



Emerging disease in melon crop associated with a mixed infection by three whitefly-transmitted viruses in La Comarca Lagunera, Mexico

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ABSTRACT

Background/Objective. Cucurbits are an economic pillar of global and national agriculture. However, their productivity is threatened by the increasing incidence of viral plant diseases. Consequently, coinfections with members of the *Begomovirus* and *Crinivirus* genera have been associated with exacerbated symptoms and high incidence rates in crops, leading to emerging disease patterns. The aim of this study was to identify and molecularly characterize the begomoviruses and criniviruses associated with an emerging disease in melon crops in a municipality of the Comarca Lagunera region, Mexico.

Materials and Methods. A targeted sampling of nine melon plants exhibiting symptoms such as severe interveinal chlorosis, mottling, yellow mosaic patterns, and fruit deformation was carried out. For viral detection, DNA extraction was performed using 3% CTAB and RNA extraction using the TRIzol reagent. Begomoviruses and criniviruses were detected using PCR and RT-PCR with specific primers. Complete begomovirus genomes were enriched by rolling circle amplification (RCA). Amplicons derived from Open Reading Frame (ORF) of the capsid protein (CP) of criniviruses were generated using RT-PCR. Subsequently, the genomes and CP amplicons were cloned and sequenced for phylogenetic and evolutionary analysis.

Results. Molecular identification revealed the prevalence of mixed infections in seven of nine samples, comprised of two begomovirus species, cucurbit leaf crumple virus (CuLCrV, *Begomovirus cucurbitae*) and watermelon chlorotic stunt virus (WmCSV, *Begomovirus citrulli*), and the crinivirus cucurbit yellow stunting disorder virus (CYSDV, *Crinivirus cucurbitae*). Genomic analyses confirmed that CuLCrV and WmCSV possess genomic structure typical of begomoviruses. Significant divergence was observed at specific *loci* (CuLCrV C4 gene, WmCSV NSP gene, and DNA-B intergenic regions of both species), suggesting their potential involvement in evolutionary adaptation processes. Phylogenetically, although the CuLCrV isolate showed close relationship to isolates from Arizona and Sonora, it was placed in a clade distinct from those previously reported in the region, indicating an independent evolutionary course. Furthermore, phylogenetic analysis based on the nucleotide sequence of the CYSDV capsid protein (CP) gene revealed a close relationship with isolates previously reported in Jordan and Mexico.

Conclusions. This study represents the first report in Mexico of a disease associated with WmCSV, CuLCrV, and CYSDV coinfection in cucurbits. The findings emphasize the complexity of emerging diseases and highlight the importance of strengthening molecular epidemiological surveillance to mitigate the impact of these mixed infections on Mexican agriculture.

Keywords: Cucurbits, Begomovirus, Crinivirus, Mixed infections



INTRODUCTION

Cucurbits (*Cucurbitaceae* family) are among the most important horticultural crops worldwide and the most economically relevant species include cucumber (*Cucumis sativus*), pumpkin (*Cucurbita pepo*), melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) (Radouane *et al.*, 2021). The crop and the production of cucurbits in Mexico are of great socioeconomic importance, since they generate many jobs in all chains of the production chain (farmers, technical advisors, day-laborers, packers, industrials and salespeople). In addition, they are an important source of income through the sale of fresh fruit. According to data provided by SIAP-SADER (2024), the Comarca Lagunera region is noteworthy, particularly in the production and sale of melon, with over 4,000 ha and a production value of over 800 million pesos MXN. It is currently being affected by emerging viral diseases.

In the past three decades, the global expansion of the plant viruses transmitted by the whitefly (*Bemisia tabaci*), due to the combination of factors such as climate change and globalization, has favored the introduction of viral species into new environmental niches, which threatens cucurbit crops (Navas-Castillo *et al.*, 2011). Among the viruses transmitted by the whitefly that affect cucurbits, the viruses with DNA genomes of the *Begomovirus* genus (*Geminiviridae* family) and with RNA genomes of the *Crinivirus* genus (*Closteroviridae* family) represent one of the most threatening groups of viral agents from an epidemiological and economic point of view (Abrahamian and Abou-Jawdah, 2014; Rojas *et al.*, 2018). Begomoviruses have single-stranded DNA genomes (ssDNA) of approximately 2.7 kb, which can consist of a single segment (monopartite, DNA-A) or two segments (bipartite, DNA-A and DNA-B) (Fondong, 2013). Based on their genomic phylogeny, begomoviruses are geographically into two groups: Old World begomoviruses (OW; found in Europe, Africa, Oceania and the Indian subcontinent) and the New World begomovirus (NW; found in the Americas) (Rybicki, 1994).

The Cucurbit leaf curl disease (CuLCD) is one of the main viral threats for cucurbits. Currently, at least 18 begomovirus species, mostly bipartite and belonging to both the Old World (OW) and the New World (NW) clades, have been associated with this disease (Fiallo-Olivé *et al.*, 2021). Among the most devastating viruses associated with the CuLCD in the OW, some of the most noteworthy are the tomato leaf curl New Delhi virus (ToLCNDV, *Begomovirus solanumdelhiense*), squash leaf curl China virus (SLCCNV, *Begomovirus cucurbitachinaense*), squash leaf curl Philippines virus (SLCuPV, *Begomovirus cucurbitachinaense*) and watermelon chlorotic stunt virus (WmCSV, *Begomovirus citrulli*) (Chen *et al.*, 2021; Lapidot *et al.*, 2014), whereas the NW is home to the cucurbit leaf crumple virus (CuLCrV, *Begomovirus cucurbitae*), melon chlorotic leaf curl virus (MCLCuV, *Begomovirus chlorocucumis*) and squash leaf curl virus (SLCuV, *Begomovirus cucurbitapeponis*) (Brown and Stenger, 2002). On the one hand, the SLCuV was first found in California (United States) in the late 1970s, and its introduction into Israel in 2002 was the first recorded entry of a begomovirus from the NW (Abudy *et al.*, 2010). On the other hand, the WmCSV was initially isolated in the Arabic Peninsula and Yemen (Walkey *et al.*, 1990) and later identified in North America, including Sonora, Mexico (Domínguez-Durán *et al.*, 2018) and in Arizona, United States (Fomentenele *et al.*, 2021), making it the first begomovirus of the OW to infect cucurbits and that was introduced into the Americas.

Criniviruses are characterized by having bipartite, exceptionally, tripartite genomes, composed of positive-sense single-stranded RNA (ssRNA) with a total size ranging from 15.3 to 17.7 kb. In the case of economically important cucurbits, four criniviruses have been reported, including the cucurbit yellow stunting disorder virus (CYSDV, *Crinivirus cucurbitae*), the cucurbit chlorotic yellows virus (CCYV), the beet pseudo yellows virus (BPYV, *Crinivirus pseudobetae*) and the lettuce infectious yellows virus (LIYV, *Crinivirus lactucaflavi*) (Tzanetakis *et al.*, 2013). CYSDV is the crinivirus that affects cucurbits, the most widely distributed and the one with the greatest global economic impact. Since it was initially found in the United Arab Emirates in 1989, it has quickly spread throughout Asia, Europe, North Africa and North America, including the southwestern United States and northwestern Mexico (Wintermantel *et al.*, 2017, 2024).

It is currently known that in the field, mixed infections between plant viruses are the rule rather than the exception, and that important diseases are a consequence of complex interactions that include the antagonism and synergism between the viral etiological agents, which frequently results in unpredictable consequences (Syller, 2012). In the case of begomoviruses and criniviruses, different combinations of mixed infections at an intraspecies level (Abrahamian *et al.*, 2013; Abudy *et al.*, 2010; Al-Musa *et al.*, 2011; Ali-Shtayeh *et al.*, 2014; Bananej *et al.*, 2013; Fontenele *et al.*, 2021; Gil-Salas *et al.*, 2012; Sufrin-Ringwald and Lapidot, 2011) or an interspecies level (Abudy *et al.*, 2010; Al-Musa *et al.*, 2011; Ali-Shtayeh *et al.*, 2014; Gadhav *et al.*, 2018; Kavalappara *et al.*, 2021; Kuo *et al.*, 2007; Polston *et al.*, 2008; Sufrin-Ringwald and Lapidot, 2011) have been described globally and recently in Mexico (Hernández-Pérez *et al.*, 2025; Arce-Leal *et al.*, 2026). However, in the case of introduced viral species, such as the begomovirus WmCSV, their timely monitoring and detection are key to understanding their infection dynamics and epidemiology, in combination with other viral agents transmitted by the whitefly that affect cucurbits in the Americas.

This investigation aimed to find and molecularly characterize begomoviruses and criniviruses associated with an emerging disease observed in the Comarca Lagunera region, Mexico. Identifying the agents associated with new diseases of a viral etiology in diverse geographic areas, ecological niches and agroclimatic regions of production areas, including the Comarca Lagunera, is essential. This will help implement timely and efficient detection methods, thus ensuring the long-term sustainability of agricultural production in Mexico.

MATERIALS AND METHODS

Begomoviruses and criniviruses detection. A total of nine samples (M1-M9) of leaf tissue were collected, along with fruits from the melon plants cultivated in the open in the municipal area of Tlahualilo, Durango, which belongs to the Comarca Lagunera (CL) region, which presented symptoms associated with virosis and infestation by whiteflies. At least approximately 100 mg of leaf tissue, ground in liquid nitrogen using a mortar and pestle, was used for the independent extraction of total DNA using the CTAB method at 3% (Doyle and Doyle, 1987) and total RNA using TRIzol reagent (Thermo Fisher Scientific) following the instructions by the manufacturer. To detect begomoviruses and criniviruses, primers targeting species previously identified as infecting cucurbit crops in the Americas were used (Supplementary Table 1). For begomoviruses: specific primers for the cucurbit leaf crumple virus (CuLCrV, *Begomovirus cucurbitae*), watermelon

chlorotic stunt virus (WmCSV, *Begomovirus citrulli*), squash leaf curl virus (SLCuV, *Begomovirus cucurbitachinaense*) and the melon chlorotic leaf curl virus (MCLCuV, *Begomovirus chlorocucumis*). For criniviruses: specific primers for the yellow stunting disorder virus (CYSDV, *Crinivirus cucurbitae*), cucurbit chlorotic yellows virus (CCYV), beet pseudo yellows virus (BPYV, *Crinivirus pseudobetae*) and the lettuce infectious virus (LIVY, *Crinivirus lactucaflavi*). The molecular detection of the begomovirus was carried out by PCR with 100 ng of DNA from each duplicate of the nine samples collected as template, using the Taq DNA Polymerase Recombinant enzyme (Thermo Fisher Scientific), following the recommendations by the manufacturer. For the criniviruses, molecular detection was carried out using RT-PCR from one microgram of total RNA from an equimolar pool of the duplicate RNA extracts from each of the nine samples. Complementary DNA (cDNA) was synthesized with the enzyme M-MLV Reverse Transcriptase (Thermo Fisher Scientific), with random primers (Thermo Fisher Scientific) as reverse transcription primers. Approximately 100 ng of each cDNA was used as a template for the PCR, using the enzyme Taq DNA Polymerase Recombinant (Thermo Fisher Scientific). The resulting amplicons were separated by electrophoresis in 1% agarose gel, stained with ethidium bromide and visualized under ultraviolet light in Chemidoc equipment (BIO-RAD).

Obtaining complete begomovirus genomes associated with cucurbits. To obtain the complete genomes of the begomoviruses previously found using PCR (CuLCrV and WmCSV), the protocol described by (Inoue-Nagata *et al.* 2004) was used. Approximately 100 ng of genomic DNA from a selected sample (M2) was used as template to enrich circular viral molecules by rolling circle amplification (RCA) with phi29 DNA polymerase (kit TempliPhi™, Cytiva®). Using sequences from isolates of CuLCrV (accession numbers: AF256200 and AF327559 for DNA-A and DNA-B, respectively) and WmCSV (accession numbers: KY124280 and KY124281 for DNA-A and DNA-B, respectively), detected earlier in Mexico and available in the NCBI data base (GenBank) and in the program SnapGene (GLS Biotech LLC, Chicago, United States), it was predicted that enzymes SacI and XbaI could linearize both genomic segments (DNA-A and DNA-B) of CuLCrV and WmCSV, respectively. One microgram of the RCA product was digested with SacI or XbaI; the product of digestion was separated by electrophoresis on 1% agarose gel, and the putative complete viral segments (~2.7 kb) were excised from the gel and ligated to the vector pGreen0029 (Hellens *et al.* 2000), previously digested with the corresponding enzyme. The ligation products were introduced by thermal shock into *E. coli* strain DH10B, and the plasmid DNA from independent clones was corroborated by enzymatic digestion and complete sequencing using Sanger's method, with the strategy of "gene walking" (Clark *et al.*, 2019) using specific primers (Supplementary Table 1).

Cloning CYSDV's CP gene. From the same sample selected (M2) that presents a mixed infection by begomovirus and crinivirus, the complete Open Reading Frame (ORF) encoding the coat protein (CP) of CYSDV was amplified by RT-PCR using the primers CYSCPf/CYSCPr (Supplementary Table 1). The expected 753 bp amplicon was purified using an agarose gel with the PureLink Quick Gel Extraction Kit (Fisher Scientific) following the manufacturer's instructions. The purified band was ligated to vector pGEM-T Easy (Promega). The ligation product was transformed into the strain of *E. coli* DH10-B by thermal shock; the positive clones were selected by digestion of the resulting

plasmid DNA with the enzyme EcoRI, and two independent clones were completely sequenced using Sanger's method.

Processing sequences and molecular analyses. The generated sequences were assembled using the program SeqMan (DNASTAR Inc., Madison, U.S.A.), the viral genetic maps were created using the program SnapGene, and the identity of the viral species was verified by comparison with the NCBI database (GenBank). The molecular and phylogenetic analyses were carried out using the program MEGA 7 (Kumar *et al.* 2016), and for this purpose, phylogenetic trees were created from nucleotide sequences of the complete DNA-A segments of the begomoviruses CuLCrV and WmCSV, and of the coding region of the CP gene of CYSDV. Additionally, gene-by-gene similarity matrices were created for begomoviruses CuLCrV and WmCSV. The recombination analyses of the DNA-A and DNA-B segments of the begomoviruses CuLCrV and WmCSV and species of begomoviruses that infect cucurbits in the Americas were carried out using the program RDP4, with the predetermined configuration, using the seven methods: RDP, GENCONV, BooScan, MaxChi, SiScan, Chimaera and 3Seq (Martin *et al.*, 2015).

RESULTS AND DISCUSSION

Identification of begomoviruses and criniviruses in coinfection. In 2023, melon plants grown in the municipal area of Tlahualilo, Durango, in the Comarca Lagunera region, presented symptoms related to virosis, such as intervenial chlorosis, yellowing, curling of leaves and infestation with whitefly. These suggest the presence of viral species transmitted by the vector insect. In addition, size reduction, severe deformation and cracking in the melon fruits (Figure 1) were also observed. A total of nine samples were collected for their subsequent analysis.

A molecular scrutiny was carried out using PCR and RT-PCR with specific primers for begomovirus and crinivirus. The results indicate that the mixed triple infection by begomoviruses CuLCrV and WmCSV and the crinivirus CYSDV was the most frequent, found in 7 out of 9 samples (M1, M2, M4, M5, M6, M7 and M9). In the two remaining samples (M3 and M8), a mixed infection was found, caused by CuLCrV and CYSDV (Table 1 and Supplementary Figure 1).

Neither the other begomoviruses (SLCuV and MLCuV) nor the additional criniviruses evaluated (CCYV, BPYV and LIYV) were found in any of the samples analyzed (Table 1 and Supplementary Figure 1). The pathological importance of viral mixed infections in plants is often underestimated, except for a few, well characterized synergistic interactions in crops (Moreno and Jos, 2020). Additionally, mixed infections can alter the acquisition, retention and inoculation of viruses by the vector insect, in comparison with simple infections (Gautam *et al.*, 2020). In this sense, given that the CuLCrV and CYSDV viruses are established species in the NW and the WmCSV is a virus from the OW recently introduced into the Americas, it is important to confirm this finding with the molecular characterization of the sequences resulting from the isolates identified.



Figure 1. Melon plants and fruits showing viral symptoms collected in the La Comarca Lagunera (CL) region. A total of nine samples (M1-M9) of melon with viral symptoms were collected in the municipality of Tlahualilo. Symptoms observed in the leaf tissue were severe interveinal chlorosis, mottling, and yellow mosaic, while in the fruits, chlorosis, deformation, and cracking.

Table 1. Molecular detection of begomoviruses and criniviruses in melon leaf tissue. Nine samples were analyzed by PCR for the detection of begomoviruses: cucurbit leaf crumple virus (CuLCrV), watermelon chlorotic stunt virus (WmCSV), squash leaf curl virus (SLCuV), and melon chlorotic leaf curl virus (MCLCuV), and RT-PCR for the criniviruses: cucurbit yellow stunting disorder virus (CYSDV), cucurbit chlorotic yellows virus (CCYV), beet pseudo yellows virus (BPYV), and lettuce infectious virus (LIVY). (+) positive detection, (-) negative detection.

Sample	Begomovirus				Crinivirus			
	CuLCrV	WmCSV	SLCuV	MCLCuV	CYSDV	CCYV	BPYV	LIVY
M1	+	+	-	-	+	-	-	-
M2	+	+	-	-	+	-	-	-
M3	+	-	-	-	+	-	-	-
M4	+	+	-	-	+	-	-	-
M5	+	+	-	-	+	-	-	-
M6	+	+	-	-	+	-	-	-
M7	+	+	-	-	+	-	-	-
M8	+	-	-	-	+	-	-	-
M9	+	+	-	-	+	-	-	-

Molecular confirmation of the begomovirus and crinivirus. To verify the identity of the viral species, a strategy involving the full-length genome cloning of begomoviruses CuLCrV and WmCSV, along with the cloning of the capsid (CP) gene of CYSDV, was employed using sample M2, which presented a mixed (triple) infection by these viral species. In the case of the begomoviruses, the strategy used was to amplify circular viral molecules using RCA, followed by cloning, to isolate both genomic segments (DNA-A and DNA-B) of CuLCrV and WmCSV. The sequences obtained were assembled using the program SeqMan and compared with the viral sequences available in NCBI.

According to the nucleotide-level BLAST analyses, clones SacI-2 (2631 bp) and SacI-3 (2602 bp) displayed the highest nucleotide identities with the DNA-A of CuLCrV (accession number: AF256200) with an identity of 97.76%, and with the DNA-B of CuLCrV (accession number: AF327559) with 93.7% of identity, respectively, corresponding to the Arizona isolate. On the other hand, clones XbaI-2 (2758 bp) and XbaI-9 (2766 bp) displayed a high identity with the DNA-A of WmCSV (accession number: KY24280), reaching an identity of 99.24%, and with the DNA-B of WmCSV (accession number: KY24281), with an identity of 97.98%, respectively, corresponding to the Sonora isolate. The sequences obtained were deposited in the NCBI database (accession numbers: PX761486, PX761487, PX761484 and PX761485, corresponding to CuLCrV-A, CuLCrV-B, WmCSV-A and WmCSV-B, respectively).

Subsequently, the assembled sequences were used to create circular maps of the genomes of CuLCrV and WmCSV obtained, as well as for the annotation of the corresponding viral *loci* with the program SnapGene (Figure 2). The genome of CuLCrV exhibits a typical arrangement of the bipartite begomovirus of the NW. Segment DNA-A encodes the CP in the chain of virion; the genes that encode proteins AC4, Rep, TrAP and REn in the complementary virion chain; whereas DNA-B encodes the cell-cell and long distance movement proteins, NSP and MP, in the sense of the virion and in the complementary chain of the virion, respectively. Additionally, both segments contain the intergenic region (IR), which participates in the regulation of the replication and viral transcription (Figure 2A). The WmCSV genome presents a typical genomic organization of the begomoviruses of the OW. In addition to the genes described for the viruses of the NW, proteins AV2 and AC5 are encoded in the segment DNA-A, in the sense and complementary to, the virion, respectively (Figure 2B).

In the case of crinivirus CYSDV, amplification by RT-PCR, the cloning and sequencing of the complete ORF of the viral CP gene resulted in a 753 bp sequence, with an identity of 99.87% in comparison with the Arizona isolate (accession number: FJ492808) (Figure 3C). The sequence obtained was deposited in the NCBI database (accession number: PX761488). Altogether, the results confirm the identity of the begomoviruses and criniviruses found, which, from now onwards, will be called CuLCrV-CL, WmCSV-CL and CYSDV-CL, respectively. Likewise, the simultaneous detection of these viruses in melon plants with characteristic symptoms confirms the presence of an emerging disease related to a mixed infection by these viral pathogens in the region of study, which underscores its potential phytosanitary importance for this crop.

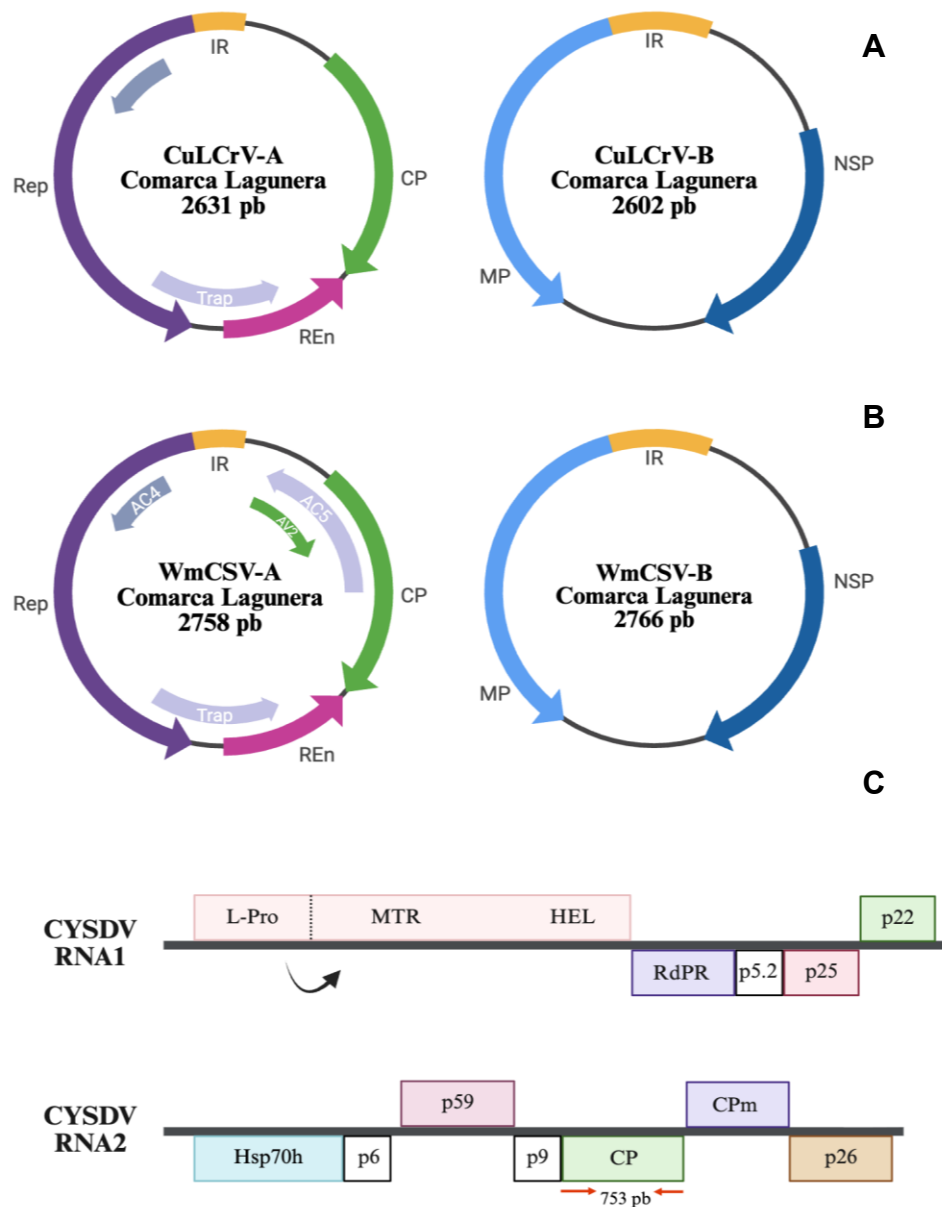


Figure 2. Complete genomic description of begomoviruses and criniviruses isolated from melon plants in the Comarca Lagunera region. **A)** Complete genome of CuLCrV-CL (DNA-A of 2631 bp and DNA-B of 2602 bp); **B)** Complete genome of WmCSV-CL (DNA-A of 2758 bp and DNA-B of 2766 bp). The open reading frames of the DNA-A (CP, AC4, Rep, TrAP, and REn) and DNA-B (NSP and MP) segments, as well as the intergenic regions (IR) of all viral segments, are shown. In the case of WmCSV, the DNA-A segment additionally encodes the AV2 and AC5 genes. **C)** Genomic organization of RNA segment 2 of CYSDV (7976 bp) (accession number: NC_004810). The open reading frames of Hsp70h, P6, P59, P9, CP, CPm, and p26 are displayed. The region corresponding to the CP gene (753 bp), used for molecular detection of CYSDV, is indicated with red arrows. Created in BioRender.

Emerging and reemerging plant diseases are caused by new or reappearing pathogens that quickly increase their incidence due to intrinsic characteristics. In addition, the appearance of new viral variants can broaden the host range, increase symptom severity and modify the geographical distribution of these diseases (Parrella *et al.*, 2022).

Emerging diseases have been reported in recent years in cucurbit crops, related to begomovirus and crinivirus in the southern United States and the Sonora desert (Devendran *et al.*, 2023; Fontenele *et al.*, 2021; Wintermantel *et al.*, 2024; Arce-Leal *et al.*, 2026). This situation suggests the existence of a potential area of dispersion of these viral species towards the Comarca Lagunera region.

Reconstruction of the evolutionary history of the viral isolates by phylogenetic analyses. In the case of begomoviruses, the phylogenetic trees were based on the nucleotide sequences of their respective DNA-A segments. For CuLCrV, three different clades were identified: the first contains the isolates from the United States (Arizona and California), and the second clade contains isolates from Mexico that have been reported to date (Campeche and Baja California Sur). Finally, and interestingly, isolate CuLCrV-CL is in the third clade (Figure 3A, left panel). This finding demands a more detailed analysis of the viral genome to identify in which *loci* the modifications associated with these different phylogenetic clades would be located. The phylogenetic analysis located isolate WmCSV-CL in a clade that groups the isolates reported in Mexico, including the one found in Sonora in 2012 (Domínguez-Durán *et al.*, 2018) (Figure 3B). This group reflects the broad genetic relation between the Mexican isolates, including the sample of this study.

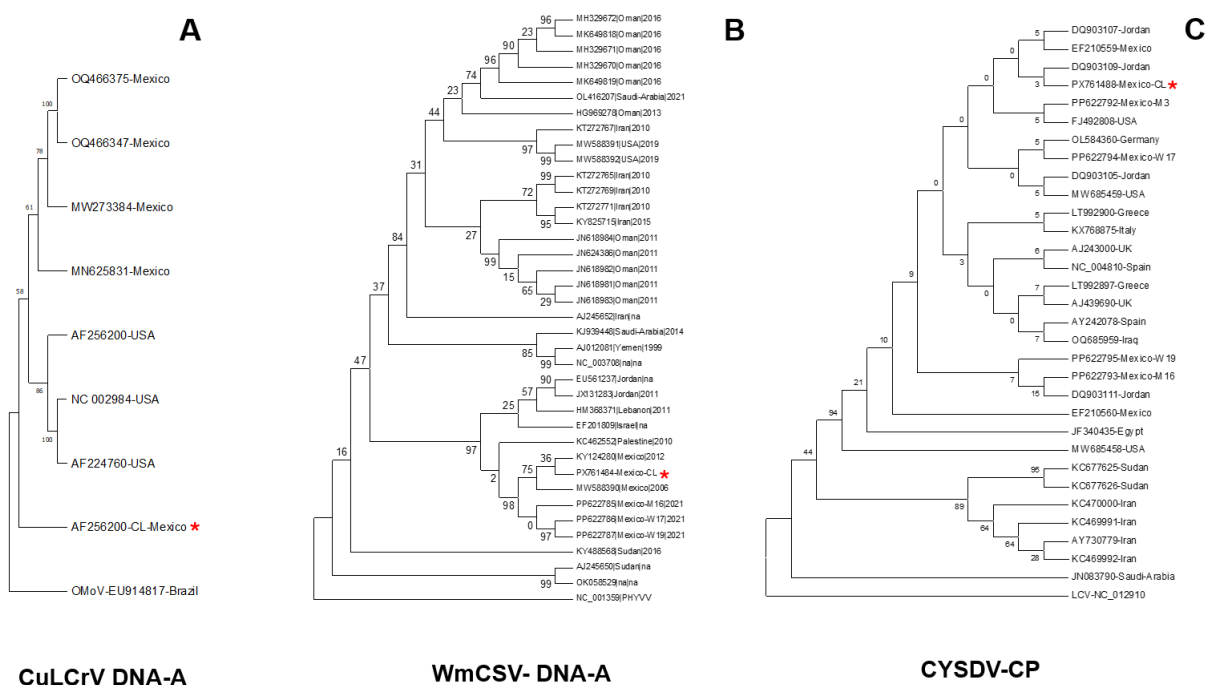


Figure 3. Phylogenetic trees corresponding to the DNA-A segments of begomoviruses and the CP gene of criniviruses. Neighbor-Joining (NJ) phylogenies were constructed by multiple alignments of the DNA-A segments of the begomoviruses CuLCrV-CL and WmCSV-CL (panels A and B, respectively), as well as by alignments of the complete ORF of the capsid protein (CP) of the crinivirus CYSDV (C). For the NJ phylogenetic trees, the Tamura-3 model with 1000 replicates was used. Isolates derived from this research are marked with a red asterisk. The accession numbers of the different isolates are indicated. The DNA-A of the begomovirus okra mottle virus (OMoV) and the CP of the crinivirus lettuce chlorosis virus (LCV) were used as outgroup sequences.

In the case of crinivirus CYSDV, the phylogenetic tree based on the nucleotide sequence of gene CP showed that the CYSDV isolates are grouped in the clades of divergent populations which display a high genetic stability: the population of the East, which is composed of isolates from Sudan, Saudi Arabia and Iran, and the population from the West, which covers the rest of the isolates worldwide, including isolate CYSDV-CL (Figure 3C). In Mexico, CYSDV was first found in Tamaulipas in the year 2000 (Kao *et al.*, 2000) and later extended to Sonora (Brown *et al.*, 2007), Nuevo León, Coahuila and Durango, causing important diseases in various cucurbits, including watermelon, melon, pumpkin and cucumber (Álvarez-Ojeda *et al.*, 2012), and recently, in a mixed infection in Jalisco (Hernández-Pérez *et al.*, 2025) and Sonora (Arce-Leal *et al.*, 2026). These results confirm the relevance of CYSDV as a virus that adapts efficiently to the agroclimatic conditions and to the varieties of cucurbits cultivated in Northwestern Mexico.

Molecular characterization of begomoviral genomes and identification of divergent loci potentially associated with virulence and/or pathogenesis. In the case of CuLCrV-CL, a gene-by-gene comparative analysis, using all viral isolates available in the NCBI, revealed a high conservation (>96% of identity in nucleotides and amino acids) in the *loci* of genes CP, Rep, TrAP and REn in the DNA-A segments, as well as in those of the MP and NSP in the DNA-B segments. However, interestingly, divergences were found in the sequence of amino acids of gene C4 (<89% identity) and in the intergenic region of DNA-B (<90 % identity) in comparison with the isolate from Campeche from 2019 and with two isolated from Baja California Sur from 2018 (Supplementary Table 2).

The protein encoded by gene C4 of the geminiviruses overlaps with the gene associated with replication (Rep), and it is one of the least conserved proteins of the viral family. C4 has multifunctional characteristics that include movement, determinant of pathogenesis, manipulation, and cell division and differentiation, as well as the suppression of silencing (Fondong, 2013). On the other hand, the intergenic region of the genomic components of the bipartite begomoviruses contains the regulatory elements of viral replication and transcription. In the case of the DNA-B segment, there must be a high identity with the regulatory elements located in the corresponding DNA-A segment in order for an efficient transreplication to take place (Hanley-Bowdoin *et al.*, 1999). In addition, the differences observed in the IR of the DNA-B segment could be related to an alteration of the levels of transcription of the NSP and MP movement genes. In the particular case of the NSP, it has been reported as a factor of virulence and suppressor of the antiviral response (Martins *et al.* 2020), which could have a direct influence on the infection process.

In the case of WmCSV-CL, the gene-by-gene analysis carried out with the Mexican isolates and with two of the original isolates from the Middle East (Palestine and Israel) revealed that the WmCSV-CL isolate shared homology (>97% identity of nucleotides and amino acids) in all the *loci* of both viral segments with the Mexican isolates. However, interestingly, a divergence was observed (<91% identity) in the intergenic regions and the gene MP of the DNA-B segment at the level of nucleotides and amino acids, respectively, in comparison with the isolates from the Middle East (Supplementary Table 3). Similarly to the case of CuLCrV-CL, the divergence observed in the intergenic region of the DNA-B segment could be related to changes in the efficiency and specificity of the transreplication mediated by the genetic elements found in the DNA-A segment, as well

as the levels of transcription of the viral movement genes. The MP protein is related to the viral movement from cell to cell, and this mechanism has been reported to be related to the affinity of the protein for the viral DNA (Hehnle *et al.*, 2004). On the other hand, it would be interesting to determine, in future investigations, whether the genetic changes observed in the MP protein of WmCSV-B could have an influence on the viral movement.

Additionally, because of a recombination analysis between isolates CuLCrV-CL and WmCSV-CL and the sequences of the most closely related viral species, no possible events of this phenomenon were identified in the analyzed sequences. This suggests that the evolution of the new viral isolates could have been produced with other mechanisms, or that the corresponding parental have not been identified.

The molecular and phylogenetic results of the DNA-B segments of both CuLCrV-CL and WmCSV-CL, are correlated with the fact that the evolution of the DNA-B segments follows different pathways to those of the DNA-A segments. This is because they undergo different selective pressures that result in mutation or recombination, which gives rise to the appearance of new viral isolates with increased pathogenicity and virulence characteristics (Briddon *et al.*, 2010).

In Mexico, mixed begomovirus infections have been documented, specifically by SLCuV and CuLCrV in pumpkin in Baja California Sur (Vargas-Salinas *et al.*, 2024), as well as begomovirus–crinivirus combinations between WmCSV and CYSDV in watermelon in Jalisco (Hernández-Pérez *et al.*, 2025) and Sonora (Arce-Leal *et al.*, 2026). Nevertheless, this study is the first report of a concurrent mixed infection by CuLCrV, WmCSV and CYSDV in cucurbit crops in Mexico. Future biological studies that use infectious clones of the identified viruses will help experimentally characterize the symptoms and quantify the physiological and agronomic impact of mixed and triple infections in commercial varieties of cucurbits cultivated in the main producing areas in Mexico.

Monitoring and early detection are essential to understanding the dynamics of the infection and the epidemiology of viral species introduced, such as begomovirus WmCSV, especially in its interaction with other phytopathogens transmitted by the whitefly. In this sense, this vector insect of cosmopolitan distribution and polyphagous has caused significant agricultural productivity losses, not only in the *Cucubitaceae* family, but also in *Solanaceae* and *Fabaceae* families. (Polston *et al.*, 2013; Sani *et al.*, 2020). Thus, its management is key to guaranteeing the security and profitability of the crops. The exclusive use of insecticides has favored the appearance of resistance in the whitefly populations and has increased risks for human health and the environment. Therefore, it is crucial to implement Integrated Pest Management Strategies that include epidemiological surveillance, agronomic practices, biological control, physical barriers, the rational use of chemical products, and regional coordination to reduce viral transmission, maintain the efficiency of management measures, and ensure productive sustainability (Abubakar *et al.*, 2022)

CONCLUSIONS

This is the first report on the identification of a viral complex associated with symptomatology in the melon crop in the Comarca Lagunera region, Mexico, consisting of the coinfection by the begomoviruses CuLCrV, WmCSV and the crinivirus CYSDV. Since CuLCrV and CYSDV are endemic viral species and established in the Americas,

respectively, and WmCSV is a begomovirus recently introduced in the region of North America (Mexico and United States), this is, to date, the first report of a disease associated with these three viruses in coinfection. The molecular and phylogenetic analyses of the new viral isolates highlight the need to continue with integral epidemiological investigations to help understand and anticipate diseases caused by viruses transmitted by the whitefly (*Bemisia tabaci*) in Mexico.

Limitations. This study describes the identification of a viral complex associated with symptomatology in melon, composed of begomoviruses CuLCrV and WmCSV and the crinivirus CYSDV. Complete and partial viral genomes were obtained, which present potential risks resulting from the interaction between these agents. Nevertheless, the study has the following limitations: (i) the complete sequence for the CYSDV genome was not obtained, making the phylogenetic and functional analysis of this agent was less thorough than the one carried out for the begomoviruses; (ii) the use of a targeted diagnosis (PCR/RT-PCR) cannot rule out the presence of other non-target viruses, since no massive, non-directed sequencing strategies were implemented; and (iii) the lack of infectious clones prevented the performance of the experimental characterization of the symptomatology and the direct quantification of the physiological and agronomic impact of the mixed and triple infections in commercial varieties. Infectious clones are currently being developed, and non-targeted sequencing approaches are being planned to address these limitations and experimentally validate bioinformatic inferences. On the other hand, although the sample size was limited and the sampling was limited to a single municipal area in the Comarca Lagunera, these results are a preliminary basis to broaden future samplings to other municipal areas of the region.

Conflict of interests. The authors declare no conflict of interest regarding the investigation, authorship and/or publication of this article.

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