Virology 413 (2011) 216–225

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

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 ☆

Craig G. Webster [a], Stuart R. Reitz [b], Keith L. Perry [c], Scott Adkins [a,*]

[a] United States Department of Agriculture-Agricultural Research Service (USDA-ARS), U.S. Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA
[b] USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Tallahassee, FL 32308, USA
[c] Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 9 December 2010
Returned to author for revision
3 February 2011
Accepted 9 February 2011
Available online 6 March 2011

Keywords:
Bunyaviridae
Tospovirus
Groundnut ringspot virus
Tomato chlorotic spot virus
Tomato spotted wilt virus
Thrips

ABSTRACT

Reassortment allows multicomponent viruses to exchange genome segments, a process well-documented in the vertebrate- and arthropod-infecting members of the family *Bunyaviridae* but not between distinct species of the plant- and insect-infecting members of the genus *Tospovirus*. Genome sequence comparisons of a virus causing severe tospovirus-like symptoms in Florida tomato with *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) demonstrated that reassortment has occurred, with the large (L) and small (S) RNAs coming from GRSV and the medium (M) RNA coming from TCSV (i.e. $L_GM_TS_G$). Neither parental genotype is known to occur in the U.S. suggesting that $L_GM_TS_G$ was introduced as a reassortant. $L_GM_TS_G$ was transmitted by western flower thrips (*Frankliniella occidentalis* [Pergande]), and was not able to overcome the *Sw5* resistance gene of tomato. Our demonstration of reassortment between GRSV and TCSV suggests caution in defining species within the family *Bunyaviridae* based on their ability to reassort.

Published by Elsevier Inc.

Introduction

Viruses in the family *Bunyaviridae* include some of the most important medical and agricultural pathogens. Members of the family are categorized into genera and species based on a combination of characteristics such as: vertebrate, arthropod and plant hosts; serological cross-reactivity to other members; and identity of protein sequences, particularly those of the nucleocapsid (N) protein (Fauquet et al., 2005). Currently 95 species in five vertebrate-, plant- and arthropod-infecting genera (*Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus* and *Tospovirus*) are recognized (ICTV Master Species list v7, available at http://talk.ictvonline.org/files/ictv_documents/m/msl/1231.aspx). A feature common to the family is the presence of three genomic RNAs [termed small (S), medium (M) and large (L)] which encode the N protein, two glycoproteins (G_N and G_C) and an RNA-dependent RNA polymerase (L) [reviewed by Elliott (1997), Schmaljohn and Hooper (2001) and Nichol (2001)]. Two nonstructural proteins, NSs and NSm, are encoded on the S and M RNA, respectively, of the orthobunyaviruses, phleboviruses and tospoviruses. In the tospoviruses, both the M and S RNAs utilize an ambisense strategy with the non-structural proteins encoded in the viral sense and the structural proteins encoded in the viral complementary sense. For viruses in the genus *Tospovirus*, NSs has been shown to function as a suppressor of silencing (Takeda et al., 2002; Schnettler et al., 2010) and NSm has been shown to function as a movement protein (Lewandowski and Adkins, 2005; Li et al., 2009).

Apart from the hantaviruses, for which arthropod vectors are not known, all other viruses in the family *Bunyaviridae* are vectored by one or more arthropod species. Tospoviruses are transmitted by thrips, which must acquire the virus as larvae to become transmitters as adults (Sakimura, 1962; Wijkamp and Peters, 1993), whereas the other genera are transmitted by mosquitoes, phlebotomine sandflies, culicoid flies or ticks that feed on vertebrates (reviewed by Nichol, 2001).

The exchange of genetic material between viruses can occur in nature during cellular co-infections by two or more virus lineages either by recombination or reassortment. Such genetic exchange is presumably an underlying reason for the existence of segmented viral genomes which allows unique or novel combinations of distinct mutations to be combined, while undesirable changes are removed from the gene pool (Pringle, 1996). The creation of chimeric nucleic acid molecules derived from segments of each parental donor, termed recombination, is one mechanism for this type of exchange. However,

☆ The use of trade, firm or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

* Corresponding author. Fax: +1 772 462 5986.
E-mail address: scott.adkins@ars.usda.gov (S. Adkins).

0042-6822/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.virol.2011.02.011

to the best of our knowledge, recombination within the family *Bunyaviridae* has only been observed in the genus *Hantavirus* (Sibold et al., 1999). Reassortment is the exchange of complete genome segments in multisegmented viruses, and is another mechanism for genetic exchange. Reassortment of genomic RNAs has been reported within the vertebrate, plant or arthropod host for viruses of all genera in the *Bunyaviridae*, including orthobunyaviruses (e.g. Gentsch et al., 1980; Ushijima et al., 1981; Beatty et al., 1985; Borucki et al., 1999; Cheng et al., 1999; Briese et al., 2006; 2007), hantaviruses (e.g. Henderson et al., 1995; Rodriguez et al., 1998; McElroy et al., 2004), phleboviruses (e.g. Saluzzo and Smith, 1990; Turell et al., 1990; Sall et al., 1999), nairoviruses (e.g. Hewson et al., 2004) and tospoviruses (e.g. Best, 1961; Best and Gallus, 1955; Qiu et al., 1998). Studies of bunyavirus reassortment have proven useful because they have allowed mapping of attributes and functions to specific genomic RNA segments. For instance, encoding of the N protein by the S RNA was determined using reassortants (Gentsch et al., 1977) prior to the development of reverse genetics systems for bunyaviruses (e.g. Bridgen and Elliott, 1996; Flick and Pettersson, 2001; Billecocq et al., 2008).

Within the plant- and insect-infecting tospoviruses, indirect evidence of reassortment was first obtained from deliberate co-infection of plants with two strains of *Tomato spotted wilt virus* (TSWV) with subsequent observation of mixed phenotypic characters (Best and Gallus, 1955; Best, 1961). More recent direct evidence of reassortment has come from nucleic acid sequencing of local lesions derived from plants co-infected with two strains of either TSWV (Qiu et al., 1998) or *Watermelon silver mottle virus* (Okuda et al., 2003). The resulting isolates were identified as containing most, but not all, possible reassortment combinations. As with the vertebrate-infecting viruses, these reassorted tospovirus isolates facilitated the identification of regions associated with specific functions such as symptom determinants (Okuda et al., 2003). Additionally, a reassortant was observed to overcome transgenic host resistance (TSWV N gene-derived), a biological characteristic not present in either parental genotype (Qiu and Moyer, 1999).

Members of the genus *Tospovirus* collectively cause diseases in hundreds of plant species (Parrella et al., 2003) including many economically important crops plants such as tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*). *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV) and TSWV are three genetically distinct species [based on N gene sequences (de Ávila et al., 1993)] but produce similar and often visually indistinguishable symptoms on tomato in South America (Gracia et al., 1999; Williams et al., 2001). GRSV was first isolated and described from both peanut (*Arachis hypogaea*) in South Africa and tomato in Brazil, whereas TCSV was first isolated from tomato in Brazil (de Ávila et al., 1990; 1993). Subsequently both GRSV and TCSV have been reported on tomato in Argentina (Dewey et al., 1995; Gracia et al., 1999; de Borbón et al., 2006; de Breuil et al., 2007). Other tomato-infecting tospoviruses, including Tomato zonate spot virus and Capsicum chlorosis virus, are only distantly related to TSWV, GRSV and TCSV (Dong et al., 2008; McMichael et al., 2002).

Based on serology and N gene sequence we recently identified GRSV from tomato plants with severe tospovirus symptoms in south Florida (Webster et al., 2010), a finding which extends the known distribution of this tospovirus beyond South America and South Africa (de Ávila et al., 1990, 1993) to North America. In the current study, we have determined and analyzed the full genome sequence of this virus. We demonstrate that the Florida GRSV isolate is actually an M RNA reassortant, with the L and S RNA segments indeed coming from GRSV but with the M RNA segment coming from TCSV to yield an $L_GM_TS_G$ genotype. We established that the $L_GM_TS_G$ genotype was widespread in tomato in south Florida and characterized some of its biological properties including vector transmission and plant resistance gene interactions. These data extend the current knowledge of the potential for reassortment within the family *Bunyaviridae*.

Results

Determination and analysis of $L_GM_TS_G$ genome sequence

The genome of a representative $L_GM_TS_G$ isolate collected in February 2010 from tomato in south Florida (Miami-Dade county) was completely sequenced. The genome was typical of a tospovirus with the three RNAs of 3067 nucleotides (nt) (S RNA, HQ644140), 4848 nt (M RNA, HQ644141) and 8876 nt (L RNA, HQ644142), and five predicted open reading frames (N = 777 nt, NSm = 912 nt, NSs = 1404 nt, G_NG_C = 3405 nt and L = 8625 nt). The conserved sequence motif 5′-AGAGCAAT-3′ or its reverse complement was present at the termini of each segment.

Nucleotide and amino acid (aa) comparisons of $L_GM_TS_G$ were made with previously sequenced and closely related tospoviruses (GRSV, TCSV, and TSWV). Comparisons of the $L_GM_TS_G$ N gene and deduced amino acid sequences with GRSV isolates in GenBank showed that identities were greater than or equal to 94.1% or 96.1% respectively, but were less than or equal to 83.3% or 88.0% at the nt or deduced aa level, respectively, with TCSV (Table 1). However, comparisons with the $L_GM_TS_G$ M RNA showed 97.6% and 91.7% nt identity to TCSV and GRSV, respectively, opposite to the trend observed with the N gene encoded on the S RNA. Similar values were seen across both coding regions (NSm and G_NG_C) of the M RNA. No comparisons to the L RNA of GRSV and TCSV could be made due to a lack of sequence information available in GenBank. Consistent low identity to TSWV (<80.7% nt and 89.6% aa; Table 1) was also seen for all three RNAs.

Sliding window analysis was also used to compare the level of nt identity between 200 nt segments of the M and S RNAs of $L_GM_TS_G$ and those of TCSV and GRSV isolates in GenBank (Fig. 1). The highest level of identity of the $L_GM_TS_G$ M RNA was observed with the TCSV M RNA across the entire NSm and G_NG_C coding regions in an individual window, as compared to GRSV and TSWV (Fig. 1A). Corresponding comparisons for the S RNA could not be made because of a lack of TCSV sequence data. However, a high level of identity (>94%) of the $L_GM_TS_G$ S RNA was observed with the GRSV S RNA (GenBank accession L12048; Dennis Gonsalves and Fuy-Jyh Jan, personal communication) across the entire NSs and N coding regions in an individual window, as compared to TSWV (Fig. 1B).

Table 1
Percentage identity of genomic RNAs and coding regions of $L_GM_TS_G$ with known isolates of GRSV, TCSV and TSWV.

Region	Identity with GRSV[a]	Identity with TCSV[a]	Identity with TSWV[a]
S RNA	**98.3% (1)**	–[b]	**76.1%**
N	**94.1–98.2% (4)**	**82.3–83.3% (2)**	**77.4%**
	96.1%–99.2% (4)	*84.5–88.0% (2)*	*79.4%*
NSs	**98.8% (1)**	–	**76.5%**
	99.8% (1)		*78.4%*
M RNA	**91.7% (1)**	**97.6% (1)**	**76.7%**
NSm	**91.8–93.5% (2)**	**98.1–98.2% (2)**	**80.7%**
	95.0–97.4% (2)	*99.3% (2)*	*86.1%*
G_NG_C	**91.6% (1)**	**97.6% (1)**	**76.0%**
	96.3% (1)	*98.4% (1)*	*81.3%*
L RNA	–	–	**77.3%**
L	–	–	**78.4%**
			89.6%

[a] Average percentage nucleotide (**bold**) and amino acid (*italics*) identity of $L_GM_TS_G$ to other full length tospovirus sequences in GenBank. *Groundnut ringspot virus* (GRSV): AF215271, AF213220, AF213673, AF513219, L12048, and S54327; *Tomato chlorotic spot virus* (TCSV): AF213674, AF282982, AF282983 and S54325; and *Tomato spotted wilt virus* (TSWV): NC_002051 (S RNA), AY744481 (M RNA) and AB198742 (L RNA). Percentages were determined in MEGA 4.1 with the numbers in parentheses indicating the number of sequences used for comparison.

[b] – = no sequence data available for comparison.