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Characterisation of Groundnut Ringspot Virus and a Related Reassortant Orthotospovirus Infecting *Adenium obesum* Plants in Brazil

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ABSTRACT

Genomic reassortment is a well-documented process in multisegmented viruses, including members in the genus *Orthotospovirus*. Comparative analysis of partial fragments of the large (L), medium (M) and small (S) segments of two orthotospovirus isolates from *Adenium obesum* (known as desert rose) collected in Brazil showed a higher nucleotide similarity with groundnut ringspot virus (GRSV). However, genome sequence analysis of another isolate of orthotospovirus from *A. obesum* revealed a reassortment event involving GRSV and tomato chlorotic spot virus (TCSV) with L and M segments assigned to GRSV and S to TCSV (i.e., $S_{TC}M_GL_G$). The two GRSV isolates from *A. obesum* were not transmissible to any tested plants by mechanical inoculation, including *A. obesum* itself. In contrast, the $S_{TC}M_GL_G$ isolate was mechanically transmitted to a number of indicator plants but not to *A. obesum*. Furthermore, neither $S_{TC}M_GL_G$ nor GRSV isolates were able to overcome the *Sw5* resistance gene in tomato.

1 | Introduction

Mixed infections of plant viruses are common in nature and often result in severe plant diseases due to synergic effects of the co-infecting viruses (Moreno and López-Moya 2020). When two or more viruses of the same genus, or even the same family, infect the same host cell, they can exchange genetic material through recombination or reassortment events. Recombination is a biological process that generates chimeric molecules by combining genetic segments from different parental donors (Pérez-Losada et al. 2015). In contrast, reassortment occurs exclusively in multisegmented viruses and involves the exchange of entire genome segments (Briese et al. 2013).

Orthotospoviruses (genus *Orthotospovirus*, family *Tospoviridae*) are among the most economically significant plant viruses worldwide, causing substantial losses in vegetable, legume and ornamental crops. Their virions are pleomorphic and spherical, measuring approximately 80–120 nm in diameter. The genome consists of three distinct linear, single-stranded RNA segments (ssRNA), which are negative or ambisense: L (large), M (medium) and S (small) (Mumford et al. 1996). The S segment

(~2900 nucleotides [nt]) encodes the nucleoprotein (N) in the negative sense and the nonstructural protein (NSs) in the positive sense. The NSs protein is known to be a suppressor of plant RNA silencing (Hedil and Kormelink 2016). The M segment (~4800 nt) encodes a precursor polyprotein in the negative sense, which is cleaved into the glycoproteins Gn and Gc, both of which play a role in virus–vector interactions (Nagata et al. 2000). In the positive sense, it encodes the nonstructural movement protein (NSm), which facilitates viral cell-to-cell movement (Storms et al. 1995). The L segment (~8900 nt) encodes the RNA-dependent RNA polymerase (RdRp) in the negative sense, an enzyme essential for RNA replication (Oliver and Whitfield 2016).

In nature, orthotospoviruses are transmitted by thrips (Thysanoptera: Thripidae) in a persistent, propagative manner. Among the vector species, the western flower thrip (*Frankliniella occidentalis*) is considered the most important due to its polyphagous nature and ability to transmit multiple orthotospoviruses (Gilbertson et al. 2015). Tomato spotted wilt virus (TSWV, *Orthotospovirus tomatomaculae*), tomato chlorotic spot virus (TCSV; *Orthotospovirus tomatoflavi*) and groundnut ringspot virus (GRSV; *Orthotospovirus arachianuli*) are known for their relatively wide host ranges (Oliver and Whitfield 2016; Webster et al. 2015).

The broad host range of several orthotospoviruses can lead to mixed infections wherever the viruses and vectors coexist. Such cases have been reported with TCSV + GRSV co-infecting tomato and pepper plants in the United States (Webster et al. 2015). Reassortment events among orthotospoviruses have been primarily observed in TSWV, using biological experiments and molecular studies demonstrating intraspecific reassortment of their segments (Butković et al. 2021; Lian et al. 2013; Qiu et al. 1998; Ramesh and Pappu 2016; Zhang et al. 2016). Additionally, evidence of natural reassortment between two orthotospoviruses was reported in the United States, where the L and S segments originated from GRSV, while the M segment came from TCSV (Webster et al. 2011). Similarly, phylogenetic and nucleotide diversity analyses suggest that a TCSV isolate from Brazil may have incorporated the M segment of GRSV through reassortment (Silva et al. 2019). Furthermore, reassortment events during mixed infections may contribute to the emergence of a novel orthotospovirus with enhanced characteristics, such as improved virus movement in the plants and/or replication in new hosts or increased transmission efficiency by their vectors (Butković et al. 2021).

In this study, the main goal was the identification of the virus(es) associated with mosaic and chlorotic ringspot symptoms exhibited by *Adenium obesum* (known as desert rose) collected from three Brazilian regions.

2 | Materials and Methods

2.1 | Sample Collection

In 2023, two *A. obesum* plants showing mosaic symptoms were collected in a greenhouse in the municipality of Fortaleza, Ceará State, Brazil (03°83′63.9″ S, 38°47′57.5″ W) (Figure 1A). Additionally, two *A. obesum* plants exhibiting chlorotic ringspot symptoms were obtained from a flower shop in the municipality of Piracicaba, São Paulo (SP) State (22°44′27.6″ S, 47°36′37.9″ W). Another plant with similar symptoms was collected from a flower shop in the municipality of Arujá, SP (23°22′58.0″ S, 46°21′14.3″ W) (Figure 1B).

2.2 | Virus Identification by Transmission Electron Microscopy and Reverse Transcription-PCR

Small pieces of all symptomatic *A. obesum* leaves collected in Fortaleza, Piracicaba and Arujá were processed for virus identification by transmission electron microscopy (TEM) analysis of negatively stained ultrathin tissue sections. Leaf samples (~2 mm^2) were fixed in a modified Karnovsky solution, post-fixed

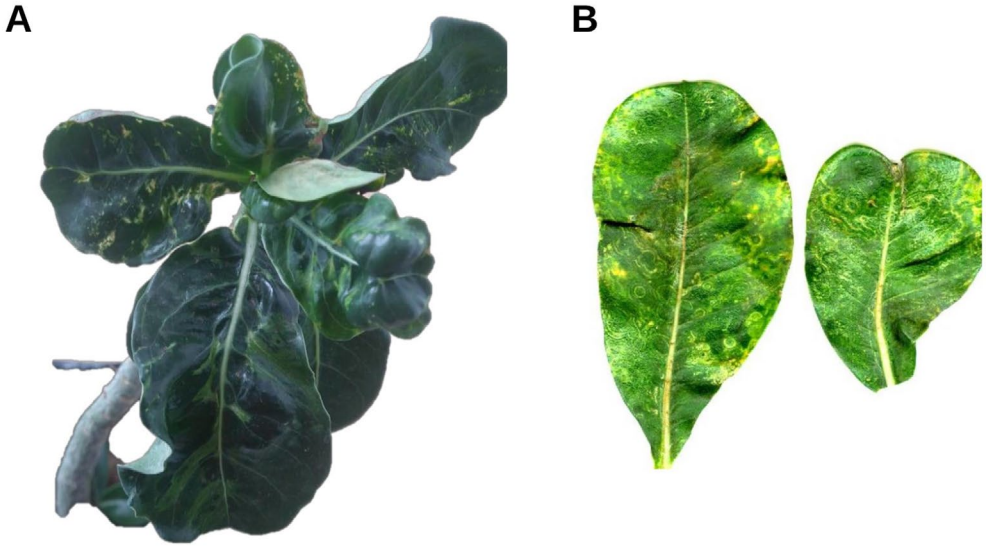

FIGURE 1 | Symptoms of mosaic and blistering (A), and chlorotic ringspots (B) on young leaves of *Adenium obesum* plants from Fortaleza and Arujá, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

with osmium tetroxide, and embedded in Spurr's low viscosity epoxy resin (Kitajima and Nome 1999). The preserved tissues were then sectioned using an ultramicrotome and examined under a JEM1011 transmission electron microscope (JEOL).

Total RNA was extracted from all symptomatic leaves collected from the field plants using the PureLink Viral RNA/DNA Mini Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. One-step reverse transcription-PCR (RT-PCR) was performed using the orthotospovirus universal primers BR60/BR65, which generate a 453 bp amplicon containing part of the nontranslated region and a portion of the N gene of the S segment (Eiras et al. 2001). RT-PCR was also performed with orthotospovirus universal primers TospoM-F/TospoM-R and TospoL-F/Tospo-L-R, which generate an 849 bp amplicon containing part of the NSm gene from the M segment and a 450 bp amplicon containing part of the RdRp gene from the L segment, respectively (Batuman et al. 2014). Additionally, RT-PCR was performed using GRSV-specific primers GRSVL-F (5′-GATARTGGCAAGAACCCAG-3′) and GRSVL-R (5′-TGGCTTCTTTAACCCACATG-3′), which generate an 800 bp amplicon containing part of the RdRp gene from the L segment. The amplicons obtained with the BR60/BR65 primer pair for all samples were sequenced at Macrogen, Korea. Then, only Fortaleza and Piracicaba isolates were also sequenced using the universal and specific primer pairs for the M and L segments, respectively. The obtained nucleotide sequences were compared with those deposited in GenBank using the Blastn algorithm (http://www.ncbi.nlm.nih.gov/Blast.cgi).

2.3 | High-Throughput Sequencing

The Arujá isolate was selected for high-throughput sequencing (HTS) based on preliminary results of mechanical transmission assays (see below). Total RNA was extracted from a symptomatic leaf using the RNeasy Plant Mini Kit (Qiagen), according to the manufacturer's instructions. Ribosomal RNA (rRNA) was then removed using Ribo-Zero Kit for plants (Illumina) following the manufacturer's protocol, and a cDNA library was prepared (TruSeq Stranded Total RNA Library Prep Plant Kit; Illumina) and sequenced on an Illumina NovaSeq6000 system with 100-bp paired-end reads (Macrogen Inc. Seoul, Korea). The HTS reads were trimmed using BBDuk (https://github.com/BioInfoTools/BBMap), and contigs were generated by de novo assembly using MEGAHIT v. 1.3.1. The resulting contigs were used for Blastn and Blastx searches against the virus nucleotide and protein database (downloaded from NCBI Virus on 15 March 2024) using Geneious Prime 2023.0.4.

2.4 | Phylogenetic Analysis

The full-length genome sequences of GRSV, TCSV and TSWV retrieved from GenBank on 8 July 2024 were included in the dataset alongside the newly characterised isolate (Arujá). The dataset consisted of the full-length genome sequences for each segment (S, M and L) and the corresponding protein-coding genes (N, NSs, NSm, GnGc and RdRp). Nucleotide sequence alignments of the full-length genomes and individual proteins were performed using ClustalW in Geneious Prime. Maximum-likelihood trees of the full-length genome sequences of each segment and proteins of individual genes were constructed using MrBayes v. 3.2.7 in the CIPRES Science Gateway. Two independent runs were conducted simultaneously for 10 million generations, with 25% of the resulting trees excluded as burn-in. Pairwise nucleotide and amino acid sequence identities were calculated using ClustalW in Geneious Prime.

2.5 | Recombination Analysis

Recombination Detection Program-4 (RDP4 v. 4.101) (http://web.cbio.uct.ac.za/~darren/rdp.html) was used to detect potential recombination events of the L, M and S segments of orthotospoviruses included in the dataset (GRSV, TCSV and TSWV) alongside the newly characterised isolate (Arujá). Recombination detection analysis was performed with all methodologies available in RDP4: RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, 3Seq, LARD and PhyIpro.

2.6 | Host Range

The Fortaleza, Piracicaba and Arujá isolates were mechanically inoculated onto *A. obesum* leaves and other indicator plants from 28 species belonging to four families, including two tomato hybrids with the *Sw5* resistance gene (Table 1). Leaves were macerated in 0.01 M potassium phosphate buffer (pH 7.0) containing 0.1% sodium sulphite and calcium chloride (0.1 M). The young plant leaves were predusted with 600-mesh carborundum and then sap-inoculated with the leaf extract. All plants were kept under greenhouse conditions to monitor symptoms for up to 60 days post-inoculation (dpi). Mock-inoculated plants of each species were treated with buffer solution only and kept under the same conditions for comparison. RT-PCR was performed using the BR60/BR65 (Eiras et al. 2001) primer pair to confirm virus infection.

3 | Results

3.1 | Virus Identification

Analysis of ultrathin sections revealed the consistent presence of dense masses of spherical virus particles (80–100 nm) in the cytoplasm of epidermal, parenchymal and vascular tissue cells of symptomatic *A. obesum* plants (Figure 2).

RT-PCR amplification of a 453 bp fragment from the S segment confirmed the presence of an orthotospovirus in all *A. obesum* samples. Comparison of the nucleotide sequences obtained from the Fortaleza samples (PV052240, PV052239) with those available in GenBank showed a high degree of similarity (99.49%) with the corresponding nucleotide sequences of a GRSV and TCSV reassortant from *Solanum lycopersicum* (NC_015467, HQ644140) and a GRSV sequence from *Plumeria pudica* (OK539547). The nucleotide sequences obtained from the Piracicaba samples (PV052238, PV052237) exhibited greatest sequence similarities of 100% and 99.72% with the corresponding sequences of GRSV from *Arachis hypogaea*

TABLE 1 | Reaction of different plant species mechanically inoculated with orthotospovirus isolates from *Adenium obesum* collected in Piracicaba, Fortaleza and Arujá.

		Piracicaba		Fortaleza		Arujá	
Family	**Species**	**No. of infected plants/no. of tested plants**	**Symptoms**	**No. of infected plants/no. of tested plants**	**Symptoms**	**No. of infected plants/no. of tested plants**	**Symptoms**
Amaranthaceae	*Chenopodium amaranticolor*	0/9	—	0/8	—	0/3	—
	Chenopodium quinoa	0/12	—	0/12	—	0/3	—
	Gomphrena globosa	0/9	—	0/6	—	1/2	Necrotic local lesion
Apocynaceae	*Adenium obesum*	0/20	—	0/8	—	0/8	—
	Plumeria pudica	0/5	—	0/5	—	0/4	—
Cucurbitaceae	*Cucurbita pepo* 'Caserta'	0/5	—	0/5	—	nt	
Solanaceae	*Capsicum annuum* 'Dahra'	0/5	—	0/5	—	nt	
	C. annuum 'Magali'	0/11	—	0/5	—	nt	
	C. annuum 'Magda'	0/6	—	0/6	—	—	—
	C. annuum 'Ikeda Casca Dura'	0/6	—	nt		6/6	Ringspot, mosaic
	Nicotiana benthamiana	0/2	—	0/2	—	0/4	—
	Nicotiana clevelandii	0/6	—	0/6	—	4/6	Mosaic
	Nicotiana glauca	0/3	—	0/3	—	nt	
	Nicotiana rustica	0/3	—	0/3	—	0/4	—
	Nicotiana occidentalis	0/3	—	0/3	—	0/4	—
	Nicotiana tabacum 'Havana'	0/9	—	0/9	—	nt	—
	N. tabacum 'Sansum'	0/3	—	0/3	—	0/2	—
	N. tabacum 'TNN'	0/9	—	0/9	—	nt	
	N. tabacum 'Turkish'	0/9	—	0/9	—	0/6	—
	N. tabacum 'Xanthi'	0/2	—	0/2	—	0/2	—
	N. tabacum 'Virginia'	0/3	—	0/3	—	1/6	Necrotic local lesion
	Solanum aethiopicum 'Esmeralda'	0/6	—	0/6	—	nt	—
	S. aethiopicum 'Morro Grande'	nt	—	nt	—	2/4	Mosaic

(Continues)

TABLE 1 | (Continued)

Family	Species	Piracicaba		Fortaleza		Arujá	
		No. of infected plants/no. of tested plants	Symptoms	No. of infected plants/no. of tested plants	Symptoms	No. of infected plants/no. of tested plants	Symptoms
	S. melongena 'Napoli'	0/9	—	0/9	—	0/7	—
	S. lycopersicum 'Santa Clara'	0/22	—	0/22	—	2/9	Purpling leaves
	S. lycopersicum 'Compack'[a]	0/9	—	0/9	—	0/9	—
	S. lycopersicum 'Caeté'[a]	0/10	—	0/10	—	0/10	—
	Petunia × *hybrida*	nt		nt		2/2	Chlorotic ringspot

Note: Viral infection was confirmed by reverse transcription-PCR using the BR60/BR65 primer pair.
Abbreviations: –, no symptoms; nt, not tested.
[a]Tomato hybrid with *Sw5* gene.

FIGURE 2 | Transmission electron micrographs of symptomatic leaf tissue of *Adenium obesum* from Arujá (A) and Fortaleza (B, C). Typical orthotospovirus-like particles (black arrows) can be seen in the lumen of the endoplasmic reticulum. Dense masses of viral particles are present in the cytoplasm (*).

(KY400110) and *Solanum melongena* (MK820664), respectively (Table S1). Similarly, the sequences obtained from the Arujá sample (PV052236) showed 100% similarity with a GRSV sequence from *P. pudica* (OQ656767).

Regarding the M segment sequences, the nucleotide sequences obtained from the Fortaleza samples (PV05535, PV052234) exhibited greatest sequence similarities of 97.23% and 99.18% with the corresponding sequences of GRSV from *Nicotiana tabacum* (AF513220) and *A. hypogaea* (KY350137), respectively. Similarly, the sequences from the Piracicaba samples (PV052233, PV052232) exhibited greatest sequence similarities of 96.67% and 97.37%, respectively, with a GRSV sequence (KY350317) (Table S1).

The two L segment sequences were obtained from the Fortaleza samples (OR756296, OR756297) only with specific primers for GRSV and exhibited greatest sequence similarities of 98.83% and 98.69% with the corresponding sequence of a GRSV and TCSV reassortant from *S. lycopersicum* (NC_015469). Similarly, the sequences from the Piracicaba samples (OR756298, OR756299) exhibited 97.79% and 97.67% similarity with a GRSV sequence from *Citrullus lanatus* (MN364670) (Table S1).

In addition, a comparison of the partial S and M segment nucleotide sequences from the Fortaleza and Piracicaba isolates revealed high similarity (96.05% to 100%) with GRSV and TCSV sequences. In contrast, the L segment from both isolates was the most conserved, exhibiting high nucleotide similarity exclusively with GRSV sequences (Table S1).

3.2 | High-Throughput Sequencing

A total of 64,106,552 raw reads were obtained through HTS. Only contigs related to orthotospovirus were detected in the

HTS data from the Arujá sample. Subsequently, the longest assembled contig corresponding to the L segment, containing 8878 nt, was aligned with 169,540 reads with a mean coverage of 1789. A second assembled contig corresponding to the M segment, containing 2078 nt, was extended using the Geneious map-to-reference command (NC_035482). The final extended contig reached 4839 nt with 379,279 mapped reads and a mean coverage of 11,900. A third assembled contig corresponding to the S segment contained 3306 nt and was aligned with 379,279 reads, with a mean coverage of 7739.

The complete genome of the orthotospovirus from Arujá consisted of three single-stranded, negative-sense RNA segments: L (~8900 nt), M (~4800 nt) and S (~3300 nt). The nearly complete sequence genome of the L segment (PQ299152) was 8878 nt long, including a 220-nt 5′ untranslated region (UTR) and a 33-nt 3′ UTR. The nearly complete sequence genome of the M segment (PQ299151) was 4839 nt long, including a 93-nt 5′ UTR and an 81-nt 3′ UTR. Similarly, the nearly complete sequence genome of the S segment (PQ299150) was 3312 nt, with an 87-nt 5′ UTR and a 151-nt 3′ UTR. Blastn searches revealed that the L and M RNA segments exhibited the highest sequence similarities of 99.16% and 97.85%, respectively, to GRSV (KY350136) (Table S2). In contrast, the S RNA segment exhibited the highest similarity of 98.01% to TCSV (MH742961) (Table S2). No other contigs related to the L and M segments of orthotospovirus other than GRSV were detected. For the S segment, no other contigs other than TCSV were detected. Segment L encodes the RdRp protein (nt 8845–221, 2874 amino acids [aa]) in a negative sense, which exhibited the highest similarity of 99.65% to GRSV (KY350136) according to Blastx search (Table S2). Segment M encodes the glycoprotein precursors Gn and Gc (nt 4758–1354, 1134 aa) in the negative sense, and these exhibited the highest similarity of 98.73% to GRSV (KY350136). In the positive sense, segment M encodes the NSm protein (nt 94–1005, 303 aa), which exhibited the highest similarity of 99.67% to multiple amino acid sequences of GRSV and TCSV (Table S2). Segment S encodes the N protein (nt 3161–2385, 258 aa) in the negative sense and the NSs protein (nt 88–1497, 469 aa) in the positive sense, which exhibited the highest similarities of 99.61% and 99.15% to TCSV (MH742961) (Table S2).

3.3 | Phylogenetic Analysis

Phylogenetic analysis of the Arujá isolate based on the nucleotide sequence of the L segment (GenBank PQ299150) grouped it with a genome sequence of GRSV (KY350136) (Figure S1). The M segment (GenBank PQ299151) grouped with the genome sequence of GRSV (MH986277) and that of TCSV (MH742960) (Figure S1). In contrast, the S segment (GenBank PQ299152) grouped with two genome sequences of TCSV (NC035484, MH742961) (Figure S1). In the phylogenetic analysis using the complete amino acid sequences of the RdRp gene, the isolate from Arujá grouped with the amino acid sequence of GRSV (KY350136) (Figure 3). Furthermore, the protein encoded by the NSm gene grouped with the corresponding amino acid sequences of GRSV and TCSV, while the protein encoded by the GnGc gene grouped with an amino acid sequence of TCSV (MH742960) and another amino acid sequence of GRSV (MH686277). The protein encoded by the N and NSs genes grouped with two corresponding amino acid sequences of TCSV (MH742961, NC035484) (Figure 3).

3.4 | Recombination Analysis

Recombination analysis using RDP4 did not detect any intramolecular recombination signals in the L, M or S segments between Arujá and other isolates (GRSV, TCSV and TSWV).

3.5 | Host Range

No plant exhibited symptoms for up to 60 dpi with the Fortaleza and Piracicaba isolates. In contrast, the Arujá isolate induced symptoms including necrotic local lesions on *Gomphrena globosa* and *N. tabacum* 'Virginia', ringspot and mosaic on *Capsicum annuum* 'Ikeda Casca Dura', mosaic on *Nicotiana clevelandii* and *Solanum aethiopicum* 'Morro Grande', purpling on *S. lycopersicum* 'Santa Clara', and chlorotic ringspots on *Petunia* × *hybrida* (Table 1). It is also important to emphasise that none of the three isolates could be mechanically transmitted to the tested tomato hybrids containing the *Sw5* resistance gene (Table 1). RT-PCR performed with total RNA extracted from all mechanically inoculated plants confirmed virus infection exclusively in symptomatic plants.

4 | Discussion

Reassortment events can occur naturally within a virus species during co-infection of a single host by different isolates or between different virus species. In this study, it was demonstrated that GRSV and a reassortant orthotospovirus composed of GRSV+TCSV (named $S_{TC}M_GL_G$) are probably the causal agents of mosaic/chlorotic ringspot symptoms in *A. obesum* plants in Brazil. As no additional plant viruses were detected in the analysed samples, it is likely that the symptoms observed were the result of the infection with the orthotospoviruses described here.

Analysis of ultrathin sections of symptomatic leaf tissue from *A. obesum* plants collected from different localities in Brazil consistently revealed the presence of spherical particles in all samples, characteristic of viruses in the genus *Orthotospovirus* (German et al. 1992). RT-PCR followed by Sanger sequencing of partial fragments of the L, M and S segments from samples collected in Fortaleza and Piracicaba revealed the occurrence of GRSV. In addition, HTS analysis revealed a reassortant orthotospovirus named $S_{TC}M_GL_G$ infecting an *A. obesum* plant. As far as we know, TSWV was the only orthotospovirus previously reported infecting *A. obesum* plants in the United States in 2007, confirmed by ELISA and RT-PCR followed by Sanger sequencing (Baker et al. 2007). In Brazil, virome studies using HTS analysis revealed desert rose mottle virus (DRMoV, genus *Potexvirus*), cucumber mosaic virus (CMV, genus *Cucumovirus*) and pelargonium flower break virus (PFBV, genus *Alphacarmovirus*) infecting *A. obesum* plants (Bello et al. 2023, 2024, 2025).

The partial host range results revealed that GRSV isolates from *A. obesum* (Fortaleza and Piracicaba isolates) could not be mechanically transmitted to any of the tested plants. A study

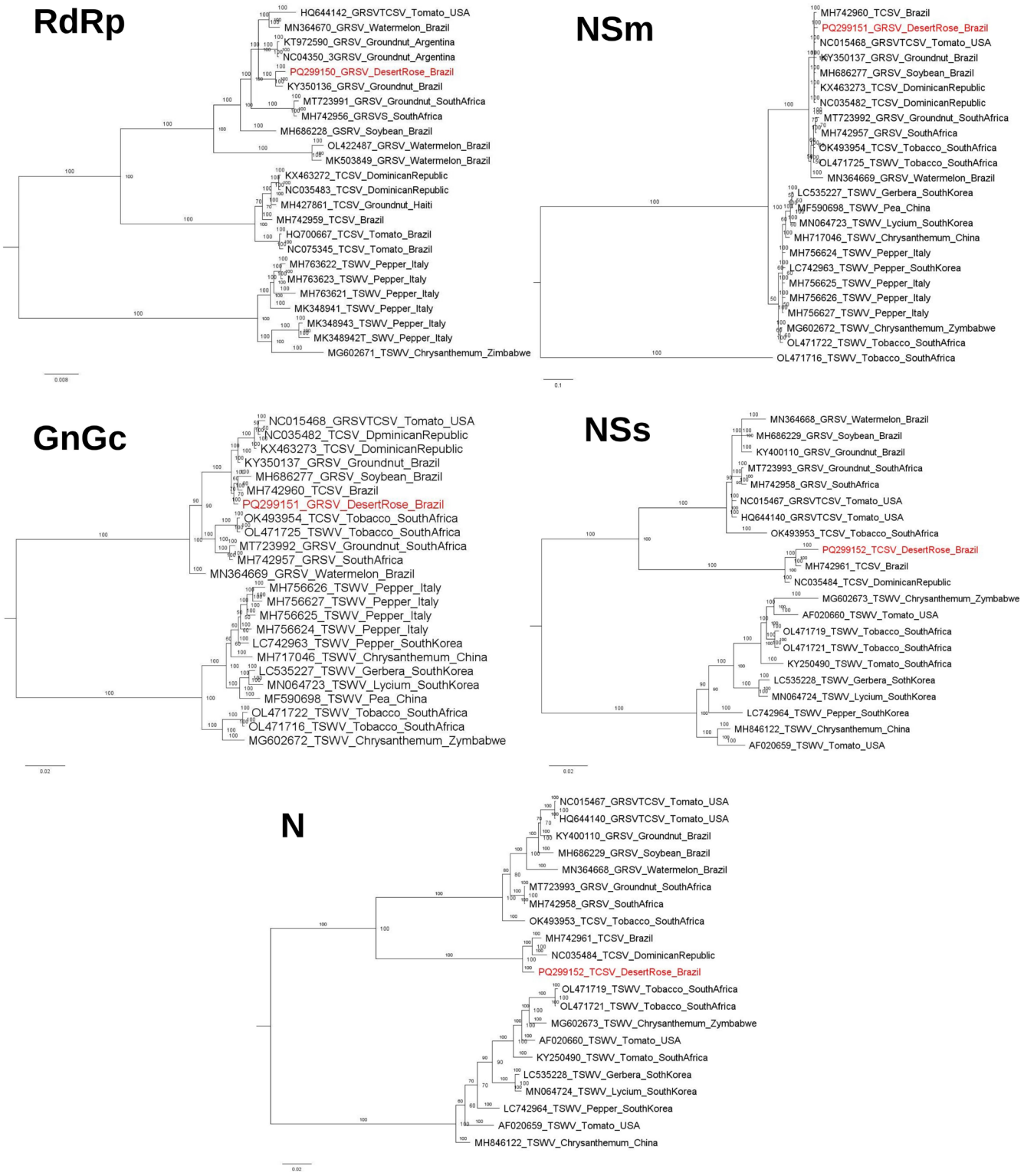

FIGURE 3 | Maximum-likelihood phylogenetic trees based on amino acid sequences of the proteins RdRp, NSm, GnGc, N and NSs of $S_{TC}M_GL_G$ (Arujá isolate) and other viruses belonging to the genus *Orthotospovirus*. Nucleotide sequences of this study are highlighted in red. Groundnut ringspot virus, GRSV; tomato chlorotic spot virus, TCSV; and tomato spotted wilt virus, TSWV. [Colour figure can be viewed at wileyonlinelibrary.com]

conducted in Brazil with a GRSV isolate from *P. pudica* similarly demonstrated that this virus could not be mechanically transmitted to three sweet pepper cultivars, *Nicandra physalodes*, *Physalis peruviana* and tomato (Favara et al. 2023). In contrast, the reassortant orthotospovirus $S_{TC}M_GL_G$ (Arujá isolate) was mechanically transmitted to some of the tested plants, including tomato and sweet pepper. However, none of the three orthotospovirus isolates could be transmitted to *A. obesum* plants. It is also important to highlight that all three isolates were unable to overcome the *Sw5* resistance gene in tomato, as was previously

observed for a reassortant orthotospovirus from tomato in the United States, in which the S and L segments were derived from GRSV and the M segment from TCSV (Webster et al. 2011).

HTS analysis of the reassortant $S_{TC}M_GL_G$ isolate further confirmed that the entire L and M segments originated from GRSV, while the entire S segment was derived from TCSV. Furthermore, nucleotide and amino acid sequences of the complete $S_{TC}M_GL_G$ genome were above 97.85% in similarity to the sequences of each parental genomic segment, confirming that each segment was derived from its respective parental virus (Table S2). It is also important to mention that the M segment shares nucleotide and protein similarities above 96.78% to some GRSV and TCSV isolates although there is no recombination signal. A lower diversity of M segments of GRSV and TCSV in comparison with the S and L segments was previously reported for two Brazilian isolates (Silva et al. 2019). Thus, this study provides the first evidence of a natural reassortant between GRSV and TCSV infecting *A. obesum* plants.

GRSV, TCSV and TSWV are the main orthotospoviruses occurring in Brazil. However, co-infection with at least two orthotospoviruses, which is required for a reassortment event, has been reported at extremely low frequencies (Martínez et al. 2019). Recent studies on orthotospoviruses have primarily focused on investigations of their host range. These studies often rely on sequencing a single gene (e.g., the N gene) or a single genome segment (e.g., the S segment) to identify or characterise the orthotospoviruses (Jorge et al. 2022; de Oliveira et al. 2022). According to Plyusnin et al. (2012), members of the same orthotospovirus species share more than 90% amino acid sequence identity in their N gene. Sanger sequencing of partial S (corresponding to the N gene) and M segments demonstrated that the GRSV isolates from *A. obesum* share nucleotide identities higher than 95.01% with GRSV and TCSV. In contrast, the partial L segment exhibited sequence identities higher than 97.08% only with GRSV (Table S1) due to the use of the specific primer for GRSV.

Full-length genome analysis (amino acid and nucleotide) of the $S_{TC}M_GL_G$ isolate confirmed that the S segment shares at least 67.54% and 74.71% identity with GRSV and TCSV, respectively (Table S2). The M segment of the $S_{TC}M_GL_G$ isolate demonstrated sequence identities of at least 90.72% and 91.74% with GRSV and TCSV, respectively (Table S2). In contrast, the L segment of the $S_{TC}M_GL_G$ isolate was the most conserved, displaying at least 89.77% and 80.23% identity with GRSV and TCSV, respectively (Table S2). Phylogenetic analysis supports the results by grouping S, M and L segments of the $S_{TC}M_GL_G$ isolate into clades with only TCSV (S), with GRSV and TCSV (M) and with only GRSV (L) (Figure S1, Figure 3).

These findings provide further evidence that identifying orthotospoviruses based solely on N gene sequences is insufficient to accurately distinguish virus species and/or reassortant viruses, as also demonstrated in previous reports (Oliver and Whitfield 2016; Silva et al. 2019; Webster et al. 2011). Additionally, it is important to emphasise that classifying a novel virus as a distinct species within this family must be based on a combination of biological data (e.g., plant hosts and vectors) and sequence analysis (nucleotide and amino acid sequences) of the L, M and S segments (Siddell et al. 2023).

It is worth noting that the significance of TCSV has increased considerably in other regions of the Americas in recent years (González-Alvarez et al. 2017; Poudel et al. 2018; Sui et al. 2018), possibly due to the biological advantage conferred by reassortment events, as previously reported (Silva et al. 2019; Webster et al. 2011). Nevertheless, it is important to highlight that neither $S_{TC}M_GL_G$ nor GRSV isolates from *A. obesum* were able to overcome the *Sw5* resistance gene in tomato.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available upon request from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.