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Prevalence of Mild T30, T3, and Severe VT Strains of *Citrus tristeza* Virus in Central-Northern Veracruz, Mexico

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Abstract

The presence and distribution of *Citrus tristeza virus* (CTV) strains on plants of four citrus species required analysis of plants showing small fruits, yellowing, and twig dieback. Typical *citrus tristeza* symptoms showed in citrus plantations in Veracruz, Mexico, and caused gradual deterioration, low yield, and death of the plants. Seven citrus-growing municipalities in Central and Northern Veracruz presented the severe VT strain: the incidence in seven of the eight sampled municipalities ranged from 7% in Alamo to 50% in Tihuatlan. As for the T30 strain, its presence and distribution ranged from 17% in Tihuatlan to 83% in Cuitlahuac. Values for the T3 strain went from 20% in Castillo de Teayo to 50% in Papantla. In Tihuatlan, two samples were positive for the T36 strain. Only *Citrus sinensis* has historical recording infections by severe strains of CTV, unlike other important citrus species. The incidence of orchards quickly declining increases rapidly in Veracruz, the largest citrus producer in Mexico. It becomes critical as the predominant and sensitive rootstock is sour orange (*Citrus aurantium*). It is important to note that the specific primers used could not be identified in 29% of the samples, suggesting that they could be other than T30, T3, VT, and T36 strains.

Keywords

Citrus, Incidence, Strains, Quick Decline, Severity

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1. Introduction

The Mexican citrus industry has various phytosanitary challenges like *Citrus tristeza virus* (CTV), *Citrus psorosis virus* (CPsV), *Citrus leprosis virus* (CiLV), *Citrus exocortis viroid* (CEVd), *Citrus viroid* II (CVd-II), *Candidatus liberibacter asiaticus* (CLas), *Xanthomonas axonopodis citri*, and *Xylella fastidiosa* subsp. *pauca* [1] and other unknown alterations such as blight and wood pocket. They all affect production, and it is difficult to define the most important disease in Mexico. Nonetheless, as CTV has caused damage to citrus in various countries since 1900, to this date, it continues to be the most devastating viral disease.

Citrus tristeza virus belongs to the genus *Closterovirus*, family *Closteroviridae*, with filamentous particles 2000 nm long × 11 nm in diameter. The genome consists of a 19.3 kb positive-sense RNA molecule with 12 open reading frames (ORFs) encoding up to 19 proteins [2] [3] [4]. *Aphis gossypii*, *Aphis spiraecola*, *Toxoptera aurantii*, and *Toxoptera citricida* are four aphid species that can transmit CTV. The virus transmits readily by grafting and the aphid species mentioned above in a semi-persistent manner [5] [6]. Mexico's main citrus growing areas contain *A. gossypii*, *A. spiraecola*, *Aphis fabae*, and *T. aurantii* [7].

CTV has been a recurrent citrus disease worldwide. Between 1930 and 1950 in Argentina, Brazil, Uruguay, Colombia, and Peru, CTV killed 20 million trees grafted onto sour orange (*Citrus aurantium*), a CTV susceptible species when grafted. A similar epidemic occurred in Venezuela and Jamaica in 1980 [8]. Since 1952, CTV emerged in Florida, USA [9], which has caused considerable damage [10].

In Mexico, *Citrus sinensis* trees showed CTV for the first time in Tamaulipas in 1983. Later, from 1986 to 1993 orchards and nurseries in different municipalities of Veracruz detected positive plants, while it spread to Yucatán, Quintana Roo, Campeche, Morelos, Michoacán by 2000, and to Colima, and Nuevo León by 2005 [10] [11] [12].

The three main symptoms associated with this pathogen are: 1) quick decline of sweet orange on sour orange (*C. aurantium* L.); 2) stem pitting in grapefruits (*Citrus paradisi* Macf.) and oranges (*Citrus sinensis* L); 3) seedling yellows on sour orange [13]. Several CTV strains have been identified: VT, T36, T30, T3, RB, and T68 [4] [14]. T36 and VT have been biologically described as severe strains, while T30 is mild [11] [15].

In 2017, in Northern Veracruz, orchards of *C. sinensis*, *Citrus latifolia*, *Citrus reticulata*, and *C. paradisi* showed trees with combined symptoms such as defoliation and yellowing, twig dieback, and eventual tree death. This growing problem causes gradual deterioration and low yield, shortening orchards' productive life.

The pathogen is gaining importance as a limiting factor for the Mexican citrus industry due to the extensive use of the highly vulnerable *C. aurantium*. The incidence of plants with symptoms associated with CTV is increasing. This research evaluated the presence and distribution of CTV strain in citrus-producing municipalities in Central-Northern Veracruz, Mexico.

2. Materials and Methods

2.1. Sampling Area

The study comprised the main citrus-producing areas of Central-Northern Veracruz in commercial orchards of *C. sinensis*, *C. latifolia*, *C. reticulata*, and *C. paradisi* of different ages, located in the municipalities of Alamo, Castillo de Teayo, Cazones, Cuitlahuac, Martinez de la Torre, Papantla, Tihuatlan, and Tuxpan. Sampling focused on trees with yellowing symptoms, decline, death of annual branches, and small fruits. From up to two trees, samples included four vegetative shoots in active growth from each orchard (one shoot per cardinal direction).

2.2. RNA Extraction

The number of samples per municipality varied: six in Cuitlahuac, nine in Martinez, 10 in Papantla, 15 in Alamo, 18 in Tihuatlan, 18 in Castillo de Teayo, 22 in Cazones, and 22 in Tuxpan. Samples per species divided as seven of *C. paradisi*, 22 of *C. latifolia*, 24 of *C. reticulata* and 67 of *C. sinensis*, as in some sampling areas only one citrus species is grown.

RNA extraction followed this protocol: 0.2 g of the midrib of leaves was macerated in a mortar with liquid nitrogen until obtaining a fine powder; the powder was transferred into a 2 mL microcentrifuge tube with 750 µL of EB1 and 75 µL of 20% SDS and mixed on a vortex (BenchMixerTM). The tubes were incubated at 65˚C for 20 min in a Dry Block Heater (Select BioProducts), mixing them with inversion every 5 min; 250 µL of 5M KOAC were added and incubated at 4˚C for 20 min. Subsequently, they were centrifuged at 13,500 rpm at 10˚C for 20 min; 600 µL of the supernatant was transferred to a 1.5 mL microcentrifuge tube with 540 µL of isopropanol, incubated at −20˚C for 30 min, then centrifuged at 13,500 rpm at 10˚C for 15 min. The supernatant was discarded, and the pellet was allowed to dry. The pellet was then dissolved with 700 µL of EB2 at 4˚C for 12 h. The tubes were centrifuged at 13,500 rpm at 4˚C for 15 min. 600 µL of the supernatant were transferred into a 1.5 mL microcentrifuge tube containing 500 µL of isopropanol and 75 µL of 3M NaOAC. The tube was mixed by inversion five to 10 times and centrifuged at 13,500 rpm at 4˚C for 10 min. The supernatant was discarded, 1 ml of 75% ethanol was added to the formed pellet, and the mixture was centrifuged at 13,500 rpm at 4˚C for 10 min. The supernatant was removed, and the pellet was allowed to dry; once dried, the pellet was resuspended in DNase-free water. Nucleic acid concentration and purity were verified with a NanoDropTM 2000 spectrophotometer.

2.3. Amplification of CP Genomic Region by RT-PCR

Two-step RT-PCR was performed. Synthesis of cDNA was performed using M-MLV reverse transcriptase (PROMEGA) with modification in the reverse transcription reaction (RT), 0.5 µL of each forward and reverse primer (**Table 1**) [12] [16] were added into a 0.2 mL microcentrifuge tube with 4 µL water and 2 µL

Table 1. Genotype-specific primer sequences were used for reverse-transcription polymerase chain reaction to amplify *Citrus tristeza virus* (CTV) strains.

Strains	Polarity	Sequences from 5' to 3'	Product size
CTV	Forward (F)	AAC GCC CTT CGA GTC TGG GGT AGG A	273
	Reverse (R)	TCA ACG TGT GTT GAA TTT CCC AAG C	
T30	Forward (F)	TGT TGC GAA ACT AGT TGA CCC TAC TG	206
	Reverse (R)	TAG TGG GCA GAG TGC CAA AAG AGA T	
T3	Forward (F)	GTT ATC ACG CCT AAA GTT TGG TAC CAC T	409
	Reverse (R)	CAT GAC ATC GAA GAT AGC CGA AGC	
VT	Forward (F)	TTT GAA AAT GGT GAT GAT TTC GCC GTC A	302
	Reverse (R)	GAC ACC GGA ACT GCY TGA ACA GAA T	
T36	Forward (F)	TTC CCT AGG TCG GAT CCC GAG TAT A	836
	Reverse (R)	CAA ACC GGG AAG TGA CAC ACT TGT TA	

of RNA (200 $\mu g \cdot \mu L^{-1}$) from each sample. The tubes were incubated at 72˚C for 5 min and then placed on ice for 10 min. Into each microcentrifuge tube, 4 µL of the mix containing 2 µL Buffer 5X of M-MLV (PROMEGA), 1 µL of DTT 0.1 M (PROMEGA), 0.5 µL of dNTP's Mix (PROMEGA), and 0.15 µL of M-MLV reverse transcriptase (PROMEGA). Reverse transcription was performed at 42˚C for 60 min, followed by inactivation at 72˚C for 10 min.

PCR was performed in a total reaction volume of 9 µL of the reaction mix. This mix contained 2 µL of Green buffer GoTaq DNA polymerase (PROMEGA), 0.4 µL of $MgCl_2$, 0.2 µL of dNTP's mix, 0.6 µL of each primer F and R [12] [16], 0.1 µL of GoTaq DNA polymerase (PROMEGA), 5.1 µL of DNase-free water, and 2 µL of cDNA. The PCR conditions proposed by [16] were followed, and the five strains (T36, VT, T3, B165, and T30) were those proposed by Roy [12]. The products were visualized on a 2% agarose gel with ethidium bromide.

2.4. Sequencing and Phylogenetic Analysis

The samples that tested positive were sent to Macrogen Corp. for Sanger sequencing. The sequences were assembled with the DNA BASER software and compared with those deposited in the National Center for Biotechnology Information (NCBI). Five sequences obtained in this study were deposited in the GenBank database (MN545966, MN545967, MN545968, MN545969, and MN545970). The phylogenetic analysis was performed with the Neighbor-Joining (NJ) method and the nucleotide substitution model obtained was K2 (Kimura 2 parameters); a bootstrap of 500 repetitions was performed with the MEGA software.

3. Results and Discussion

3.1. *Citrus tristeza Virus* Detection

In six out of the eight municipalities, the percentage of samples positive for CTV was higher than 50% (**Figure 1**). The reason might be that *C. sinensis* dominates

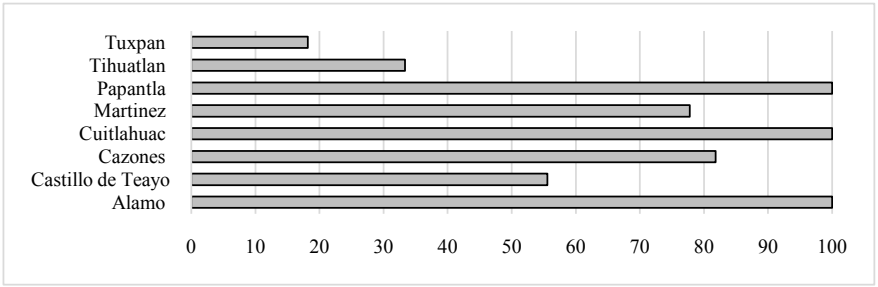

Figure 1. Incidence (%) of *Citrus tristeza virus* in eight municipalities in Central and Northern Veracruz, Mexico.

in Alamo, Castillo de Teayo, Cazones, Papantla, and Tihuatlan, and most of the cultivars from this species produce three vegetative sprouting per year. In contrast, *C. reticulata* (in Tuxpan) produces a single vegetative sprouting per year, while in Cuitlahuac, *C. latifolia*, the prevalent species, has three to four vegetative sprouting affected by the weather. These species then become the preferred hosts for the main vectors (aphids). Our results agree with [17], who mentioned that CTV is semi-persistently transmitted by *A. gossypii*, *T. citricida*, and *T. auranti*, among other aphids. The results for Martinez de la Torre are enigmatic as *C. latifolia* dominates, similarly to Cuitlahuac, yet their outcomes differ; a more intensive technology usage that increases the number of pest control applications might be the reason for this discrepancy.

It is essential to indicate that since 1986, [18] mentions CTV detection in Ixtacuaco, Veracruz, a community located on the limits of Martinez de la Torre. The time between studies (32 years) presumes a higher incidence percentage. This situation occurred in Brazil and Argentina with the brown citrus aphid (*T. citricida*) as a vector, and the rapid spread of the *Citrus tristeza virus* caused the death of millions of trees grafted onto *C. aurantium* in the 1930s and 1940s [19]. In Mexico, CTV was first detected in 1983 and then the primary vector in 2000; it is probably for this reason that it took longer for the disease to spread.

The results obtained in Veracruz coincide with findings in Spain, where the disease took 54 years to become an epidemic as there was no vector; while in Brazil, where a vector existed, the process took five to 10 years [20]. The detection of *T. citricida* in Quintana Roo [21] resulted in predictions of an increase in strains and severe CTV symptoms as in other countries [22]; however, the reasons for the lack of correlation between vector dispersion in Mexico and disease spread are unknown.

The virus may have already infected most producing orchards in the state of Veracruz, but it is necessary to verify this. Extensive surveys are necessary for all citrus-producing areas in Mexico as the virus might be present in other citrus-producing states.

This study found CTV in 100% of the *C. paradisi* samples, followed by *C. latifolia* in 82% of the samples, *C. sinensis* in 60%, and *C. reticulata* in 46% (**Figure 2**). Differences in cultivar incidences may depend on various factors that influence the CTV transmission rate, such as cultivars that produce more shoots per year,

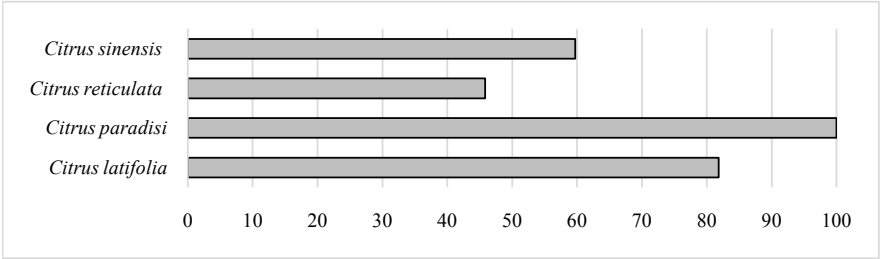

Figure 2. Percentage of positive samples for *Citrus tristeza virus* by species.

aphid species and their reproduction rates changing with the local environmental conditions [23], use of non-certified vegetative material, and orchard management (e.g., pruning) with infected tools [24].

The presence of CTV in all the municipalities and cultivars sampled may result from using infected material as most producers acquired their plants in non-certified nurseries and with sour orange (*C. aurantium*) rootstock. Thus, certification programs are essential to prevent the introduction of severe CTV strains or other pathogens associated with citruses. Besides the main insect vectors, four species of aphids have shown the ability to transmit CTV: *A gossypii*, *A. spiraecola*, *T. aurantia*, and *T. citricida*. The last species mentioned is a highly efficient transmission vector for CTV, up to 25 times. Where *T. citricida* is absent, *A. gossypii* becomes the primary vector of CTV [25] [26] [27].

Nevertheless, in Mexico, the distribution and transmissibility of CTV, or the main citrus-associated aphids in Central and Northern Veracruz, is not known. A pest currently widely in most citrus-producing areas, *Diaphorina citri*, acts as a CTV vector [28]. In some states of Mexico, given the conditions of humidity, temperature, and alternate hosts, *D. citri* can be found throughout the year [29].

3.2. *Citrus tristeza Virus* Strain Detection

This study employed RT-PCR for five different strains with specific primers: T30, T3, B165, VT, and T36. Out of the 120 samples analyzed, 76 were positive for CTV. All eight municipalities studied contained the T30 strain (**Figure 3**): the incidence ranged from 17 in Tihuatlan to 83% in Cuitlahuac. Thus, all municipalities studied have the disease and, indeed, most other municipalities of Veracruz. Notoriously, visible symptoms do not appear under the T30 strain, even in key lime (*C. aurantifolia*) indicator plants [30]. The lack of symptoms may explain why no tree deaths have occurred in Mexico.

The VT strain, considered severe [31], was found in seven municipalities, with incidence from 7% in Alamo to 50% in Tihuatlan (**Figure 3**). This prevalence might explain the observed tree deaths in the north of Veracruz and put the country's citrus industry at risk since *C. aurantium* is the principal rootstock. Epidemics related to CTV have occurred at different times in Ghana (1938), California (1939), Florida (1951), Spain (1957), Israel (1970), and Venezuela (1980); those countries had *C. aurantium* as a rootstock [32]. We highlight the above as the observed symptoms in the field coincide with those reported in the literature

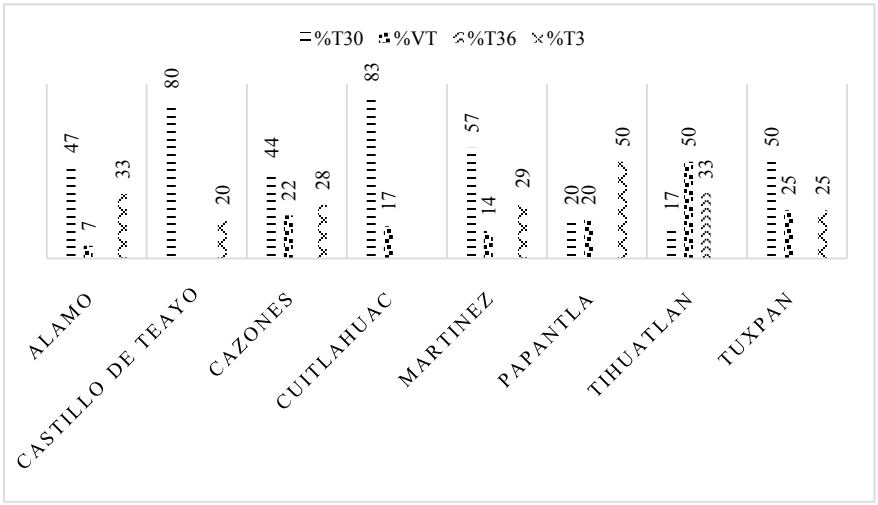

Figure 3. Incidence of *Citrus tristeza virus* strains in eight municipalities of Veracruz, Mexico.

by [12] [33]: small fruits attached to the plant, defoliation, and quick tree death. The T3 strain was detected in six municipalities, from 20% in Castillo de Teayo to 50% in Papantla.

In Tihuatlan, CTV-positive samples for T30 and VT strains (**Figure 4**), also tested positive for T36 (33%), which is considered the most aggressive type worldwide [14] [31]. All strains are associated with severe leaf symptoms (vein clearing or leaf cupping), mild to moderate stem pitting on all replicates of key lime, as well as little or no stunting (depending on replicate) on this host [34]. Our results conclude that this strain should be present in other municipalities; thus, more exhaustive studies are required.

The results indicate rapid progression affecting Veracruz and other producing states; NOM-031-FITO-2000 and NOM-079-FITO-2002 standards, CTV continues spreading. Moreover, in the Mexican citrus orchards, sour orange (*C. aurantium*) is the prevailing rootstock, making it even more vulnerable to CTV.

In most municipalities, sampled trees showed yellowing of shoots, leaves, and small fruits, dieback of twigs, and debarking of the trunk and branches. In Cazones and Tihuatlan, the cultivars sampled corresponded to *C. sinensis* and *C. reticulata*, in most cases grafted onto *C. aurantium*; current knowledge indicates that CTV symptoms vary depending on the virus strain and the cultivar/rootstock combination [35]. The most often described symptoms are deterioration and death in cultivars of *C. sinensis*, *C. reticulata*, and *C. paradisi*. In *C. aurantium*, "stem pitting" is commonly observed resulting from aberrant phloem development. "Yellowing" is characterized by growth retardation and leaf chlorosis in *C. sinensis* and *C. paradisi* [36]. Aggressive strains and the cultivar/rootstock combination cause tree death and substantially decrease plant yield and vigor, resulting in cumulative economic losses. The CTV positive trees in Cazones, Cuitlahuac, and Tihuatlan showed yellowing of shoots, leaves, and small fruits, dieback of twigs, and rapid deterioration.

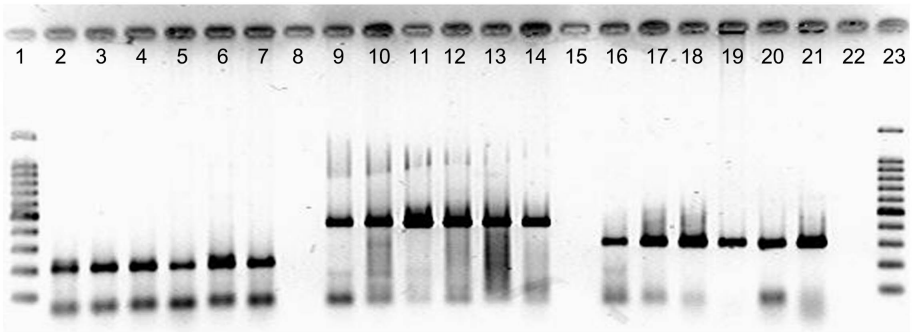

Figure 4. Simplex reverse-transcription polymerase chain reaction (RT-PCR) amplicons were obtained from *Citrus tristeza virus* (CTV)-T30 (206 bp), CTV-T3 (409 bp), and CTV-VT (302 bp). Lanes 1 and 22 are 100-bp molecular marker ladder; lanes 2, 9, and, 16 were positive controls; lines 3 to 7, 10 - 14, and 17 to 21 citrus samples; and lanes 8, 15, and 22 were negative controls.

3.3. *Citrus tristeza Virus* Strain Phylogeny

The sequences of each isolate were read in Bioedit, aligned, and compared with GenBank and showed a similarity of up to 100% with CTV strains. For phylogenetic analysis, three sequences (AF260651.1, EU857538.1, and MN545966) were selected. Therefore, based on this analysis, strains of the virus can be detected with specific primers as proposed by Roy *et al.* [11] for an endpoint RT-PCR. The dendrogram (**Figure 5**) shows three groupings: CTV type T30, T3, and VT. The *Citrus tristeza virus* has caused epidemics in citrus cultivation worldwide for two centuries. Stain T36 and VT mainly caused quick tree decline. Stem pitting, often caused by T3 or T68, substantially reduces the growth and fruit quality of *C. paradisi*, *C. sinensis*, and *C. aurantifolia* trees, regardless of the rootstock used, which limits the type of rootstock and varieties that can be grown commercially where these strains are present [12] [25] [26].

The high incidence of CTV in the prominent citrus-producing municipalities in the state of Veracruz allows us to conclude that: 1) Producers generally do not use certified material; 2) The required prophylactic measures are not applied in the handling of pruning tools; 3) The nursery certification program to produce citrus plants used in Mexico is only partially applied. It is vital to acknowledge the current situation in Veracruz, where citrus farming is the basis for the livelihood of more than 50,000 families; furthermore, *C. latifolia* is the primary fruit exported to other countries.

Our results can explain the causes of the accelerated decline of citrus farming in Central-Northern Veracruz: most sampled plants had damaged trunks, as well as damaged main branches from second to fifth or sixth order, showed psosoris (CPsV), exocortis (CEVd), cachexia (CVd-II), and HLB (CLas). Additional factors like associated diseases caused by *Phytophthora*, *Lasiodiplodia*, and *Colletotrichum* species and the lack of pruning and fertilization further promote the decline. The effects show particularly on Persian lime, mandarin, orange orchards, and to a lesser extent, grapefruit.

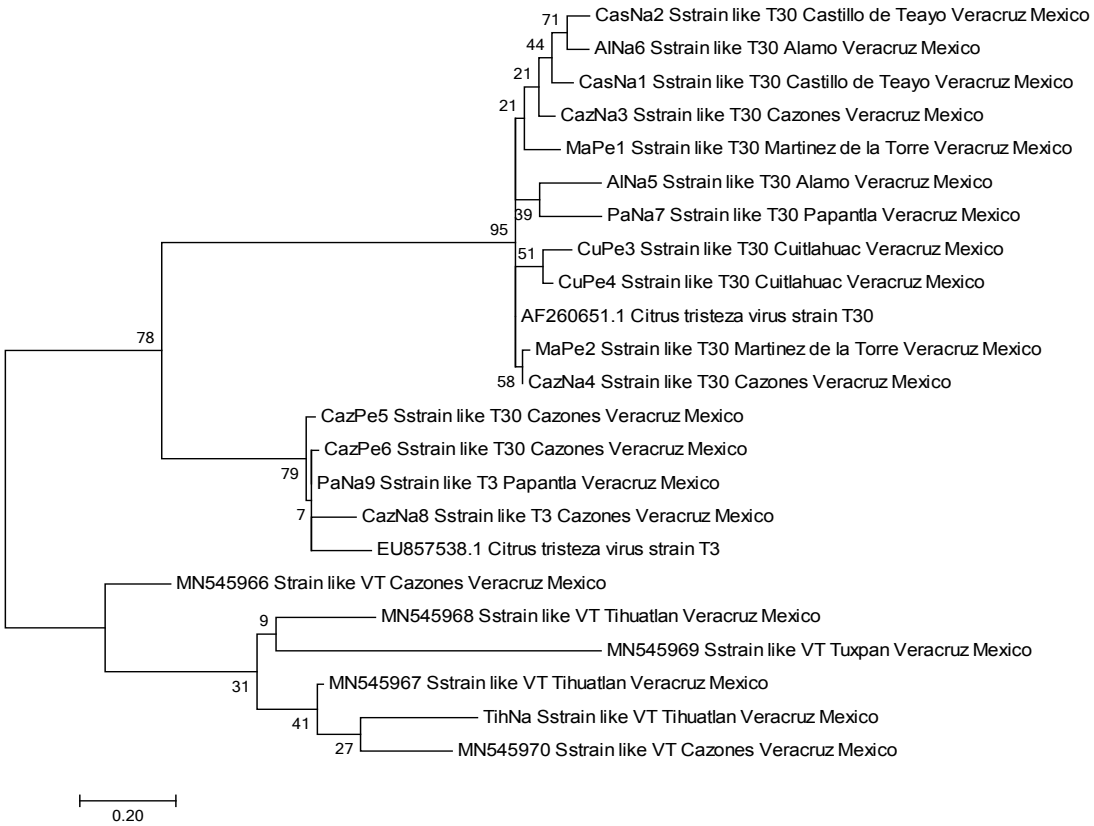

Figure 5. Phylogenetic reconstruction of the *Citrus tristeza virus* was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model, with 500 Bootstrap of 23 sequences, 21 from this study, and two CTV sequences from the Gen Bank.

The potential for future citrus losses from CTV is more significant than previously recorded. Therefore, pre-immunization using attenuated strains is the only means to deal with severe *Citrus tristeza virus* strains. According to [37], it has been the best way to control CTV in Brazil, South Africa, Australia, and Japan. Pre-immunization as a management strategy for CTV could resolve one of the phytosanitary problems in Mexican citrus farming. Additionally, the production must be done in certified nurseries and on a scientific and professional basis. In addition, the understanding of the relationship between CTV isolates, plants, and vectors, which in Mexico is scarce, should be strengthened. The combination of the use of certified and pre-immunized buds is undoubtedly the best way to control the losses induced by CTV. Before you begin to format your paper, first write and save the content as a separate text.

4. Conclusion

The presence of strains like VT in seven of the eight municipalities studied shows the high risk of citrus farming in Central-Northern Veracruz, Mexico. On the other hand, it is necessary to carry out more exhaustive studies to proactively find strains more severe than the VT and T36 strains found in Central-Northern Veracruz.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sáenz Pérez, C.A., Osorio Hernández, E., Estrada Drouaillet, B., Poot, W.A.P., Delgado Martínez, R. and Rodríguez Herrera, R. (2019) Principales enfermedades en cítricos. *Revista Mexicana de Ciencias Agrícolas*, **10**, 1653-1665. https://doi.org/10.29312/remexca.v10i7.1827

[2] Satyanarayana, T., Gowda, S., Ayllon, M.A., Albiach-Marti, M.R., Rabindram, R. and Dawson, W.O. (2002) The p23 Protein of *Citrus tristeza* Virus Controls Asymmetrical RNA Accumulation. *Journal of Virology*, **76**, 473-483. https://doi.org/10.1128/JVI.76.2.473-483.2002

[3] Biswas, K.K., Palchoudhury, S., Sharma, S.K., Saha, B., Godara, S., Ghosh, D.K. and Keremane, M.L. (2018) Analyses of 3' Half Genome of *Citrus tristeza* Virus Reveal Existence of Distinct Virus Genotypes in Citrus Growing Regions of India. *Virus-Disease*, **29**, 308-315. https://doi.org/10.1007/s13337-018-0456-2

[4] Harper, S.J. (2013) *Citrus tristeza* Virus: Evolution of Complex and Varied Genotypic Groups. *Frontiers in Microbiology*, **4**, Article No. 93. https://doi.org/10.3389/fmicb.2013.00093

[5] Roistacher, C.N. and Bar-Joseph, M. (1987) Aphid Transmission of *Citrus tristeza* Virus: A Review. *Phytophylactica*, **19**, 163-167.

[6] Çevik, B., Yard, N. and Korkmaz, S. (2013) The First Identified *Citrus tristeza* Virus Isolate of Turkey Contains a Mixture of Mild and Severe Strains. *The Plant Pathology Journal*, **29**, 31-41. https://doi.org/10.5423/PPJ.OA.09.2012.0141

[7] Gaona, G.G., Ruíz, C.E. and Peña, M.R. (2000) Los pulgones (Homoptera: Aphididae) y sus enemigos naturales en la naranja, *Citrus sinensis* (L.), en la zona centro de Tamaulipas, México. *Acta Zoológica Mexicana*, **81**, 1-12.

[8] Müller, G.W. and Rezende, J.A.M. (2004) Preimmunization: Applications and Perspectives in Virus Disease Control. In: Naqvi, S.A.M.H., Ed., *Diseases of Fruits and Vegetable-Diagnosis and Management*, Springer, Dordrecht, 361-395. https://doi.org/10.1007/1-4020-2606-4_9

[9] Lee, F.L. and Keremane, L. (2013) Mild Strain Cross-Protection of Tristeza: A Review of Research to Protect against Decline on Sour Orange in Florida. *Frontiers in Microbiology*, **4**, Article No. 259. https://doi.org/10.3389/fmicb.2013.00259

[10] Herrera-Isidrón, L., Ochoa-Sanchez, J.C., Rivera-Bustamante, R. and Martínez-Soriano, J.P. (2009) Sequence Diversity on four ORFs of *Citrus tristeza* Virus Correlates with Pathogenicity. *Virology Journal*, **6**, Article No. 116.

[11] Ochoa-Corona, F.M., Rocha-Peña, M.A. and Lee, F.R. (1995) Impacto del virus de la tristeza de los citricos en la citricultura venezolana: cronología de eventos [The Impact of *Citrus tristeza* Virus on Venezuelan Citriculture. Chronology of Events]. *Revista Mexicana de Fitopatología*, **12**, 97-105.

[12] Roy, A., Ananthakrishnan, G., Hartung, J.S. and Brlansky, R.H. (2010) Development

and Application of a Multiplex Reverse-Transcription Polymerase Chain Reaction Assay for Screening a Global Collection of *Citrus tristeza* Virus Isolates. *Phytopathology*, **100**, 1077-1088. https://doi.org/10.1094/PHYTO-04-10-0102

[13] Moreno, P., Ambrós, S., Albiach-Martí, M.R., Guerri, J. and Peña, L. (2008) *Citrus tristeza* Virus: A Pathogen that Changed the Course of the Citrus Industry. *Molecular Plant Pathology*, **9**, 251-268. https://doi.org/10.1111/j.1364-3703.2007.00455.x

[14] Harper, S.J., Killiny, N., Tatineni, S., Gowda, S., Cowell, S.J., Shilts, T. and Dawson, W.O. (2016) Sequence Variation in Two Genes Determines the Efficacy of Transmission of *Citrus tristeza* Virus by the Brown Citrus Aphid. *Archives of Virology*, **161**, 3555-3559. https://doi.org/10.1007/s00705-016-3070-x

[15] Biswas, K.K., Tarafdar, A. and Sharma, S.K. (2012) Complete Genome Sequence of Mandarin Decline *Citrus tristeza* Virus of the Northeastern Himalayan Hill Region of India: Comparative Analyses Determine Recombinant. *Archives of Virology*, **157**, 579-583. https://doi.org/10.1007/s00705-011-1165-y

[16] Rivas-Valencia, P., Loeza-Kuk, E., Mora-Aguilera, G., Febres, V., Ochoa-Martínez, D.L., Gutiérrez-Espinosa, M.A., Jesus-Junior, W.C., Correia-Malvas, C. and Arno-Wulff, N. (2008) Estructura poblacional de aislamientos del *Citrus tristeza* virus y su asociación con la muerte súbita de los cítricos en Brasil [Population Structure of *Citrus tristeza* Virus Isolates and Its Association with Citrus Sudden Death in Brazil]. *Agrociencia*, **42**, 85-93.

[17] Yokomi, R., Selvaraj, V., Maheshwari, Y., Chiumenti, M., Saponari, M., Gilampetruzzi, A., Weng, Z., Xiong, Z. and Hajeri S. (2018) Molecular and Biological Characterization of a Novel Mild Strain of *Citrus tristeza* Virus in California. *Archives of Virology*, **163**, 1795-1804. https://doi.org/10.1007/s00705-018-3799-5

[18] Becerra, L.E.N. (1996) Virus de la tristeza de los citricos 2a edición. Campo Experimental Cotaxtla CIRGC, INIFAP and SARH.

[19] Knorr, L.C. and DuCharme, E.P. (1951) This Is Tristeza—Ravager of Argentina's Citrus Industry. *Citrus Magazine*, **13**, 17-19.

[20] Costa, A.S. and Müller, G.W. (1980) Tristeza Control by Cross-Protection: A U.S. Brazil Cooperative Success. *Plant Disease*, **64**, 538-541. https://doi.org/10.1094/PD-64-538

[21] Michaud, J.P. and Alvarez, R. (2000) The First Collection of Brown Citrus Aphids (Homoptera: Aphididae) is in Quintana Roo, Mexico. *Florida Entomologist*, **83**, 357-358.

[22] Leonel, W.M.S., Corazza, M.J., Zanutto, C.A., Müller, G.W., Carvalho, S.A. and Nunes, W.M.C. (2015) Stability of *Citrus tristeza* Virus Protective Isolates 'Pêra IAC' According to SSCP Analysis of Old and New Lines of Three Sweet Orange Varieties. *Summa Phytopathologica*, **41**, 8-12. https://doi.org/10.1590/0100-5405/1991

[23] Cambra, M., Gorris, M.T., Marroquín, C., Roman, M.P., Olmos, A., Martínez, M.C., Hermoso de Mendoza, A., López, A. and Navarro, L. (2000) Incidence and Epidemiology of *Citrus tristeza* Virus in the Valencian Community of Spain. *Virus Research*, **71**, 85-95. https://doi.org/10.1016/S0168-1702(00)00190-8

[24] Roistacher, C.N. (2005) Indexing for Viruses in Citrus. In: Hadidi, A., Khetarpal, R.K. and Koganezawa, H., Eds., *Plant Virus Disease Control*, APS Press, St. Paul, MN, 301-319.

[25] Hilf, M.E., Mavrodieva, V.A. and Garnsey, S.M. (2005) Genetic Marker Analysis of a Global Collection of Isolates of *Citrus tristeza* Virus: Characterization and Distribution of CTV Genotypes and Association with Symptoms. *Phytopathology*, **95**, 909-917. https://doi.org/10.1094/PHYTO-95-0909

[26] Matos, L.A., Hilf, M.E., Cayetano, X.A., Feliz, A.O., Harper, S.J. and Folimonova, S.Y. (2013) Dramatic Change in *Citrus tristeza* Virus Populations in the Dominican Republic. *Plant Disease*, **97**, 339-345. https://doi.org/10.1094/PDIS-05-12-0421-RE

[27] Campolo, O., Chiera E., Malacrino, A., Laudani, F., Fontana, A., Albanese, G.R. and Palmeri, V. (2014) Acquisition and Transmission of Selected CTV Isolate by *Aphis gossypii*. *Journal of Asia-Pacific Entomology*, **17**, 493-498. https://doi.org/10.1016/j.aspen.2014.04.008

[28] Wu, F., Huang, M., Fox, E.G.P., Huang, J., Cen, Y., Deng, X. and Xu, M. (2021) Preliminary Report on the Acquisition, Persistence, and Potential Transmission of *Citrus tristeza* Virus by *Diaphorina citri*. *Insects*, **12**, Article No. 735.

[29] Wang, J., Bozan, O., Kwon, S.J., Dang, T., Rucker, T., Yokomi, R.K., Lee, R.F., Folimonova, S.Y., Krueger, R.R., Bash, J., Greer, G., Diaz, J., Serna, R. and Vidalakis, G. (2013) Past and Future of a Century-Old *Citrus tristeza* Virus Collection: A *California citrus* Germplasm Tale. *Frontiers in Microbiology*, **4**, Article No. 366. https://doi.org/10.3389/fmicb.2013.00366

[30] Díaz-Padilla, G., López-Arroyo, J.I., Sánchez-Cohen, I., Guajardo-Panes, R.A., Mora-Aguilera, G. and Quijano-Carranza, J.Á. (2014) Áreas de abundancia potencial en México del vector del Huanglongbing, *Diaphorina citri* (Hemiptera: Liviidae). *Revista Mexicana de Ciencias Agrícolas*, **5**, 1137-1153. https://doi.org/10.29312/remexca.v5i7.836

[31] Besoain, X., Bertolini, E., Camps, R., Ramella, F., Gorris, M.T., Torres, M. and Cambra, M. (2015) Aggressive *Citrus tristeza* Virus Isolates in Chile Are MCA13-Positive and VT Type, While Mild Isolates Are MCA13-Negative and T30 Type. *Ciencia e Investigación Agraria*, **42**, 251-262. https://doi.org/10.4067/S0718-16202015000200011

[32] Davino, S., Davino, M., Sambade, A., Guardo. M. and Caruso, A. (2003) The First *Citrus tristeza* Virus Outbreak Was Found in a Relevant Citrus-Producing Area of Sicily, Italy. *Plant Disease*, **87**, 314. https://doi.org/10.1094/PDIS.2003.87.3.314A

[33] Bar-Joseph, M. and Dawson, W.O. (2008) *Citrus tristeza* Virus. In: Mahy, B.W.J. and Van Regenmortel, M.H.V., Eds., *Encyclopedia of Virology*, Elsevier Ltd., Amsterdam, 520-525. https://doi.org/10.1016/B978-012374410-4.00639-7

[34] Read, D.A., Palacios, M.F., Figueroa, J., Foguet L., Kleynhans ,J., Stein B. and Pietersen, G. (2017) Survey of *Citrus tristeza* Virus (CTV) Strains in *Citrus x Limon* (L) Burm f. (Lemon) in Tucumán Province, Argentina. *European Journal Plant Pathology*, **149**, 1029-1039. https://doi.org/10.1007/s10658-017-1245-2

[35] Dawson, W.O., Garnsey, S.M., Tatineni, S., Folimonova, S.Y., Harper, S.J. and Gowda, S. (2013) *Citrus tristeza* Virus-Host Interactions. *Frontiers in Microbiology*, **4**, Article No. 88. https://doi.org/10.3389/fmicb.2013.00088

[36] Abbas, M., Khan, M.M., Mughal, S.M., Ji, P. (2015) Comparison of Infection of *Citrus tristeza* Closterovirus in Kinnow Mandarin (*Citrus reticulata*) and Mosambi Sweet Orange (*Citrus sinensis*) in Pakistan. *Crop Protection*, **78**, 146-150. https://doi.org/10.1016/j.cropro.2015.09.006

[37] Tenorio, C.A., de Carvalho, N.W.M., Zanutto, C.A. and Müller, G.W. (2010) Stability of *Citrus tristeza* Virus Protective Isolates in Field Conditions. *Pesquisa Agropecuária Brasileira*, **45**, 693-700. https://doi.org/10.1590/S0100-204X2010000700009