*Review Article*

**A comprehensive review on *Citrus Tristiza Virus***

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ABSTRACT

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| Citrus Tristiza Virus (CTV) is an aphid-borne pathogen that causes devastating diseasesymptoms in citrus like stem pitting,quick decline and seed yellows. This disease even reach endmic,epidemic and pandemic levels.CTV belongs to Closterovirus group with largest genome size of 19.3kb.CTV variants causing stem pitting, initially restricted to regions of Asia, Australia, South Africa, and South America, have been successively reported in other citrus areas, including California, Florida, and the Mediterranean region.The virus CTV is phloem-limited and is reported to be transmitted by viruliferous aphids *Toxoptera citricida*, *Aphis gossypii*, *A. citricola*, *T. aurantia.*Detection and identification of CTV can be achieved using biological, serological, or molecular amplification tests.Biological indexing or indicator plants can be used for the extraction,diagnosis of viruses, and further detection by RT-PCR and ELISA.Trifoliate orange and its derivative cultivars can be used as a resistant rootstock against Quick Decline.Preimmunization with mild strain through cross protection is the best method of managing CTV. |

*Keywords: (Citrus Tristiza Virus,citrus,filamentous,Closterovirus,stem pitting,quick decline,cross protection,Toxopteracitricida,aphid).*

1. INTRODUCTION

Citrus Tristiza Virus (CTV) is an aphid-borne pathogen responsible for devastating disease outbreaks that occurred in many citrus-growing regions around the world during the past century and continues in the twenty-first century, causing endemics,epidemics, and pandemics, with symptoms such as stem pitting,quick decline, and seed yellowing in *Citrus* species

**2. TAXONOMY**

CTV belongs to the genus Closterovirus in the family Closteroviridae, which comprises viruses with large (15–20 kb) single-stranded positive-sense RNA genomes (1–6). Folimonova and Sun(2022)revealed that the CTV possesses the largest non-segmented genome (19.3 kb), which is packaged into a long, flexuous virion (2,000 nm × 10–12 nm).

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**3. GEOGRAPHICAL DISTRIBUTION**

Symptomless hosts for many CTV isolates suggest that CTV probably appeared many years ago in the areas of origin of citrus (South East Asia and the Malayan archipelago) and then coevolved with citrus hosts. Moreno *et al.*(2007) reported that the most destructive epidemics of tristeza occurred in Argentina (1930), Brazil (1937), California (1939), Florida (1951), Spain (1957), Israel (1970), and Venezuela (1980); however, important outbreaks have also been reported in Cyprus (1989), Cuba (1992), Mexico (1995), the Dominican Republic (1996), and Italy (2002).CTV variants causing stem pitting, initially restricted to regions of Asia, Australia, South Africa, and South America, have been successively reported in other citrus areas, including California, Florida, and the Mediterranean region. Jones(2021) reported that CTV occurs in South, Central, and North America; SSA and North Africa; Europe; the Middle East and Arabia; the Indian subcontinent; East Asia; Southeast Asia; and Oceania. Worldwide, CTD has killed more than 100 million trees in Argentina (>26 million), Brazil (>6 million), Venezuela (>6 million), the USA (>3 million), Spain (>40 million), South Africa since 1910 and Israel since the 1950s, and in many other countries with warmer climates across the globe (many millions more killed)and also reported that this CTV caused epidemics with a devastating global pandemic in orange, mandarin, grapefruit and lime orchards and destroyed entire industries.

**4. TRANSMISSION**

CTV is transmitted by *Citrus* spp. and *Fortunella* spp. which are natural hosts, can be mechanicallytransmitted and mainly by aphid vectors (Moreno*etal*.,2007).The virus CTV is transmitted by viruliferous aphids *Toxoptera citricida*, *Aphis gossypii*, *A. citricola*, *and T. aurantia*, and so on. The mode of CTV transmission by aphids is noncyclic and semi-persistent. CTV can be obtained by *T. citridus* from citrus within 1–24 h after the acquisition period, with a persistence time of 24–48 h and a transmission time of 30–100 h, depending on the feeding time. transmission efficiency of a single CTV-carrying aphid on sweet orange plants, the transmission rate of a single *T. citridus* was the highest (16%), followed by that of *A. gossypii* (14%)when compared to other aphids(Chen *et al.*,2023).they also reported that psyllid *Diaphorina citri* that transmits a citrus greening bacterium, also transmits CTV. Jones(2021) reported that the aphids *T. aurantii* (black citrus aphid) and *A. spiraecola* (green citrus aphid) are also less efficient vectors for CTV.Apart from aphids, dodder(*Cuscuta* spp.)has been found to carry and transmit CTV.Transmission probably involves an association of dodder and host cells, and directional movement of nutrients.It has long been known that dodder’s haustoria establishes cellular connections with the hostplant that could result in transmission of virus as well(Hosford,1967).

**5. HOST RANGE AND SYMPTOMATOLOGY**

Citrus Tristiza Virus (CTV), which causes citrus tristeza disease, is the most widely distributed disease that hinders citrus crop production. Strains of CTV cause symptoms in citrus, including rapid decline, stem pitting, and seedling yellows. Sour orange (*Citrus aurantium* L.), which is used as a rootstock, or sweet orange (*Citrus sinensis* (L.) Osbeck) and grapefruit (*Citrus paradisi*MacFadyen)will be infected by stem pitting and quick decline(Chen *et al.,*2023). Depending on the virus variant and citrus host scion-rootstock combination, CTV can cause two major diseases. The first disease is referred to as a quick decline and results in the death of trees grown on the sour orange (*Citrus aurantium* L.) rootstock. The second disease caused by variants of the virus is stem pitting (SP). SP affects sweet orange (*Citrus sinensis* [L.) Osbeck], grapefruit (*Citrus paradisi*MacFadyen), lime (*Citrus aurantifolia* [Christm.) Swingle], and other citrus varieties, irrespective of the rootstock. The disease manifests as the formation of pits and grooves in tree branches and trunks, resulting from abnormal vascular development induced by the virus. The affected trees show loss of vigor, stunting, low yield, and small, unmarketable fruits. It is also important to note that some CTV variants cause mild to no symptoms, at least in certain citrus hosts(Folimonova and Sun,2022).In the case of a sweet/sour orange combination, bark flaps cut across the graft union show small holes (honeycombing) on the inside face of the bark flap from the rootstock side of the union(Schrader *et al.,*2019). Quick decline trees may only have a yellow-brown stain at the bud union, without honeycombing’ The symptoms of CTV infection range from leaf cupping and epinasty, leaf vein clearing or corking, seedling yellows, and stunting to the two most damaging syndromes—quick decline and stem pitting (Dawson *et al*., 2015).The decline is the result of virus-induced phloem necrosis in the sour orange rootstock bark underneath the bud union with the scion. Due to such necrosis, carbohydrate movement to the root system from the canopy is disrupted, and after a certain period, the plant stops growing new fibrous roots because of the limited supply of energy reserves, which is followed by subsequent degeneration of the existing fibrous root system (Garnsey *et al*., 1998). Infected plants may exhibit thin dull green to yellow foliage, leaf shedding, twig dieback, chlorotic leaves, and pale colored fruits that have a low market value (Moreno *et al*., 2007). In the case of stem pitting, the virus interferes with the normal differentiation of cambial cells in different areas of the stem, leading to pit-like structures(fig1) in the trunk, branches, xylem cells, and several other parts where the virus has been present. Plants infected with steam pitting strains also show symptoms such as stunting, reduction of radial growth, yellowish small leaves, and reduced fruit size and quality (Moreno *et al*., 2007). Different loci in the viral genome are responsible for inducing decline and steam pitting syndromes. An additional symptom of seedling yellows is observed in some young sour oranges, grapefruit, and lemons, mainly under experimental conditions (Bar-Joseph *et al*., 2002). The response of seedling yellows has been characterized by a severe reduction in seedling size and severe chlorosis of the foliage in inoculated plants (Roistacher*et al*., 2010). In artificial inoculation Moreno *et al*.(2007)detected CTV in *genera Aegle, Aeglopsis, Afraegle, Atalantia, Citropsis, Clausena, Eremocitrus, Hesperthusa, Merrillia, Microcitrus, Pamburus, Pleiospermium and Swinglea*. Experimental infection was also achieved in the non-citrus species *Passiflora gracilis* and *Passiflora coerulea* using aphid vectors (Roistacher *et al*., 1988), and in *Nicotiana benthamiana* protoplasts or agroinfiltrated leaves.

**6. PATHOGENICITY/PATHOGENIC CYCLE/PATHOGEN BIOLOGY**

CTV is a phloem-limited virus that possesses the largest non-segmented RNA genome (19.3kb) among plant viruses. It is a flexuous filamentous particle with a positive-sense single-stranded RNA genome containing 12 open reading frames (ORFs) (Folimonova and Sun, 2022). Virus replication is enhanced by proteins encoded by ORF1a and ORF1b, virus assembly and movement (major coat protein, minor coat protein, p65, p61, and p6), and suppression of antiviral RNA silencing (CP, p20, and p23). Three unique proteins (13, 18, and p33) help in the host range of CTV and selection of host species(Agranovsky, 2016).

**7. ISOLATION AND IDENTIFICATION OF *Citrus Tristiza Virus***

Detection and identification of CTV can be achieved using biological, serological, or molecular amplification tests.Gosh *et al.*(2022)extracted CTV and proved Koch’s postulate successfully for CTV in acid-lime indicator plants. The virus-inoculated plants developed vein clearing, leaf cupping, temporary yellowing, and stunting of young seedlings. Purification of Citrus Tristiza Virus, extraction from liquid-nitrogen frozen midribs and cortical tissue of infected Mexican lime, precipitation with polyethylene glicol mol. wt 6000 (PEG 6000) column chromatography on Sephacryl S-300-HR columns, and the concentration of virus-containing fractions by PEG 6000, followed by a low-speed (17,200 g) centrifugation protocol can be followed(Kambegrolu *et al.,*2001).Huang *et al.*(2004)reported that the detection and differentiation of CTV isolates can be performed with SDS-Immunodiffusion, direct tissue blot immunoassay (DTBIA), restriction fragment length polymorphism (RFLP) , serologically specific electron microscopy (SSEM), enzyme-linked immunosorbent assay (ELISA),dot-immunobinding assay,radio-immunosorbent assay (RISA) , in situ immunofluorescence (ISIF), in situ immunoassay (ISIA), western blot assay, single-strand conformation polymorphism (SSCP), and reverse transcription–polymerase chain reaction (RT-PCR).Biological characterization of CTV isolates was performed by Varveri*et al.*(2014), who studied three indicator plants or plant combinations to determine the aggressiveness and pathogenicity of the isolate. Mexican lime (*C. aurantiifolia*) and sweet orange cultivar (‘Pineapple’) plants were cut down to 30 cm and chips budded with infected material. Rough lemon (*C. jhambiri*) seedlings were inoculated with isolate L192GR, and after 5 months, systemic infection was assessed by tissue print-ELISA. Two healthy sour oranges, cv. Sevillano buds were propagated on CTV-infected rough lemon plants. The combination of sour orange grafted on rough lemons was assayed to index the ability of the tested CTV isolate to induce a decline in scion varieties propagated on sour orange rootstock. Three plants were used for each indicator. Plants were periodically checked for symptom expression for a year, the time typically required for the development of stem pitting symptoms. A severe CTV isolate, T308, was used as an internal positive control, and non-inoculated sweet orange (pineapple) was used as a negative control.

**8. EPIDEMIOLOGY**

Along with CTV viral strains involved in infection, other parameters, especially rise/fall in temperature and co-infection with otherviruses or pathogens, could alter symptom development in the host plants (Garnsey *et al*., 1991).Moreno *et al.* (2008) revealed that the aphid fauna,aphid population density,environmental conditions favoring new flush and aphid population build-up (moderate temperatures, irrigation, and fertilization),susceptibility of the predominant citrus varieties, and transmissibility of the predominant CTV isolates influence the spread of CTV.

**9. ECONOMIC IMPORTANCE**

Citrus Tristiza Virus (CTV) is the most destructive citrus virus in the world. Approximately 30 million trees on sour orange rootstocks were lost in Brazil and Argentina in 1940–60, 6.6 million trees in Venezuela in the 1980s, and an estimated 10 million trees in Florida and other Caribbean Basin countries. In Spain, the CTV killed approximately 10 million trees, and several million more trees were lost in California, Israel, and other areas. In these areas, the melon aphid Aphis gossypii is spreading the CTV. When both vectors are present, as in Florida, the spread of the CTV is significantly enhanced. Yield losses have been documented in several locations, for example, in Jamaica. At two locations totaling 1159 acres, losses over five years were more than US$4 million(Lobeinstein,2008).Thus, in Brazil, production increased from 1.7 million t in 1960 to more than 20 million tons in the 1990s of the previous century.

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**10. Control measures**

* 1. **Cultural methods**

Certified budwood for propagation, control CTV transmitting vectors, and follow quarantine and legislative measures; major diseases of CTV, stem pitting, which affects citrus varieties irrespective of the rootstock, is more difficult to control. Many commonly used rootstocks, such as Cleopatra mandarin, Rough lemon, Rangpur lime, and Volkamer, are referred to as "CTV tolerant" because they are not affected by the decline in sour orange strains of CTV, which kill trees on sour orange. These rootstocks are tolerant of CTV, for example, the virus replicates, and some stem pitting strains of CTV can cause severe stem pitting. Thus, CTV-tolerant rootstocks are useful in areas where stem-pitting strains that affect these rootstocks are not widespread(Sharma ,2023).

* 1. **Chemical methods**

Susi *et al.(*2021)reported that the percentage control of *Toxoptera citricidus*was obtainedwith 1 % %tobacco extract, which is a botanical pesticide.

* 1. **Biological methods**

While rapid decline is managed by the use of tolerant rootstocks, the only viable means to protect field citrus trees against aggressive isolates of CTV causing stem pitting is pre-immunization with mild virus isolates. This approach is based on the phenomenon of viral cross-protection, in which plants pre-infected with a symptomless or mild variant of a virus are protected from more severe variants of the same virus. With CTV, this strategy has been used for more than half a century in Australia, South Africa, Brazil, and Peru, where it enabled the commercial production of sweet orange and grapefruit despite the presence of severe stem pitting isolates. To date, at least seven major strains of the CTV Citrus Tristiza Virus have been identified. Each strain combined numerous variants with a minor sequence divergence (less than 5%). Remarkably, variants of the same CTV strain can induce a range of phenotypes, with some being symptomless and others causing severe disease. It is expected that the understanding that the protecting inoculum must harbor a CTV variant of the same strain as that of the disease-causing component in the severe isolate will make the selection of protecting isolates more efficient. Similarly, the cross-protection technique could be useful against Stem Pitting isolates of the CTV(Sharma,2023)

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* 1. **Resistant/Tolerant varieties (if any)**

Trifoliate orange and its derivative cultivars can be used as resistant rootstocks against rapidly declining isolates of CTV. Similarly, the cross-protection technique could be useful against Stem Pitting isolates of the CTV(Sharma,2023). Sharma(2023) also reported that *Poncirus trifoliata* is a resistant rootstock against CTV because it is sexually compatible and the resistance ability can be transferred to citrus hybrid rootstocks via sexual crossing Swingle citrumelo (C. paradisi × P. trifoliata), Troyer, and Carrizo citranges (*C. sinensis* × *P. trifoliata*), and hybrids resulting from crossing between *C. jambhiri, C. volkameriana, C. limonia* and *P. trifoliata* were found to be tolerant against different strains of CTV. Trifoliate orange and its hybrid rootstocks are primarily used against rapidly declining strains of CTV (Roistacher *et al*., 2010). *C. latifolia, C. grandis* and some mandarins are generally considered as somewhat tolerant to stem pitting CTV strains. Transgenic lines /coat protein mediated resistance are proved to be unsuccessful with CTV.

Competing interests

“Authors have declared that no competing interests exist.”

Authors’ Contributions

‘All authors have contributed in collection of literature, wrote, read and approved the final manuscript.”



**Fig.1.Stem pitting on Citrus bark due to *Citrus Tristiza Virus.***

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