Original Research Article

METAGENOMIC ANALYSIS OF RNA VIRUSES IN WATERMELON

Abstract

Watermelon is one of the most consumed cucurbits worldwide. Its production, nevertheless, is threatened by different pathogens that compromise the development of plants and, consequently, fruits. Here, plants showing typical virus-like symptoms were collected in the Tocantins State, Brazil. Aiming for the identification of viruses, viral RNA was sequenced by Illumina. Four virus genomes were sequenced and assembled, corresponding to isolates of *Papaya ringspot virus* (PRSV) and *Zucchini Yellow Mosaic Virus* (ZYMV) from the family *Potyviridae*, *Groundnut ringspot virus* (GRSV) from the family *Bunyaviridae*, and Citrullus lanatus partitivirus (CiLPV) from family *Partitiviridae*. Although these viruses have been previously reported infecting watermelon, we have sequenced the first complete genome of GRSV and the first complete genome of a ZYMV isolate from Brazil. Symptomatic watermelon leaves from the field were also used as ~~inoculation~~ for virus mechanical transmission to indicative plants. Despite co-infecting watermelon plants in the field, only the potyviruses were mechanically transmitted to indicative plants, in which ZYMV presented the broadest host range.

Comment [W1]: Source of Inoculum

Comment [W2R1]:

Keywords: Identification, genome, transmission.

1. Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] belongs to the family Cucurbitaceae which includes other important crops for human diet like cucumber, melon, and squashes. Among these examples of cucurbits, watermelon is reported as the most produced worldwide in tons according to the United Nations' Food and Agriculture Organization (FAO), 2013. Besides a significant production, watermelon plants are threatened by various pathogens that compromise the productivity and quality of fruits [1-4].

In Brazil, the fourth highest producer of watermelon on the globe (FAO, 2013), viruses from the families *Potyviridae*, *Bromoviridae*, *Comoviridae*, and *Bunyaviridae* have been reported to infect that crop [4, 5]. While aphids mostly transmit viruses from the first three abovementioned families in a non-persistent manner [6], tospoviruses (genus *Tospovirus*, family *Bunyaviridae*) are

transmitted by thrips (order Thysanoptera) in a circulative and propagative manner [7].

Comment [W3]: Mention the recent references

With the development of Next Generation Sequencing (NGS) tools, more and more viruses have been reported infecting crops worldwide [8, 9]. Although looking for plant viruses is biased by host importance and symptomatology, most viruses seem to have a non pathological relationship with hosts [10, 11]. A select group of virus species seems to be continuously reported as disease inducers. Knowing pathogenic and non pathogenic viruses help plant breeding programs to focus on the main facts for disease resistance.

In this study, symptomatic watermelon plants were collected in three production regions in Tocantins State, Brazil. After RNA sequencing of these plants, four viruses classified within the families *Potyviridae*, *Bunyaviridae,* and *Partitiviridae* were identified. Although these viruses were previously reported to infect watermelon, we have sequenced their complete genome of them. Here, we report the first complete genome of the *Groundnut ringspot virus* (GRSV).

2. Materials and methods

2.1 Sample collection, viral enrichment, and RNA extraction.

Watermelon leaves (cv. Manchester and Top Gun) presenting viral infection symptoms were collected in three production fields in Formoso do Araguaia, Lagoa da Confusão and Porto Nacional, Tocantins State (Brazil), in 2013. Leaves were homogenized in 2 volumes (w/v) of PBS (0.25 M, pH 7.5 containing 0.1% thioglycolic acid) and filtered through sterile gauze. The flow-through was centrifuged at 8,000 x g for 10 min and the supernatant was layered on a 20% sucrose cushion which was centrifuged at 28,000 x g for 1,15 h at 4°C. The resulting pellet was used for RNA extraction. From this enriched virus fraction, total RNA was extracted using the RNeasy plant mini kit (following the manufacturer's manual).

2.2 Sample sequencing and processing.

After rRNA depletion using Ribo-Zero rRNA Removal Kit, the remaining RNA was sequenced by Illumina HiSeq 2000 platform at Macrogen, Inc., Seoul, South Korea. The sequencing library was prepared using the Illumina TruSeq RNA V2 kit (2 × 100 paired-end). The sequence data were analyzed using CLC

Genomics Workbench (CLC Bio, Aarhus, Denmark). The resulting reads (41,680,568) were filtered and trimmed to remove short and low-quality regions/reads and then assembled using CLC de novo assembler with default parameters. The assembled contigs were compared with a viral RefSeq database (6,500 genomes) from the National Center for Biotechnology Information (NCBI) using blastx. Sequences with e-values > $1E^{-5}$ were retained and compared to the entire nr database to exclude non-viral sequences. The viral-related contigs were manually inspected and annotated using Geneious [12]. The length of some viral-related contigs could be increased when the assembled reads extended beyond the contig ends. Therefore, reads were assembled back to the extended contig until the sequence could be extended no further.

2.3 Phylogenetic analysis.

The viral genomes were compared to the nearest sequences available in Genbank. All related sequences were then aligned using MAFFT [15]. Phylogenetic analysis was performed using the maximum likelihood (ML) method implemented in FastTree 2.1 [16]. To assess the reliability of the phylogenetic trees, FastTree uses a Shimodaira-Hasegawa test. Tree files were visualized and edited using FigTree v1.4.0 (available at http://tree.bio.ac).

2.4 Host-range and virus detection.

RT-PCR with specific primers was performed for detection of each potential viral genome from the samples sent to RNA sequencing. Meanwhile, the same leaf samples were used as inoculums for host-range determination. Virus presence in inoculated plants was confirmed by RT-PCR as well. Firstly, total RNA was extracted from 3g of symptomatic leaves using Trizol LS (Invitrogen). cDNA first strand was synthesized using specific primers with M-MLV Reverse Transcriptase (Promega) and then amplified by PCR with Platinum® Taq DNA Polymerase High Fidelity (Invitrogen) using different combinations of primers. The PCR products were cloned in pGEM-T easy (Promega) and sequenced using capillary based Sanger fluorescent dideoxy termination sequencing at Macrogen Corporation (Seoul, Korea). All procedures followed the manufacturers' recommendations.

3 Results and discussion

3.1 Identification of viruses infecting symptomatic watermelon plants in Brazil

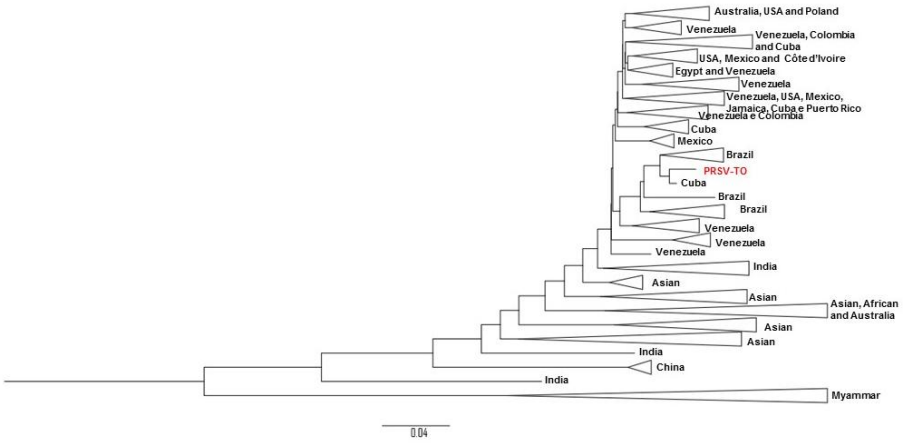
The watermelon plants collected in the fields presented symptoms like mosaic, blistering, chlorosis, and blotching on leaves and fruits, suggesting viruses as etiologic agents. Aiming the identification these viruses, the total RNA of a viral semi-purification using a pool of symptomatic leaves (material and methods) was sequenced. Approximately 42 million paired-end reads were obtained, resulting in the assembly of 49,515 contigs. Comparison of these contigs against a viral Ref-Seq and nr database revealed four complete genomes of viruses from families *Bunyaviridae, Potyviridae,* and *Partitiviridae*. Although these viruses have been previously reported infecting watermelon [4, 5, 17], not all of them had their complete genome sequenced before.

3.2 The complete genome of potyvirus isolates

Several contigs had a significant (e-value < 10^{-5}) relationship with viruses belonging to the genus *Potyvirus* (Family Potyviridae). These contigs assembled the complete genome of *Papaya ringspot virus* (PRSV) and *Zucchini Yellow Mosaic Virus* (ZYMV) isolates. These viruses are notorious for causing severe losses in cucurbits around the world [18, 19].

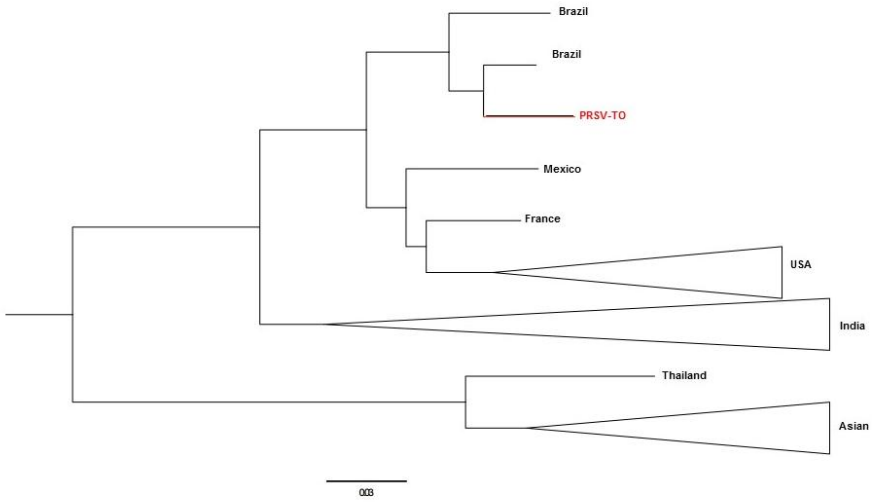
A total of 1,465,768 reads mapped to PRSV, (herein named PRSV-TO) the known sequence (GenBank Accession nº identity with PRSV-TO isolate MN364666 in the NCBI GenBank databases), assembling a positive single stranded-RNA genome of 10,326 nucleotides that codes a polyprotein of 3,344 amino acids (aa) and a small polypeptide of 72 aa (PIPO). A phylogenetic tree built on complete genomes revealed PRSV-TO highly close to other Brazilian PRSV isolates [20] (Figure 1A).

Figure 1A: Phylogenetic analysis PRSV-TO. Phylogenetic tree showing PRSV-TO and 27 complete nucleotide sequences of the PRSV viral genome. Built by the Maximum Likelihood method, PhyML 3.0 program. The bar at the bottom of the left side shows the number of substitutions/sites. The tree was rooted by *midpoint*. Some branches were collapsed for better visualization.



Nevertheless, the phylogenetic tree based on coat protein (CP) nucleotide sequences (higher number of available sequences) included a Cuban isolate in the Brazilian clade (Figure 1B).

Figure 1B: Phylogenetic analysis of PRSV-TO (CP). Phylogenic tree showing PRSV-TO (CP) and 631 nucleotide cp sequences (PRSV). Built by the Maximum Likelihood method, PhyML 3.0 program. The bar at the bottom of the left side shows the number of substitutions/sites. The tree was rooted by *midpoint*. Some branches were collapsed for better vizualization.

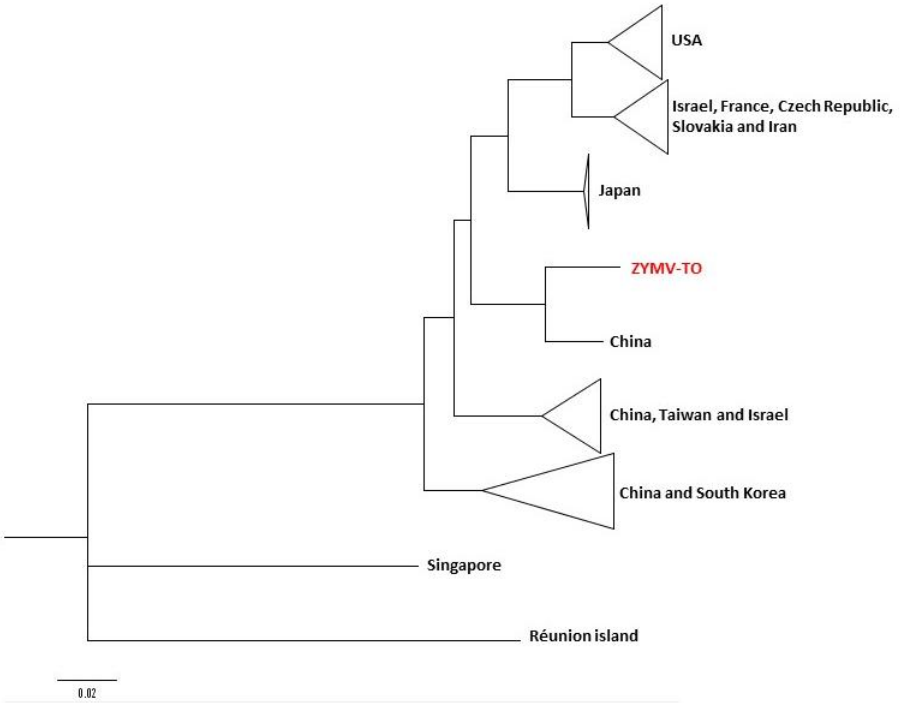


This tree suggests that PRSV isolates from Cuba and Brazil share the same origin. PRSV is known as a limiting factor for papaya production with many reports worldwide [21]. This scenario has led to breeding and transgenic

strategies aiming at the establishment of resistant papaya cultivars [22]. For watermelon, PRSV seems to be less problematical, and different resistant accessions are available for breeding programs [23, 24].

A total of 1,658,169 reads mapped to the first complete genome of a ZYMV isolate (herein named ZYMV-TO) from Brazil (GenBank Accession nº identity with ZYMV-TO isolate MN364667 in the NCBI GenBank databases). This sequence contains 9,666 nucleotides and codes a polyprotein of 3080 aa. A phylogenetic tree based on complete genomes of ZYMV isolates (63 sequences) showed ZYMV-TO grouped with a Chinese isolate (Figure 2).

Figure 2A: Phylogenetic analysis of ZYMV-TO. Phylogenic tree showing ZYNV-TO and 63 complete nucleotide sequences of the ZYMV viral genome. Built by the Maximum Likelihood method, PhyML 3.0 program. The bar at the bottom of the left side shows the number of substitutions/sites. The tree was rooted by *midpoint*. Some branches were collapsed for better visualization.



The tree based on CP nucleotide sequences kept ZYMV-TO separate from other Brazilian ZYMV isolates, suggesting that at least two independent lineages of ZYMV are circulating in Brazil. Those other isolates were found in cucurbits grown in all regions of Brazil [25]. Different levels of resistance against

ZYMV have been reported in watermelon, varying in range (specific to some isolates or multiple potyvirus species) or environmental conditions (temperature-dependent) [26, 27].

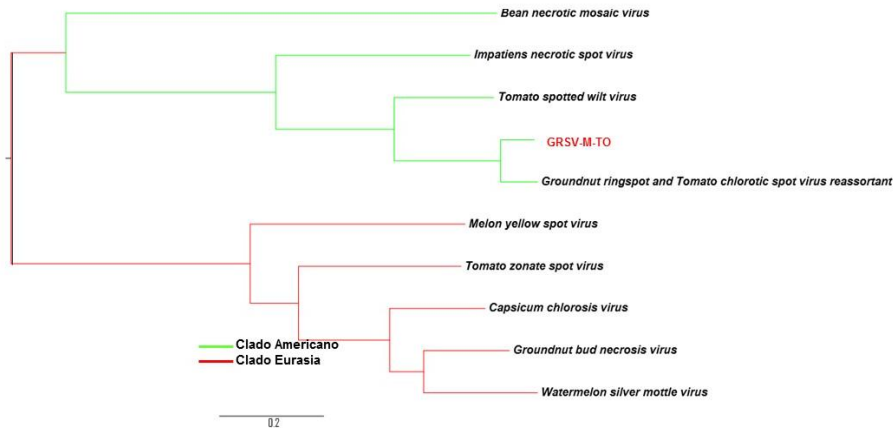
3.3 The complete genome of Groundnut ringspot virus

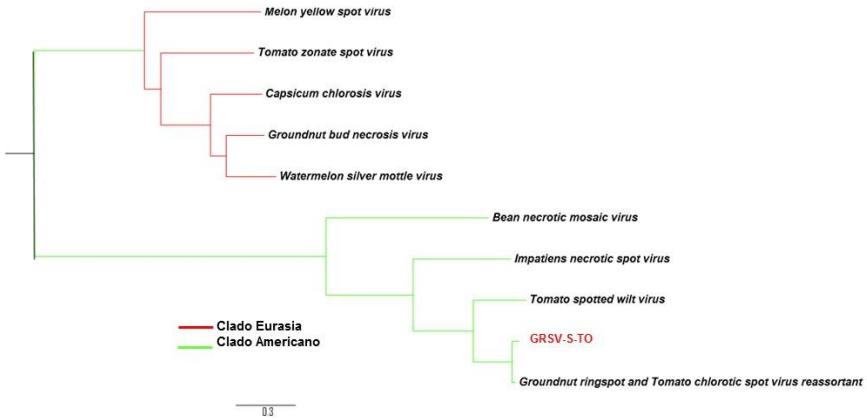
Although the *Groundnut ringspot virus* (GRSV) has been reported to infect watermelon and other crops in Brazil, only partial genetic sequences from different isolates have been sequenced so far [28-32]. GRSV belongs to the genus *Tospovirus* (family *Bunyaviridae*) which includes plant viruses with a tripartite negative and ambisense-RNA genome [33]. Here, the complete genome of a GRSV isolate was sequenced and assembled. A total of 4,585,588 reads mapped to the three segments of GRSV-TO. The L (Large) RNA segment contains an antisense orientation with 8.876 nucleotides and codes an RNA-dependent RNA polymerase (herein named GRSV-TO_L) the known sequence (GenBank Accession nº identity with GRSV-TO_L isolate MN364670 in the NCBI GenBank databases). The M (Medium) RNA segment contains 4.847 nucleotides and has an ambisense orientation (herein named GRSV-TO_M) the known sequence (GenBank Accession nº identity with GRSV-TO_M isolate MN364669 in the NCBI GenBank databases). The M RNA codes two proteins, NSm, and a glycoprotein precursor. The third segment, the S (Small) RNA, contains 3.074 nucleotides, coding a nonstructural protein (NSs) and the nucleocapsid (N) protein in an ambisense manner (herein named GRSV-TO_S) the known sequence (GenBank Accession nº identity with GRSV-TO_S isolate MN364668 in the NCBI GenBank databases).

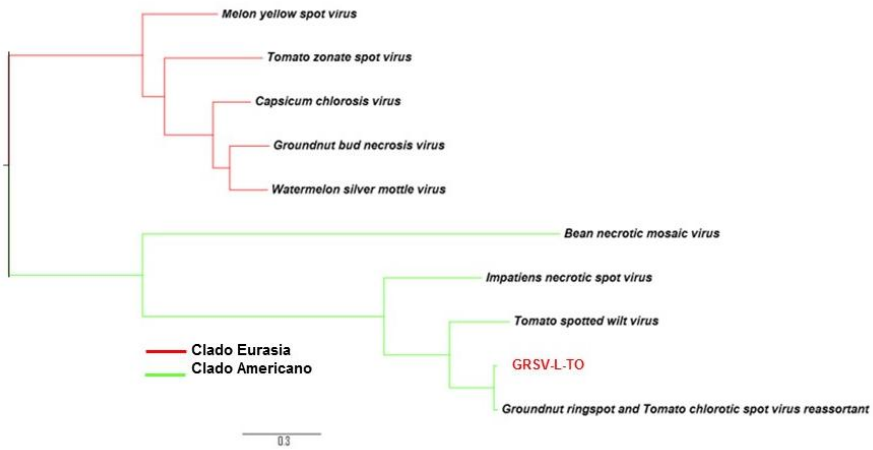
The phylogenetic analysis showed GRSV-TO clustered with other American tospoviruses (Figure 3A-C), being closest to a putative reassortant of GRSV found in the USA [32]. A higher accumulation of genetic variation between the M segments of GRSV-TO and the reassortant GRSV reinforces that the latter has S and L segments of GRSV and the M segment from *Tomato chlorotic spot virus* (TCSV), another tospovirus species.

Figure 3A; 3B; 3C: Phylogenetic analysis of the (S, M and L) of grsv encoded proteins. Phylogenetic analysis was performed between GRSV-TO (red letter) and nine nucleotide sequences of geographic isolates of each segment. A) Tree constructed from s-segment sequences; B) Tree constructed from sequences of segment M; C) Tree constructed from sequences of segment L. Constructed by

the maximum likelihood method, PhyML 3.0 program. The bar at the bottom of the left side shows the number of substitutions/sites. Aa trees were rooted by midpoint.







Watermelon has been recently reported as natural host for GRSV in São Paulo, Brazil [5]. These plants showed leaf deformation, mosaic, necrotic lesions, and necrotic spots on the fruits, somehow similar to the plants we collected in the Tocantins States. In that study, nevertheless, the authors just checked for tospoviruses, which does not rule out that those symptoms may also be due to the presence of other viruses. Usually, the tospovirus *Zucchini lethal chlorosis virus* (ZLCV) has been associated with the natural infection of cucurbits plants in Brazil [4, 34]. However, these observations are biased in the way that these plants have been just checked for ZLCV by enzyme-linked immunosorbent assay (ELISA). Since ZLCV belongs to the same evolutionary clade as GRSV (Figures 3) and serological analysis of related viruses may end up in cross reactions, other tospovirus species may have been overlooked in those cucurbits. Any strategy concerning watermelon resistance should consider a broad spectrum of tospovirus species.

3.4 Genome sequencing of the first watermelon-infecting deltapartitivirus from Brazil

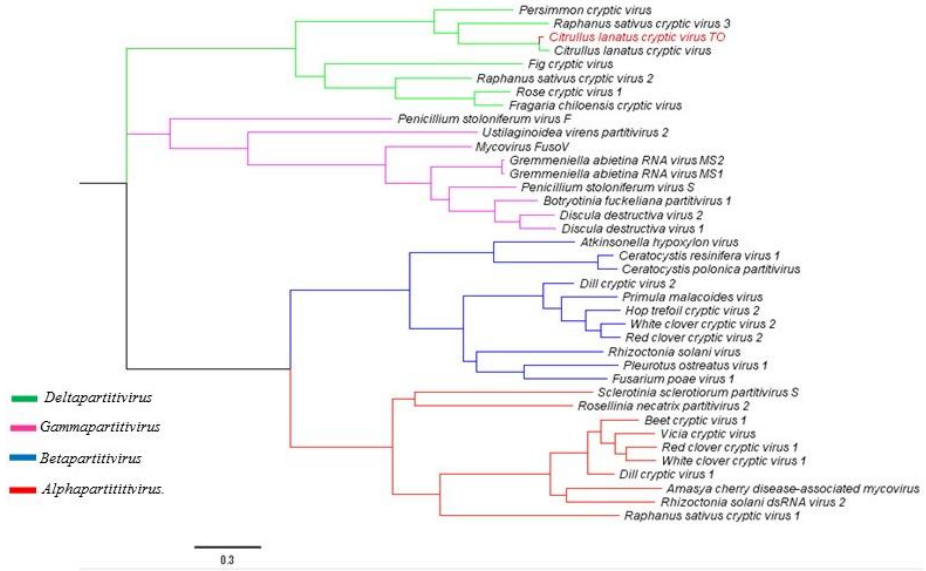
A total of 5,058 reads mapped to the bipartide double stranded-RNA genome of a deltapartitivirus isolate, *Citrullus lanatus partitivirus* (CiLPV), in which 2,982 reads corresponded to RNA-1 and 2,076 reads to RNA-2. These two RNA molecules are 1,574 (RNA-1) and 1,655 (RNA-2) bp long, encoding putative RNA-dependent RNA polymerase (RdRp) and coat protein (CP), respectively. The genome of CiLPV from Tocantins (CiLPV-TO) is longer than

the one from the first CiLPV isolate sequenced by SOLiD next-generation sequence analysis [17], which had lengths of 1,312 and 1,118bp for RNA-1 and RNA-2, respectively (GenBank Accession nº identity with CiLPV-TO_ RNA-1 isolate MN365721 and CiLPV-W-TO_RNA2 isolate MN378561 in the NCBI GenBank databases). Besides a reliable genome assembly, we could not trace back the CiLVC-TO in the field watermelon plants by RT-PCR, even upon extraction of dsRNA molecules.

Viruses from family *Partitiviridae* infect plants and fungi and, so far, no cell-to-cell movement proteins have been reported for them [35]. Only a few genomes of partitiviruses are available on public databases, which make the determination of taxonomy criteria difficult. Apart from phylogenetic analysis, the length of opening reading frames (ORFs) is taken into consideration for the demarcation of genera within the family *Partitiviridae* [35]. Within the genus *Deltapartitivirus*, the RdRp is shorter than the CP, being observed for CiLPV. CiLPV-TO shares 98% and 93% aa identities with RNA-1 and RNA-2 of CiLPV isolate from Israel, respectively [17].

The phylogenetic history of partitivirus was inferred with RdRp sequences (Figure 4), revealing four groups that correspond to the genera Alphapartitivirus, Betapartitivirus, Gammapartitivirus, and Deltapartitivirus. The First two genera contain viruses that infect plants and fungi, while the last two contain viruses that infect either fungi or plants as seen in Figure 4.

Figure 4: Phylogenetic analysis of CiLPV-TO (CP) representing RNA1 of the genus *Deltapartitivirus.* Phylogenetic analysis was performed between CiLPV-TO (red letter) with 37 nts rdrp sequences of members belonging to *deltapartitivirus genera; Gammapartitivirus*; *Betapartitivirus* and *Alphapartitivirus*. The tree was built by the Maxim likelihood method, PhyML 3.0 program. The bar at the bottom of the left side shows the number of substitutions/sites. The tree was rooted by *midpoint.*

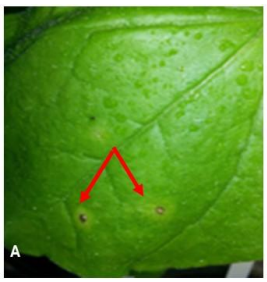


3.5 Mechanical transmission of watermelon-infecting viruses to indicative plants

All viruses found by NGS were traced back in the symptomatic watermelons by RT-PCR to confirm their presence. For GRSV-TO, amplicons of 636, 504, and 505 bp were amplified, corresponding to segments S, M, and L, respectively. Fragments of 504 bp and 706 bp were amplified to confirm the presence of PRSV-TO and ZYMV-TO, respectively.

Aiming for the isolation of each virus, the symptomatic watermelon leaves were mechanically inoculated into indicative plants. The presence of GRSV-TO, PRSV-TO, and ZYMV-TO in those plants was confirmed by RT-PCR as abovementioned. Surprisingly, only the potyviruses were transmitted, and only to plants from the family Cucurbitaceae. A total of 5 and 10 out of 24 inoculated plants were infected by PRSV-TO and ZYMV-TO, respectively. Two watermelons, 2 melons, 4 cucumber, and 2 pumpkin cultivars were infected by ZYMV. In many of these plants, ZYMV-TO caused mosaic and local injuries as listed in Figure 5.

Figure 5: Evaluation of symptoms in range of host plants in the period from 30 to 40 days after planting and mechanical inoculation. The arrows indicate the symptoms obtained. A*)* *Citrullus lanatus* cv Manchester, chlorotic rings; B) *Nicotiana rustica,* internerval chlorosis and musket*;* C) *Citrullus lanatus* cv Top Gun, with necrotic stitches; D) *Nicotiana rustica,* chlorosis and blisters.



Interestingly, co-infection of ZYMV-TO and PRSV-TO in melon and cucumber did not induce symptoms, differently melon and cucumber plants were positive for ZYMV-TO only. In this context, it was observed a kind of negative synergism between ZYMV-TO and PRSV. PRSV-TO was only transmitted by itself to a pumpkin cultivar, causing mosaic. No GRSV-TO transmission to any indicative plant indicated that ZYMV and PRSV may hijack host factors for their replication faster and more efficiently than GRSV. In this way, it suggests that those watermelon plants collected in the field may have been infected first by GRSV and then by potyviruses. From a different perspective, this outcome just occurred due to the experimental conditions. By transmission with aphids and thrips, another host range would have been obtained.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use

these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Al-Musa A, Anfoka G, Al-Abdulat A, Misbeh S, Ahmed FH, Otri I. Watermelon chlorotic stunt virus (WmCSV): a serious disease threatening watermelon production in Jordan. Virus Genes. 2011;43(1):79-89. doi: 10.1007/s11262-011-0594-8. PubMed PMID: WOS:000292158900013.

2. Kousik CS, Donahoo RS, Webster CG, Turechek WW, Adkins ST, Roberts PD. An outbreak of Cucurbit Powdery Mildew on Watermelon Fruit Caused by Podosphaera xanthii in Southwest Florida. Plant Disease. 2011;95(12):1586-7. doi: 10.1094/pdis-06-11-0521. PubMed PMID: WOS:000297274000026.

3. Adkins S, McCollum TG, Albano JP, Kousik CS, Baker CA, Webster CG, et al. Physiological Effects of Squash vein yellowing virus Infection on Watermelon. Plant Disease. 2013;97(9):1137-48. doi: 10.1094/pdis-01-13-0075-re. PubMed PMID: WOS:000323405500002.

4. de Souza Aguiar RW, Rodrigues A, Franke Portella AC, Lopes MdM, Lima MF, Resende RdO, et al. Serological Identification of Virus in Watermelon Production Fields in the Tocantins State. Brazilian Archives of Biology and Technology. 2015;58(2):192-7. doi: 10.1590/s1516-8913201400219. PubMed PMID: WOS:000354053700006.

5. Leao EU, de Almeida Spadotti DM, Goncalves Rocha KC, da Cunha Pantoja KdF, Marques Rezende JA, Pavan MA, et al. Citrullus lanatus is a New Natural Host of Groundnut ringspot virus in Brazil. Journal of Phytopathology. 2015;163(11-12):1014-8. doi: 10.1111/jph.12327. PubMed PMID: WOS:000363730100016.

6. Ng JCK, Perry KL. Transmission of plant viruses by aphid vectors. Molecular Plant Pathology. 2004;5(5):505-11. doi: 10.1111/j.1364-3703.2004.00240.x. PubMed PMID: WOS:000223814700012.

7. Whitfield AE, Ullman DE, German TL. Tospovirus-thrips interactions. Annu Rev Phytopathol. 2005;43:459-89. Epub 08/05. doi: 10.1146/annurev.phyto.43.040204.140017. PubMed PMID: 16078892.

8. Barba M, Czosnek H, Hadidi A. Historical Perspective, Development and Applications of Next-Generation Sequencing in Plant Virology. Viruses-Basel. 2014;6(1):106-36. doi: 10.3390/v6010106. PubMed PMID: WOS:000336093200006.

9. Wu Q, Ding S-W, Zhang Y, Zhu S. Identification of Viruses and Viroids by Next-Generation Sequencing and Homology-Dependent and Homology-Independent Algorithms. Annual Review of Phytopathology, Vol 53. 2015;53:425-44. doi: 10.1146/annurev-phyto-080614-120030. PubMed PMID: WOS:000359851700020.

10. Roossinck MJ. Plant virus metagenomics: biodiversity and ecology. Annu Rev Genet. 2012;46:359-69. Epub 2012/09/01. doi: 10.1146/annurev-genet-110711-155600. PubMed PMID: 22934641.

11. Roossinck MJ. Plant Virus Ecology. PLoS Pathogens. 2013;9(5):e1003304. doi: 10.1371/journal.ppat.1003304. PubMed PMID: PMC3662651.

12. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28(12):1647-9. doi: 10.1093/bioinformatics/bts199. PubMed PMID: WOS:000305419800052.

13. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nature Methods. 2012;9(4):357-U54. doi: 10.1038/nmeth.1923. PubMed PMID: WOS:000302218500017.

14. Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, et al. LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Research. 2012;40(22):11189-201. doi: 10.1093/nar/gks918. PubMed PMID: WOS:000313414800010.

15. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution.

2013;30(4):772-80. doi: 10.1093/molbev/mst010. PubMed PMID: WOS:000317002300004.

16. Price MN, Dehal PS, Arkin AP. FastTree 2 - Approximately maximum-likelihood trees for large alignments. Plus One. 2010:10.1371/journal.pone.0009490.

17. Sela N, Lachman O, Reingold V, Dombrovsky A. A new cryptic virus belonging to the family Partitiviridae was found in watermelon co-infected with the Melon necrotic spot virus. Virus Genes. 2013;47(2):382-4. doi: 10.1007/s11262-013-0937-8. PubMed PMID: WOS:000325034400025.

18. Guner N, Strange EB, Wehner TC, Pesic-VanEsbroeck Z. Methods for screening watermelon for resistance to papaya ringspot virus type-W. Scientia Horticulturae. 2002;94(3-4):297-307. doi: 10.1016/s0304-4238(02)00007-9. PubMed PMID: WOS:000176656000009.

19. Gal-On A. Zucchini yellow mosaic virus: insect transmission and pathogenicity - the tails of two proteins. Molecular Plant Pathology. 2007;8(2):139-50. doi: 10.1111/j.1364-3703.2007.00381.x. PubMed PMID: WOS:000244279400001.

20. Inoue-Nagata AK, Franco CdM, Martin DP, Marques Rezende JA, Ferreira GB, Dutra LS, et al. Genome analysis of a severe and a mild isolate of Papaya ringspot virus-type W found in Brazil. Virus Genes. 2007;35(1):119-27. doi: 10.1007/s11262-006-0032-5. PubMed PMID: WOS:000246521900015.

21. Olarte Castillo XA, Fermin G, Tabima J, Rojas Y, Tennant PF, Fuchs M, et al. Phylogeography and molecular epidemiology of Papaya ringspot virus. Virus Research. 2011;159(2):132-40. doi: 10.1016/j.virusres.2011.04.011. PubMed PMID: WOS:000294943300008.

22. Gonsalves D. Control of papaya ringspot virus in papaya: A case study. Annual Review of Phytopathology. 1998;36:415-37. doi: 10.1146/annurev.phyto.36.1.415. PubMed PMID: WOS:000076116000018.

23. de Azevedo SM, Maluf WR, Faria MV, Vilela de Resende JT, de Menezes CB, do Nascimento IR. Inheritance of resistance to the Papaya ringspot virus-watermelon strain (PRSV-W) from watermelon accession 'PI 595201'. Crop Breeding and

Applied Biotechnology. 2012;12(1):67-75. PubMed PMID: WOS:000304450200009.

24. Levi A, Coffey J, Massey L, Guner N, Oren E, Tadmor Y, et al. Resistance to Papaya ringspot virus-Watermelon Strain (PRSV-W) in the Desert Watermelon Citrullus colocynthis. Hortscience. 2016;51(1):4-7. PubMed PMID: WOS:000370114300001.

25. de Almeida Spadotti DM, Wassano DT, Marques Rezende JA, Aranha Camargo LE, Inoue-Nagata AK. Biological and molecular characterization of Brazilian isolates of Zucchini yellow mosaic virus. Scientia Agricola. 2015;72(2):187-91. doi: 10.1590/0103-9016-2014-0197. PubMed PMID: WOS:000352544600010.

26. Xu Y, Kang D, Shi Z, Shen H, Wehner T. Inheritance of resistance to zucchini yellow mosaic virus and watermelon mosaic virus in watermelon. Journal of Heredity. 2004;95(6):498-502. doi: 10.1093/jhered/esh076. PubMed PMID: WOS:000224482500005.

27. Inheritance of resistance to Zucchini yellow mosaic virus-Florida strain in watermelon. Hortscience. 2005;40(4):1039-40. PubMed PMID: WOS:000230494700225.

28. Deavila AC, Dehaan P, Kormelink R, Resende RD, Goldbach RW, Peters D. CLASSIFICATION OF TOSPOVIRUSES BASED ON PHYLOGENY OF NUCLEOPROTEIN GENE-SEQUENCES. Journal of General Virology. 1993;74:153-9. PubMed PMID: WOS:A1993KL08800001.

29. Lovato FA, Nagata T, de Oliveira Resende R, de Avila AC, Inoue-Nagata AK. Sequence analysis of the glycoproteins of Tomato chlorotic spot virus and Groundnut ringspot virus and comparison with other tospoviruses. Virus Genes. 2004;29(3):321-8. Epub 2004/11/20. doi: 10.1007/s11262-004-7435-y. PubMed PMID: 15550772.

30. Silva MS, Martins CR, Bezerra IC, Nagata T, de Avila AC, Resende RO. Sequence diversity of NS(M) movement protein of tospoviruses. Arch Virol. 2001;146(7):1267-81. Epub 2001/09/15. PubMed PMID: 11556705.

31. Hallwass M, Leastro MO, Lima MF, Inoue-Nagata AK, Resende RO. Sequence determination and analysis of the NSs genes of two tospoviruses. 2012;157:591-6.

32. Webster CG, Reitz SR, Perry KL, Adkins S. A natural M RNA reassortant arising from two species of plant- and insect-infecting bunyaviruses and comparison of its sequence and biological properties to parental species. Virology. 2011;413(2):216-25. doi: 10.1016/j.virol.2011.02.011. PubMed PMID: WOS:000290062400008.

33. de Oliveira AS, Melo FL, Inoue-Nagata AK, Nagata T, Kitajima EW, Resende RO. Characterization of bean necrotic mosaic virus: a member of a novel evolutionary lineage within the Genus Tospovirus. PLoS One. 2012;7(6):e38634. Epub 2012/06/21. doi: 10.1371/journal.pone.0038634. PubMed PMID: 22715400; PubMed Central PMCID: PMCPmc3371012.

34. Giampan JS, Rezende JAM, Piedade SMS. Yield loss caused by Zucchini lethal chlorosis virus (ZLCV) on zucchini squash 'Caserta'. 2009. p. 223-5.

35. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. Family - Partitiviridae. Virus Taxonomy. San Diego: Elsevier; 2012. p. 523-34.