *Banana virus X* and *Banana mild mosaic virus*, are unassigned members of the *Betaflexiviridae* family, while *Cucumber mosaic virus* is a *Cucumovirus* (family

Bromoviridae). The molecular diversity within and among virus species has been elucidated through full-genome sequencing and phylogenetic analyses, which have also informed taxonomic revisions and diagnostic strategies (Tripathi *et al*.2016).

Pathogen Diversity

The global banana virome is diverse, comprising both episomal and endogenous viruses with distinct genome structures and replication strategies. The coexistence of DNA and RNA viruses in banana complicates detection and control, especially when multiple infections occur. Endogenous BSV sequences can reactivate under stress, mimicking infection by episomal forms and confounding diagnosis. Continued detection of new virus strains in diverse regions underscores the dynamic nature of banana virus evolution and spread, particularly in the absence of strong genetic resistance in *Musa* germplasm (Hanafi, 2023).

Diagnostics

The diagnosis of banana viral diseases has evolved significantly with advancements in molecular tools. ELISA has traditionally been used due to its simplicity and cost-effectiveness, but PCR-based assays have become the preferred diagnostic tools due to their specificity and sensitivity. Reverse transcription PCR (RT-PCR), real-time PCR, and multiplex PCR enable the simultaneous detection of multiple viruses and are widely used for certification and surveillance programs. Isothermal amplification methods, such as recombinase polymerase amplification (RPA), Lateral Flow Immunoassay (LFIA) offer rapid, field-deployable alternatives that do not require thermal cyclers. Moreover, next-generation sequencing (NGS) has revolutionized virus discovery, enabling the identification of novel viruses and complete viral genomes from infected samples. These diagnostic advances have greatly improved disease surveillance, epidemiological studies, and the certification of virus-free planting material in banana production systems (Varma *et al*. 2022, Selvarajan *et al*. 2020).

Development of disease diagnostics for clean planting material of horticultural crops

Early and accurate detection of graft-transmissible pathogens is essential for the effective management of viral and viroid-associated diseases in horticultural crops. A variety of serological and molecular techniques have been employed to diagnose major pathogens affecting citrus, apple, banana, and grapes. Table 1 summarizes the host crops, key associated viruses and viroids, diagnostic tools used, and relevant references supporting the application of these methods in India. Serological methods such as ELISA remain useful for high-throughput detection, particularly in banana and citrus, though they are limited in sensitivity and specificity. Molecular diagnostics including RT-PCR, real-time PCR, multiplex PCR, and isothermal amplification methods like RT-RPA and RT-LAMP have emerged as the primary tools due to their high sensitivity and specificity. In recent years, high-throughput sequencing (HTS) has revolutionized the field by enabling unbiased detection of known and novel pathogens. These diagnostics have been critical for plant certification, epidemiological surveillance, and germplasm exchange programs. Workflow of a Clean Plant Program for Generating Certified Disease-Free Planting Material outlining the stepwise process aimed at producing certified disease-free planting material for growers has been depicted in Fig.2.

CONCLUSION

Graft-transmissible pathogens pose a major constraint to the sustainable production of horticultural crops, especially high-value fruit crops such as citrus, apple, banana, and grapes. These pathogens, including a wide array of viruses, viroids, and phloem-limited bacteria, are often systemic, latent, and difficult to manage once established. Their impact is exacerbated by the widespread use of vegetative propagation, which facilitates their rapid and persistent spread across generations and regions. In the absence of curative treatments, the development and implementation of robust diagnostic systems are central to disease surveillance, certification of planting material, and effective management strategies.

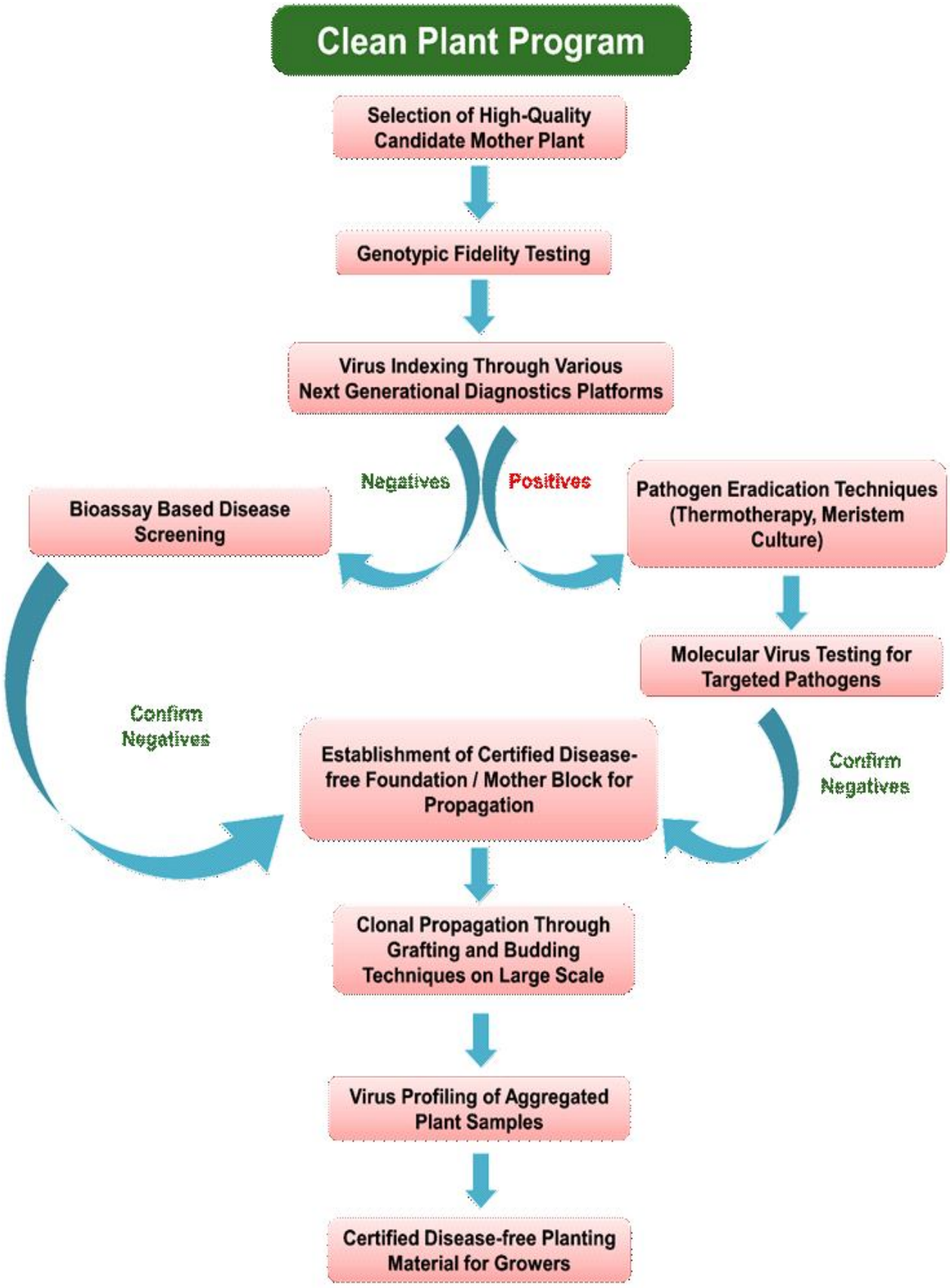

Fig 2 : Workflow of a Clean Plant Program for Generating Certified Disease-Free Planting Material. This flowchart outlines the stepwise process of a Clean Plant Program aimed at producing certified disease-free planting material for growers

Recent advancements in molecular diagnostics—including RT-PCR, real-time PCR, multiplex PCR, and isothermal amplification technologies like RT-RPA and RT-LAMP—have greatly enhanced our ability to detect multiple pathogens with high sensitivity and specificity. Moreover, the adoption of high-throughput sequencing (HTS) has revolutionized pathogen discovery and diversity analysis, enabling the identification of both known and novel viruses and viroids in complex host-pathogen systems. These tools have been instrumental in informing breeding programs, quarantine regulations, and integrated disease management practices.

Despite these advances, challenges remain in translating laboratory diagnostics to field-level applications. There is an urgent need to develop cost-effective, user-friendly, point-of-care diagnostic kits and to standardize certification protocols across nurseries and germplasm repositories. Additionally, integrating molecular diagnostics with clean plant programs, breeding for virus resistance, and strengthening phytosanitary regulations will be key to reducing the burden of graft-transmissible pathogens. Overall, a coordinated approach that combines diagnostic innovation, certified clean planting material, vector management, and policy support is essential for safeguarding the productivity, quality, and global competitiveness of India's horticulture sector.

ACKNOWLEDGMENT

I extend my sincere thanks to Shri RushikeshBharsakale, Shri MrugendraGubyad and Dr Ashish Warghane for their valuable contributions, insightful discussions, and technical assistance throughout the project.

DECLARATION

Conflict of Interest. Authordeclares no conflict of interest.

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