Special Report

Integrating Local Lesion Assays with Conventional RT-PCR for Detection of Interspecies Tospovirus Reassortants and Mixed Tospovirus Infections

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Abstract

Tomato spotted wilt virus (TSWV) has historically been the major tospovirus present in North America. Recent emergence of *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) in Florida and the Caribbean has complicated reliable identification of tospoviruses in this region. Field symptoms of these three tospoviruses are indistinguishable in most host plants, and commercially available TSWV lateral-flow immunoassay reagents cross react with GRSV and TCSV, leading to incorrect diagnoses of GRSV or TCSV as TSWV. Reliable diagnosis of TSWV, GRSV, and TCSV is further confounded by the fact that all currently known isolates of GRSV in the United States are reassortants containing

one genomic RNA segment derived from TCSV. To address these practical challenges, we developed and validated genome segment-specific primers for conventional reverse-transcription polymerase chain reaction (RT-PCR) detection of the large, medium, and small RNA segments of TSWV, GRSV, and TCSV. When used in conjunction with local lesion-passaged virus isolates, the genome segment-specific RT-PCR assays developed in this study will facilitate high-throughput screening of plant or thrips samples for interspecies reassortants in epidemiological studies and reliable identification of these three tospoviruses in mixed infections commonly observed in the field.

Tospoviruses infect plants and insects, and cause significant economic losses in major horticultural, field, and agronomic crops worldwide (Oliver and Whitfield 2016). The type member, *Tomato spotted wilt virus* (TSWV), was first described over 100 years ago (Scholthof et al. 2011) and remains the major tospovirus pathogen of vegetable, peanut, and tobacco crops in many regions of the world, including the southeastern United States (Culbreath and Srinivasan 2011; Culbreath et al. 1991; Demirozer et al. 2012). Since 2009, two additional tospoviruses emerged in Florida and the Caribbean: *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) (Londoño et al. 2012; Webster et al. 2010, 2015). Previously reported only from South America and Africa, GRSV and TCSV have become significant pathogens of many of the same crops historically infected by TSWV in Florida and the Caribbean (Adegbola et al. 2016; Almeida et al. 2014; Batuman et al. 2014; de Jensen and Adkins 2014; de Jensen et al. 2017; González-Alvarez et al. 2017; Martínez-Zubiaur et al. 2016; Raid et al. 2017; Webster et al. 2013, 2015). Sporadic findings elsewhere in the continental United States of GRSV (South Carolina and New York) and TCSV (Ohio) have been reported

(Baysal-Gurel et al. 2015; Webster et al. 2015). The ubiquitous presence and fecundity of thrips vectors, especially the western flower thrips (*Frankliniella occidentalis*), have contributed to the geographic range expansion of TSWV and emergence of other tospoviruses (Gilbertson et al. 2015). Additionally, global trade and export of produce, and environment-driven changes to agricultural cropping systems, create many opportunities for tospoviruses and thrips vectors to spread to new locations and crops, triggering disease epidemics, with devastating consequences to crop production (Jones 2016; Szostek et al. 2017).

All recognized tospoviruses have tripartite genomes consisting of three genomic RNA segments known as large (L), medium (M), and small (S) RNAs (Oliver and Whitfield 2016). The L RNA is negative sense, approximately 9 kb in size, and encodes the multifunctional L protein. The M RNA is ambisense, approximately 5 kb in size, and encodes the glycoprotein precursor ($G_N G_C$) and nonstructural movement proteins. The S RNA is also ambisense, approximately 3 kb in size, and encodes the nucleocapsid (N) and nonstructural silencing suppressor proteins. From an evolutionary point of view, tospoviruses, like other multipartite viruses infecting plants, insects, and animals, have the propensity to undergo intraspecies and interspecies reassortment, leading to the emergence of novel viral progeny with potentially significant epidemiological implications (Sicard et al. 2016).

For instance, all GRSV isolates identified to date in the United States (Florida, South Carolina, and New York) represent a unique strain of this virus that is an interspecies reassortant between GRSV and TCSV (Webster et al. 2011, 2015). This strain has been previously designated with an $L_G M_T S_G$ genotype to indicate that it contains L and S RNAs from GRSV, whereas the M RNA is from TCSV (Webster et al. 2011). Although $L_G M_T S_G$ is currently the only known interspecies tospovirus reassortant, TSWV isolates have long been documented to undergo intraspecies reassortment (Qui and Moyer 1999; Qiu et al. 1998; Tentchev et al. 2010). Animal-infecting relatives of the tospoviruses have also been documented to undergo both interspecies and intraspecies reassortment (Briese et al. 2013).

Occurrence of reassortants and mixed infections of tospoviruses confounds standard means of tospovirus detection and identification. Therefore, it is imperative to develop robust and reliable methods to

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track the dissemination of tospoviruses and potential novel reassortants for practical applications in crop improvement (germplasm screening and plant breeding) and quarantine programs. Indistinguishable symptoms make differentiation of TSWV, GRSV, and TCSV impossible by visual means in most host plants (Webster et al. 2015). Serological tests based on the N protein are frequently used in diagnostic laboratories. Cross reaction of common TSWV commercial lateral-flow immunoassay reagents with GRSV and TCSV (Adkins et al. 2015) adds value for broad-spectrum tospovirus detection but limits use for specific identification of TSWV, particularly in locations such as south Florida, where all three tospoviruses have occurred sympatrically. Enzyme-linked immunosorbent assay (ELISA) offers greater specificity, although commercial reagents currently available are often not able to distinguish between GRSV and TCSV. Furthermore, one ELISA test is not able to identify reassortants because it, too, is based on detection of a single virus protein. For that same reason, ELISA can also be confounded by mixed infections unless multiple ELISA tests based on detection of multiple viral proteins encoded on different genomic RNAs are used. Nucleic acid detection techniques are likewise commonly based on partial N gene sequences (Adkins and Rosskopf 2002). Use of the complete N gene or other virus gene sequences for these techniques does not eliminate confounding by reassortants and mixed infections because it, too, is based on detection of a single virus gene. A method for amplification, cloning, and sequencing of complete tospovirus M and S RNA sequences was recently demonstrated (Marshall et al. 2017). Full genome sequencing has also been described (Lian et al. 2013). Although these techniques are powerful for phylogenetic and evolutionary studies with limited numbers of samples, they are not practical for high-throughput screening of large numbers of samples for detection of tospovirus reassortants and mixed infections.

Local lesion isolation of plant viruses is a classic technique for separation of virus mixtures into individual viruses and biological variants (Hull 2002). We have previously reported the use of local lesion passing to ensure highly homogenous tospovirus isolates prior to biological characterization and thrips transmission studies (Webster et al. 2011, 2015). Primers currently used are not sufficient for conventional reverse-transcription polymerase chain reaction (RT-PCR) detection of each of the eight unique genomic RNA segments of TSWV, GRSV, and TCSV isolates in the United States and the Caribbean. Thus, the purpose of the current study was to develop and validate a standardized RT-PCR technique using virus genome segment-specific primers that, especially when used in conjunction with local lesion-passaged virus isolates, permits reliable detection of interspecies reassortants and mixed infections of TSWV, GRSV, and TCSV.

Materials and Methods

Collection and maintenance of tospoviruses. TSWV, GRSV, and TCSV isolates collected from Florida tomato plants were used to mechanically inoculate *Nicotiana glutinosa* plants using 0.5% (wt/vol) sodium sulfite, as previously described (Webster et al. 2011). Individual local lesions were subsequently used for mechanical inoculation of 'Florida 47' tomato plants using sodium sulfite as above or for total RNA extraction using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Following symptom development at 2 weeks postinoculation, total RNA was extracted as above from upper, noninoculated Florida 47 leaves and used for development of reassortant detection protocols described below. Additional Florida 47 tomato plants were mock-inoculated with 0.5% sodium sulfite for use as negative controls. Total RNA was extracted from leaves corresponding to those of infected tomato plants at the same time and by the same method.

Field samples of tomato ($n = 469$), pepper ($n = 55$), and other ($n = 50$) plants with typical tospovirus symptoms of necrotic flecks, spots, and ringspots were collected from 2013 through 2017 in south Florida, where TSWV, GRSV, and TCSV occurred sympatrically (Webster et al. 2015). Representative south Florida field samples were used to mechanically inoculate *N. glutinosa* plants to generate local lesions, as described above. Additional field samples of tomato ($n = 118$), pepper ($n = 25$), and other ($n = 34$) plants with typical

tospovirus symptoms were collected under an Animal and Plant Health Inspection Service Plant Protection and Quarantine permit during this same time period from Georgia, South Carolina, North Carolina, Virginia, New York, Maine, California, and Puerto Rico. Total RNA was extracted directly from field samples and also from *N. glutinosa* local lesions as described above, and used for development of mixed-infection detection protocols described below.

Development of RT-PCR for detection of tospovirus reassortant and mixed infections. For the current study, eight primer pairs were needed—one primer pair for conventional RT-PCR amplification of a portion of each of the eight unique L, M, and S RNAs that constitute the genomes of TSWV, GRSV, and TCSV. Primers for the L and M RNAs of TSWV were designed and validated as part of the current study. Primers useful for differentiating the S RNA of TSWV, and the L, M, and S RNAs of GRSV and TCSV have been designed, validated, and previously reported (Adkins and Rosskopf 2002; Webster et al. 2011, 2013). For each of the three viruses, primers for the L RNA target the L protein gene, primers for the M RNA target the $G_N G_C$ precursor gene, and primers for the S RNA target the N gene (Table 1). We note that, because all U.S. GRSV isolates are reassortants containing the TCSV M RNA, a single primer pair was used for detection of the M RNA of both GRSV and TCSV. Thus, there are only eight total primer pairs used in this study instead of the expected nine. All primers were selected or designed to have a melting temperature of 60°C so that simultaneous PCR reactions for all eight RNAs could take place in a single thermocycler with a common annealing temperature. In addition, primer pairs were chosen to generate RT-PCR products that could easily be distinguished visually from one another on agarose gels. Degenerate tospovirus primers J13 and UHP (Cortez et al. 2001) were also used on representative samples to ensure that we would detect the authentic GRSV M RNA in the event that a nonreassorted GRSV isolate was introduced to Florida.

To ascertain the usefulness of this approach for detection of interspecies reassortants, first-strand cDNA was synthesized from total RNA of TSWV-, GRSV-, or TCSV-infected or mock-inoculated tomato plants (extracted as described above) using random hexamers and *Moloney murine leukemia virus* reverse transcription (Promega Corp., Madison, WI) at 45°C for 45 min. Independent PCR reactions were made, one with each of the primer pairs (L, M, or S) from cDNA for each of the four templates (TSWV, GRSV, TCSV, or mock-inoculated tomato). A program of 95°C for 1 min; followed by 30 cycles at 95°C for 30 s, 60°C for 45 s, and 72°C for 45 s; and a final extension at 72°C for 10 min was used. Aliquots of each PCR reaction were analyzed by agarose gel electrophoresis on 1% (wt/vol) gels. This experiment was replicated five times.

To ascertain the usefulness of this approach for detection of mixed infections and reassortants, first-strand cDNA was synthesized from total RNA of field-collected, tospovirus-infected tomato, pepper, or other plants, as described above. Similarly, independent PCR reactions were made, one with each of the primer pairs (L, M, or S) for each of the three viruses (TSWV, GRSV, or TCSV) from cDNA for each of the field samples. TSWV-, GRSV-, or TCSV-infected or mock-inoculated tomato plants were included as controls. Aliquots of each PCR reaction were analyzed by agarose gel electrophoresis on 1% (wt/vol) gels. This experiment was replicated three times for all south Florida field samples tested.

Local lesions were also examined for direct testing for tospovirus interspecies reassortant and mixed-infection detection. Total RNA was prepared as described above from 10 individual *N. glutinosa* local lesions arising from each starting sample. These 10 total RNA preparations were then tested by RT-PCR with all primer pairs.

Representative RT-PCR products for each of the eight genomic RNAs from the three tospoviruses, and the J13 and UHP degenerate tospovirus primers, were cloned in the pGEM-T vector (Promega Corp.) using standard methods. Three clones per amplicon were sequenced in both directions on an ABI 3730XL sequencer at the United States Department of Agriculture–Agricultural Research Service U.S. Horticultural Research Laboratory DNA Sequencing Support Laboratory. A consensus sequence from forward and reverse sequences for each clone was generated and compared with the sequence of the corresponding genome region of the appropriate virus.

Results

Local lesion isolation of tospoviruses. Necrotic local lesions appeared by 7 days postinoculation on inoculated *N. glutinosa* leaves (Fig. 1A) and showed the characteristic concentric rings (Fig. 1B) typically induced by TSWV, GRSV, or TCSV. Individual local lesions were carefully excised using new single-edge razor blades (Fig. 1C). These excised local lesions (Fig. 1D) were demonstrated to be useful for mechanical inoculation of Florida 47 tomato plants and as a direct source of total RNA for further testing, as described below.

Detection of interspecies reassortants and mixed tospovirus infections. Primers used in this study were specific for one of the three genomic RNA segments of TSWV, GRSV, or TCSV. In the representative results shown in Figure 2, the TSWV L, M, and S RNA primers only amplified PCR products from TSWV-infected samples (Fig. 2A). Similarly, the GRSV L and S RNA primers only amplified PCR products from GRSV-infected samples (Fig. 2B), and the TCSV L and S RNA primers only amplified PCR products from TCSV-infected samples (Fig. 2C). We note again that a single pair of primers was used for detection of the GRSV and TCSV M RNA because all GRSV isolates in the United States are reassortants and contain the TCSV M RNA. Thus, the M RNA of both our GRSV and TCSV isolates is detected by primer pair GLY5-v and GLY4-vc (Table 1; Fig. 2B and 2C). Total RNA from tomato plants inoculated with local lesion-passaged virus isolates (Fig. 2) yielded identical results to total RNA from individual local lesions from inoculated *N. glutinosa* leaves (data not shown). None of the primer pairs amplified any products from mock-inoculated tomato samples (Fig. 2). No differences were observed between experimental replications. Sequencing of representative RT-PCR products confirmed that primer pairs were amplifying the expected region (Table 1) of individual genomic RNA segments from each virus (data not shown).

For total RNA from field-collected tospovirus-infected tomato, pepper, or other plant samples, the primers designed or selected in this study were useful for detection of reassortants and mixed tospovirus infections, including a representative mixed TSWV and TCSV infection of tomato (Fig. 3A and C, lane 2). Of the 751 plant samples tested, 421 were determined to be tospovirus infected. The reassortant GRSV strain was detected in approximately 8% of infected samples. Mixed infections of two tospoviruses were detected in approximately 2% of infected samples. All possible combinations of mixed infections of TSWV, GRSV, or TCSV were detected using these methods during this study (data not shown), similar to previous reports (Webster et al. 2015). The relative intensities of each virus species' RT-PCR products in mixed infections varied between samples without an obvious trend.

In the representative results shown in Figure 3, the TSWV L, M, and S RNA primers only amplified PCR products from TSWV-infected field samples (Fig. 3A, lanes 1, 2, and 3) and the TSWV control (Fig. 3A, lane 6). The GRSV L and S RNA primers only amplified PCR products from the GRSV control (Fig. 3B, lane 7). The TCSV L and S RNA primers only amplified PCR products from TCSV-infected samples (Fig. 3C, lanes 2, 4, and 5) and the TCSV control (Fig. 3C, lane 8). Again, we note that, because a single pair of primers was used for detection of the M RNA of our GRSV and TCSV isolates, PCR products were amplified with these primers from TCSV-infected samples (Fig. 3B and C, lanes 2, 4, and 5), and the GRSV and TCSV controls (Fig. 3B and C, lanes 7 and 8). Total RNA directly extracted from field-collected tomato samples (Fig. 3, lanes 1 to 5) yielded results consistent with those obtained from total RNA from individual local lesions of *N. glutinosa* leaves inoculated with the field samples (data not shown). About half of the individual local lesions from mixed infections (Fig. 3A and C, lane 2)

Table 1. Primers used for detection of interspecies reassortants and mixed infections of *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), and *Tomato chlorotic spot virus* (TCSV)^a

Virus	Primer	RNA	Gene	Sequence (5' to 3')	Product ^b	Location ^c	Source
TSWV	TSWV-Lv3	L	L	CACCACTGTGGGTGTGGTTCCAACATT	449	452–475	This study
TSWV	TSWV-Lvc3	L	L	AGTCCTGCATTAACAAGCTCTCTG	...	873–900	This study
TSWV	GCGN2v3	M	G _N G _C	CGGGTCTGCAGCACAATTCTG	973	3,145–3,165	This study
TSWV	GCGN3vc3	M	G _N G _C	CCCTACTAATGGAACAGTATC	...	4,097–4,117	This study
TSWV	TSWV722	S	N	GCTGGAGCTAAGTATAGCAGC	620	2,098–2,118	Adkins and Rosskopf 2002
TSWV	TSWV723	S	N	CACAAGGCAAAAGACCTTGAG	...	2,698–2,717	Adkins and Rosskopf 2002
GRSV	3RdRp-v	L	L	TTCCAGGCAGGTGTCTTTGATG	734	1,517–1,538	Webster et al. 2011
GRSV	5'Lgap-vc	L	L	GGCTGTGGAACATGGGTCTG	...	2,231–2,250	Webster et al. 2011
GRSV	GRSV-N-v	S	N	AGAGCTTCCTTAGTGTGTACTTAG	595	2,348–2,372	Webster et al. 2011
GRSV	GRSV-N-vc	S	N	GAAAGGTCTAGATCTAACTGCCAC	...	2,918–2,942	Webster et al. 2011
TCSV	TCSV-3'Lv	L	L	AAAGGCAATGCATAAATAGCATCAC	1,012	447–471	Webster et al. 2013
TCSV	TCSV-3'Lvc	L	L	GGAGACCAAGTCAAGAAAAATTCGTT	...	1,432–1,458	Webster et al. 2013
TCSV	GLY5-v	M	G _N G _C	CAGAGTGTGCTTTGAAGTTCCC	166	4,524–4,545	Webster et al. 2011
TCSV	GLY4-vc	M	G _N G _C	AGCATTTCTGTTGTTCAGGGCTAC	...	4,667–4,689	Webster et al. 2011
TCSV	TCSV-Nv2	S	N	CCTAAAGCTTCTTAGTGTATACTTCG	530	697–724	Webster et al. 2013
TCSV	TCSV-Nvc2	S	N	CTAACTCAGGCTGGAGAAATCGAG	...	195–218	Webster et al. 2013

^a Abbreviations: L, M, and S = large, medium, or small, respectively, genomic RNA; G_NG_C = glycoprotein precursor; and N = nucleocapsid protein.

^b Expected product size in nucleotides.

^c Primer location with reference to GenBank accession numbers: TSWV = D10066, S48091, and NC_002051; GRSV = NC_015469 and NC_015467; and TCSV = HQ700667, NC_015468, and S54325.

Fig. 1. Tospovirus local lesion isolation in *Nicotiana glutinosa* with *Tomato spotted wilt virus* (TSWV) shown as an example. **A**, TSWV necrotic local lesions on mechanically inoculated leaves at 7 days postinoculation. **B**, Individual TSWV local lesion with typical concentric necrotic rings. **C**, Excision of individual TSWV local lesion using single-edge razor blade. **D**, Postexcision TSWV local lesion ready for plant inoculation and/or RNA isolation.

yielded PCR products only for the first virus (e.g., TSWV) and the other half only yielded PCR products for the second virus (e.g., TCSV). Similar results were obtained for all types of mixed infections identified in this study. As with the interspecies reassortant experiment (Fig. 2), none of the primer sets amplified any products from mock-inoculated tomato samples (Fig. 3, lane 9). No differences were observed between experimental replications. Sequencing of representative RT-PCR products confirmed that primer pairs were amplifying the expected region (Table 1) of individual genomic RNA segments from each virus (data not shown).

Discussion

Given the lack of a reverse genetics system for tospoviruses, it is necessary to explore currently available methods to better understand the complex biology and the many intrigues of this virus group. For instance, interspecies genome segment reassortment in tospoviruses is less studied compared with animal-infecting bunyaviruses (Briese

et al. 2013) and other virus groups with multipartite genomes (Vijaykrishna et al. 2015). As a first step, we outline here a straightforward approach using inexpensive primers and conventional RT-PCR coupled with local lesion isolation to identify interspecies reassortants and mixed infections of TSWV, GRSV, and TCSV. A single total RNA preparation from an infected plant can be tested with all primer pairs reported in this study to determine what combination of virus genomic RNAs is present in a given sample. This approach was used successfully on 751 field-collected samples of tomato, pepper, and other plants from eight states and one territory in the United States. With design and validation of additional primers, this approach may be readily extended to other tospoviruses that may be of greater regional importance in other geographic areas.

The differences in the RT-PCR product sizes also allowed us to explore multiplex RT-PCR detection of the L, M, and S RNA segments for TSWV, GRSV, or TCSV in a single reaction. For reasons not entirely understood, multiplex RT-PCR was not consistently successful.

Fig. 2. *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), and *Tomato chlorotic spot virus* (TCSV) genomic RNA-specific primers facilitate detection of interspecies reassortants. Total RNA from TSWV-, GRSV-, or TCSV-infected or mock-inoculated (Mock) tomato plants were used as templates for reverse-transcription polymerase chain reaction (RT-PCR). Primers specific for large (L), medium (M), or small (S) genomic RNAs of **A**, TSWV; **B**, GRSV; or **C**, TCSV were used. RT-PCR products are present only in homologous template-primer combinations, with one exception: GRSV/TCSV M RNA primers yield RT-PCR products for both GRSV and TCSV templates, indicating that Florida GRSV isolates contain the TCSV M RNA. Markers present in several lanes (m) on each gel, with size in kilobases indicated to the right.

Fig. 3. *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), and *Tomato chlorotic spot virus* (TCSV) genomic RNA-specific primers facilitate detection of mixed infections in field samples. Total RNA from five representative tomato field samples (lanes 1 to 5) and controls of TSWV- (lane 6), GRSV- (lane 7), or TCSV- (lane 8) infected or mock-inoculated (lane 9) tomato plants were used as templates for reverse-transcription polymerase chain reaction (RT-PCR). Primers specific for large (L), medium (M), or small (S) genomic RNAs of **A**, TSWV; **B**, GRSV; or **C**, TCSV were used. Presence of RT-PCR products indicates TSWV infection of samples 1, 2, and 3; TCSV infection of samples 2, 4, and 5; and mixed TSWV/TCSV infection of sample 2. Markers present in several lanes (m) on each gel, with size in kilobases indicated to the right.

Thus, we ultimately decided to use each primer pair independently for detection of a single genomic RNA of a single tospovirus. Eight PCR reactions were made for each starting sample.

Although a classic technique, local lesion isolation of plant viruses remains useful for separation of virus mixtures, including tospoviruses. For instance, when *N. glutinosa* was inoculated with the tomato sample with the mixed TSWV and TCSV infection shown in Figure 3 (lane 2), about half of the local lesions tested positive only for TSWV and about half tested positive only for TCSV. This is not surprising because this is the purpose of local lesion passaging and it demonstrates the continued relevance of this technique, especially when coupled with RT-PCR using genome segment-specific primer pairs.

Additional interspecies tospovirus reassortment is likely to occur, especially given the known occurrence of mixed tospovirus infections in a variety of crop and noncrop plant species. The polyphagous nature of thrips and vegetative propagation of many ornamental crops facilitate creation and maintenance of additional mixed infections. In the case of TSWV, GRSV, and TCSV studied in this report, various combinations of mixed infections have been detected in tomato and pepper in Florida (Webster et al. 2015). Similarly, mixed infections of TSWV and *Impatiens necrotic spot virus* have been detected in tomato in Italy (Finetti Sialer and Gallitelli 2000) and *Hoya wayetti* in New York (S. Adkins and M. L. Daughtrey, unpublished).

Further extensions of the approach reported here include examination of RNA from large numbers of individual local lesions or individual thrips. This would facilitate mechanistic studies of tospovirus reassortment, population dynamics of tospoviruses when present in mixed infections, and transmission of both reassortants and mixed infections. Our approach would prove highly practical when large numbers of local lesions or thrips were being studied simultaneously. However, further studies of both interspecies reassortment and population dynamics in mixed infections will benefit from development of additional methods such as quantitative RT-PCR to facilitate more precise quantification of each of the individual genomic RNAs of each of the tospoviruses. Trade and extreme weather events could potentially increase the spread of indigenous and exotic tospoviruses, and create ideal conditions for the emergence of reassortants. The technique described in this study provides a framework to identify viable reassortants circulating in a cropping system or geographic region as part of an effort to develop a broader understanding of the population genetic structure and epidemiology of tospoviruses.

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