Characterization of Tomato yellow spot virus, a novel tomato-infecting begomovirus in Brazil

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Abstract – The objective of this work was the biological and molecular characterization of a begomovirus detected in São Joaquim de Bicas, Minas Gerais, Brazil, named TGV-[Bi2], by determining its host range, complete nucleotide sequence and phylogenetic relationships with other begomoviruses. Biological characterization consisted of a host range study using either sap inoculation or particle bombardment as inoculation methods. The yellow spot virus can infect plants in Solanaceae and Amaranthaceae, including economically importat crops as sweet pepper, and weeds as *Datura stramonium* and *Nicotiana silvestris*. For the molecular characterization, the full-length genome (DNA-A and DNA-B) was amplified, cloned and completely sequenced. Sequence comparisons and phylogenetic analyses indicated that TGV-[Bi2] constitutes a novel begomovirus species named Tomato yellow spot virus (ToYSV), closely related to *Sida mottle virus* (SiMoV).

Index terms: *Lycopersicon esculentum*, *Bemisia tabaci*, ToYSV, molecular biology, geminivirus, whitefly.

Caracterização do Tomato yellow spot virus, um novo begomovírus isolado de tomateiro no Brasil

Resumo – O objetivo deste trabalho foi a caracterização biológica e molecular de um begomovírus detectado em tomateiros em São Joaquim de Bicas, Minas Gerais, denominado TGV-[Bi2]. A caracterização biológica consistiu em teste de gama de hospedeiros, realizado por meio de inoculação via extrato foliar tamponado ou bombardeamento de partículas. O isolado TGV-[Bi2] infecta plantas das famílias Solanaceae e Amaranthaceae, inclusive espécies economicamente importantes como o pimentão, e algumas plantas daninhas como *Datura stramonium* e *Nicotiana silvestris*. A caracterização molecular consistiu na clonagem e seqüenciamento de seu genoma completo (DNA-A e DNA-B). A comparação de seqüências e análise filogenética indicaram que o TGV-[Bi2] constitui uma nova espécie de begomovírus, denominada Tomato yellow spot virus (ToYSV), filogeneticamente relacionado ao *Sida mottle virus* (SiMoV).

Termos para indexação: *Lycopersicon esculentum*, *Bemisia tabaci*, ToYSV, biologia molecular, geminivírus, mosca-branca.

Introduction

The *Geminiviridae* family is comprised of plant viruses with a circular single-stranded DNA genome, and a particle morphology of twinned incomplete icosahedra (Rojas et al., 2005). The family is divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocovirus* and *Begomovirus*) according to the type of insect vector, host range, genomic organization and phylogeny (Stanley et al., 2005). Viruses classified into the genus *Begomovirus* are transmitted by the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae), infect dicotyledoneous plants, and have mono- or (mostly) bipartite genomes. Begomoviruses are considered to be an emergent group of plant viruses, due to the high incidence and severity of diseases caused by them over the last three decades, in tropical and subtropical regions of the world (Polston & Anderson, 1997; Legg & Thresh, 2000; Morales & Anderson, 2001; Briddon, 2003).

Novel begomovirus species infecting tomato (*Lycopersicon esculentum* Mill.) were detected in the 1990's in different regions of Brazil, after the introduction and quick dissemination of B biotype of *B. tabaci* (Ribeiro et al., 2003). In the Triângulo Mineiro region of Minas Gerais State, a novel bipartite begomovirus was detected in tomato plants growing under greenhouse conditions (Fernandes et al., 2006). The comparison of the complete nucleotide sequences of the DNA-A and DNA-B

components with other begomoviruses indicated that this was a distinct species, named *Tomato rugose mosaic virus* (ToRMV). A second begomovirus, named *Tomato chlorotic mottle virus* (ToCMoV) was isolated from tomato plants in Zona Metalúrgica, a region of Minas Gerais State, and characterized molecularly and biologically (Ambrozevicius et al., 2002; Ribeiro et al., 2003). The sequence identity between ToCMoV and ToRMV is 86% for the DNA-A and 60% for DNA-B, which demonstrates they are distinct, although closely related viruses. Three begomovirus species infecting the common weed *Sida* have also been sequenced (*Sida mottle virus*, SiMoV; *Sida yellow mosaic virus*, SiYMV; and *Sida micrantha mosaic virus*, SimMV) (Jovel et al., 2004).

A third begomovirus isolate, provisionally named TGV-[Bi2] (Tomato geminivirus-[Bi2]), was obtained from a tomato plant collected near São Joaquim de Bicas, in Minas Gerais State, causing symptoms which were much more severe than those caused by ToRMV and ToCMoV (Ambrozevicius et al., 2002). Tomato plants infected by TGV-[Bi2] show intense yellow mosaic, leaf roll, reduction of leaf area and generalized dwarfing. Determining its host range may assist in the identification of alternate hosts and virus reservoirs, which could serve as inoculum sources for field outbreaks, and may indicate additional crop plants that could be infected by the virus.

The objective of the present work was to carry out the biological and molecular characterization of TGV-[Bi2], by determining its host range, complete nucleotide sequence and phylogenetic relationships with other begomoviruses.

Material and Methods

The TGV-[Bi2] isolate was obtained from a tomato plant showing yellow mosaic, leaf distortion and dwarfing, collected near São Joaquim de Bicas, at Zona Metalúrgica of Minas Gerais State (Ambrozevicius et al., 2002). The replicative form (RF) of the viral genome was extracted from the collected plant (Gilbertson et al., 1991), treated with RNase A to eliminate possible viruses occurring in mixed infections, and used for the biolistic inoculation (Aragão et al., 1996) of *Nicotiana benthamiana* plants. DNA of all inoculated plants was extracted (Dellaporta et al., 1983) and used as a template for PCR-amplification of DNA-A fragments using the universal begomovirus oligonucleotides PAL1v1978 and PAR1c496 (Rojas et al., 1993). The results were analyzed by agarose gel electrophoresis to check for the presence of amplicons with 1,100 to 1,400 nucleotides (nt); amplicons were partially sequenced from 15 independent PCR reactions, which yielded identical sequences and confirmed the presence of a single begomovirus. The isolate was maintained in *N. benthamiana* and *N. glutinosa* plants by successive sap inoculations using 0.1 M sodium phosphate pH 8 with 0.1% sodium sulfite. All plants were maintained in a greenhouse at the Plant Pathology Department, Universidade Federal de Viçosa, Viçosa, MG.

The biological characterization consisted on a host range study using species from Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae: *Capsicum annuum* 'Ikeda', *Chenopodium quinoa*, *Cucurbita pepo* 'Caserta', *Datura stramonium*, *Gomphrena globosa*, *Lycopersicon esculentum* 'Miller Early Pack', 'Rutgers' and 'Santa Clara', *Nicotiana benthamiana*, *Nicotiana clevelandi*, *Nicotiana glutinosa*, *Nicotiana rustica*, *Nicotiana silvestris*, *Nicotiana tabacum* 'Havana 425', 'Samsun', 'TNN', and 'Xanthi', *Phaseolus vulgaris* 'Ouro Negro' and 'Pérola' and *Solanum melongena*.

Plants were sap-inoculated or biolistically-inoculated. Viral DNA used for biolistics was extracted by grinding 2 g of infected leaves in liquid nitrogen. The ground tissue was transferred to 1.5 mL microfuge tubes containing 500 µL lysis buffer (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 50 mM EDTA, 10 mM 2-mercaptoethanol, 0.5% SDS). The tube was vortexed for 2 min and incubated at room temperature for 15 min. Subsequently, 150 µL phenol were added to each tube, followed by 2 min vortexing and 2 min incubation at room temperature, followed by the addition of 150 µL chloroform, 2 min vortexing and centrifugation at 10,000 *g* for 2 min. Supernatant was transferred to a new tube, and one volume of isopropanol was added, followed by incubation at -20°C for 12 hours. The solution was then centrifuged at 10,000 *g* for 5 min. The supernatant was discarded, the pellet was washed with 500 µL of 70% ethanol and resuspended in 200 µL of TE with RNase A. Then, one volume of phenol: chloroform was added and the solution was agitated for 1 min and centrifuged at 10,000 *g* for 2 min. The supernatant was collected, and 500 µL of 100% ethanol were added, followed by incubation for 10 min at -20°C. A new centrifugation was done at 10,000 *g* for 5 min, and the supernatant was discarded. The pellet was washed with 500 µL of 70% ethanol and resuspended in 30 µL of TE with Rnase A.

Plants inoculated with the virus were maintained in the greenhouse and observed for the appearance of symptoms until 35 days after inoculation. Infection was confirmed by

PCR using universal begomovirus primers as described above. The experiment was repeated three times.

Molecular characterization of TGV-[Bi2] consisted on the determination and analysis of its complete DNA sequence. Viral DNA was extracted (Dellaporta et al., 1983), and the RF was amplified using the Templiphi kit according to manufacturer's instructions. DNA concentration was visually estimated in ethidium bromide-stained agarose gels using mass standards. Approximately 500 ng of viral DNA were cleaved with each of the following restriction endonucleases: *Apa* I, *Bam*H I, *Cla* I, *Hind* III, *Not* I, *Sac* I, *Sma* I, *Xba* I and *Xho* I. An aliquot of cleaved DNA was submitted to electrophoresis in order to identify the enzyme that cleaved each DNA component in a single site, thus allowing ligation to a plasmid vector. Cloning was carried out according to standard procedures (Sambrook & Russel, 2001), with the exception that ligation reactions were prepared with a 1:12 vector:insert ratio. Clones corresponding to both DNA components were sequenced in both orientations using the BigDye Terminator Cycle Sequencing Ready Reaction kit and an ABI 310, using internal primers designed after each sequencing step.

Nucleotide and deduced amino acid sequences were compared to those of other begomoviruses deposited in the GenBank (Table 1) using DNAMan version 4.0. Nucleotide identities were calculated using the following parameters: K-tuple = 2, Gap open = 10, Gap penalty = 7, and Gap extension = 5. Amino acid identities were calculated using the BLOSUM matrix with the following parameters: K-tuple = 2, Gap open = 10, Gap penalty = 4, and Gap extension = 0.1. Phylogenetic trees based on the complete nucleotide sequences of DNA-A and DNA-B were prepared using MEGA 3.1 (Kumar et al., 2004) from multiple alignments obtained with Clustal W (Thompson et al., 1994). Trees were constructed with the UPGMA method, and branches were bootstraped with 1,000 replications.

Table 1. Begomovirus sequences used for pairwise sequence comparisons and phylogenetic analysis.

Viral species	GenBank access number
Abutilon mosaic virus (AbMV)	NC_001928 (DNA-A) and NC_001929 (DNA-B)
African cassava mosaic virus (ACMV)	NC_001467 (DNA-A) and NC_001468 (DNA-B)
Bean dwarf mosaic virus (BDMV)	NC_001931 (DNA-A) and NC_001930 (DNA-B)
Bean golden mosaic virus (BGMV)[1]	NC_004042 (DNA-A) and NC_004043 (DNA-B)
Bean golden yellow mosaic virus (BGYMV)	NC_001439 (DNA-A) and NC_001438 (DNA-B)
Chino del tomate virus (CdTV)	NC_003830 (DNA-A) and NC_003831 (DNA-B)
Macroptilium mosaic Puerto Rico virus (MMPRV)	NC_004097 (DNA-A) and NC_004098 (DNA-B)
Pepper golden mosaic virus (PGMV)	NC_004101 (DNA-A) and NC_004096 (DNA-B)
Pepper huasteco yellow vein virus (PHYVV)	NC_001359 (DNA-A) and NC_001369 (DNA-B)
Potato yellow mosaic virus (PYMV)	NC_001934 (DNA-A) and NC_001935 (DNA-B)
Potato yellow mosaic Trinidad virus (PYMTV)	NC_004638 (DNA-A) and NC_004644 (DNA-B)
Sida golden mosaic virus (SGMV)	NC_002046 (DNA-A) and NC_002047 (DNA-B)
Sida golden mosaic Costa Rica virus (SGMCRV)	NC_004657 (DNA-A) and NC_004658 (DNA-B)
Sida golden mosaic Honduras virus (SGMHoV)	NC_004659 (DNA-A) and NC_004660 (DNA-B)
Sida micrantha mosaic virus (SimMV-[A2B2])[1]	NC_005330 (DNA-A) and NC_005331 (DNA-B)
Sida mottle virus (SiMoV-[A1B3])[1]	AJ557450 (DNA-A) and AJ557454 (DNA-B)
Sida yellow mosaic virus (SiYMV)[1]	NC_004639 (DNA-A)
Sida yellow vein virus (SYVV)	NC_004661 (DNA-A) and NC_004662 (DNA-B)
Squash leaf curl virus (SqLCV)	NC_001936 (DNA-A) and NC_001937 (DNA-B)
Tomato golden mosaic virus (TGMV)[1]	NC_001507 (DNA-A) and NC_001508 (DNA-B)
Tomato leaf curl New Delhi virus (TLCNDV)	NC_004611 (DNA-A) and NC_004612 (DNA-B)
Tomato chlorotic mottle virus (ToCMV -[Se1])[1]	NC_003664 (DNA-A) and NC_003665 (DNA-B)
Tomato chlorotic mottle virus (ToCMV -[Bt1])[1]	AY090557 (DNA-A)
Tomato chlorotic mottle virus (ToCMV -[Ig1])[1]	DQ336353 (DNA-A) and DQ336354 (DNA-B)
Tomato mottle virus (ToMoV)	NC_001938 (DNA-A) and NC_001939 (DNA-B)
Tomato rugose mosaic virus (ToRMV)[1]	NC_002555 (DNA-A) and NC_002556 (DNA-B)
Tomato severe rugose virus (ToSRV -[Ub2])[1]	AY029750 (DNA-A)
Tomato yellow leaf curl virus (TYLCV)	NC_004005 (monopartite)
Tomato yellow spot virus (ToYSV-[Bi2])[1]	DQ336350 (DNA-A) and DQ336351 (DNA-B)
Watermelon chlorotic stunt virus (WCSV)	NC_003708 (DNA-A) and NC_003709 (DNA-B)

[1]Viruses obtained from samples collected in Brazil.

Results and Discussion

The TGV-[Bi2] isolate was capable of systemically infecting plants of Amaranthaceae (*G. globosa*) and Solanaceae, including *C. annuum* 'Ikeda', *D. stramonium*, *L. esculentum* 'Miller Early Pack', 'Rutgers' and 'Santa Clara', *N. benthamiana*, *N. clevelandi*, *N. glutinosa*, *N. rustica*, *N. tabacum* and *N. silvestris* (Table 2). In general, symptoms induced by TGV-[Bi2] in all hosts were severe, and consisted of yellow or golden mosaic, blisters, crinkled leaves, and dwarfing (Figure 1 A, B and C). These severe symptoms were similar to those previously observed for this viral isolate (Ambrozevicius et al., 2002). *N. silvestris* and *N. tabacum* 'Havana' plants displayed a latent infection when sap-inoculated. *G. globosa* displayed a latent infection when biolistically inoculated. Infection was confirmed by PCR-amplification of a DNA-A-specific fragment (Figure 1 D).

As observed for other begomoviruses (Urbino et al., 2004; Rajeshwari et al., 2005; Rothenstein et al., 2005; Ariyo et al., 2006), a number of differences were observed between the two inoculation methods in terms of the establishment of a systemic infection. Sap inoculation was an efficient mode of transmission. All plants of *N. benthamiana*, *N. glutinosa*, *N. rustica* and *N. tabacum* 'Xhanti' were infected by sap inoculation. Interestingly, the virus could not be sap-transmitted to tomato. Latent infection was confirmed by PCR in *N. silvestris* and *N. tabacum* 'Havana', albeit at a low efficiency (only 26% of the inoculated plants were infected). The possibility of symptoms appearing later in the infection, beyond the period of evaluation used in this work, cannot be ruled out.

Efficient sap transmission has not been observed for tomato-infecting begomoviruses from Brazil such as ToRMV and ToCMoV-[Ig1] (Ambrozevicius et al., 2002; Fernandes et al., 2006), although it has been reported for ToCMoV-[Se1] (Ribeiro et al., 2007). Clearly, this property varies for each distinct begomovirus species. It is possible that TGV-[Bi2] is capable of invading the mesophyll and the epidermis of *Nicotiana* species, which could explain its sap-transmission to these hosts. Analysis of tissue tropism using immunolocalization or in situ hybridization could confirm this hypothesis.

Table 2. Plant species inoculated with Tomato yellow spot virus-[Bi2] (ToYSV-[Bi2]) in the host range experiments, and symptoms observed for each inoculation method.

Species/cultivar	Inoculation method			
	Plant sap		Biolistics	
	Infected/ inoculated[(1)]	Symptoms[(2)]	Infected/ inoculated	Symptoms
Capsicum annuum	4/15	ym, ld	12/15	ym, ld, df
Chenopodium quinoa	0/15	-	0/15	-
Cucurbita pepo 'Caserta'	0/15	-	n.i.[(3)]	n.i.
Datura stramonium	13/15	ym, ld, df	0/15	-
Gomphrena globosa	0/15	-	3/15	-
Lycopersicon esculentum 'Miller Early Pack'	0/45	-	34/45	ym, ld
L. esculentum 'Rutgers'	0/24	-	17/24	ym, ld
L. esculentum 'Santa Clara'	0/24	-	16/24	ym, ld
Nicotiana benthamiana	24/24	ym, ld, df	36/45	ym, ld
N. clevelandi	12/15	ym, ld, df	0/15	-
N. glutinosa	15/15	ym, ld, df	12/24	ym, ld, df
N. rustica	15/15	ym, ld, df	0/15	-
N. sylvestris	2/15	-	0/15	-
N. tabacum 'Havana'	6/15	-	0/15	-
N. tabacum 'Samsun'	3/15	ym, ld	3/15	ym, ld
N. tabacum 'TNN'	2/15	ym, ld, b	3/15	ym, ld, b
N. tabacum 'Xanthi'	15/15	ym	8/15	ym
Phaseolus vulgaris 'Ouro Negro'	0/15	-	0/15	-
P. vulgaris 'Pérola'	0/15	-	0/15	-
Solanum melongena	0/15	-	0/15	-

[(1)]Total number of plants inoculated with ToYSV-[Bi2] in three independent experiments. [(2)]Symptoms in systemic (noninoculated) leaves are represented by: b, blisters; df, dwarfing; ld, leaf distortion; ym, yellow mosaic; -, no symptoms during the evaluation period; infections were visually assayed at 30 days postinoculation, and confirmed by PCR with universal begomovirus primers. [(3)]Noninoculated.

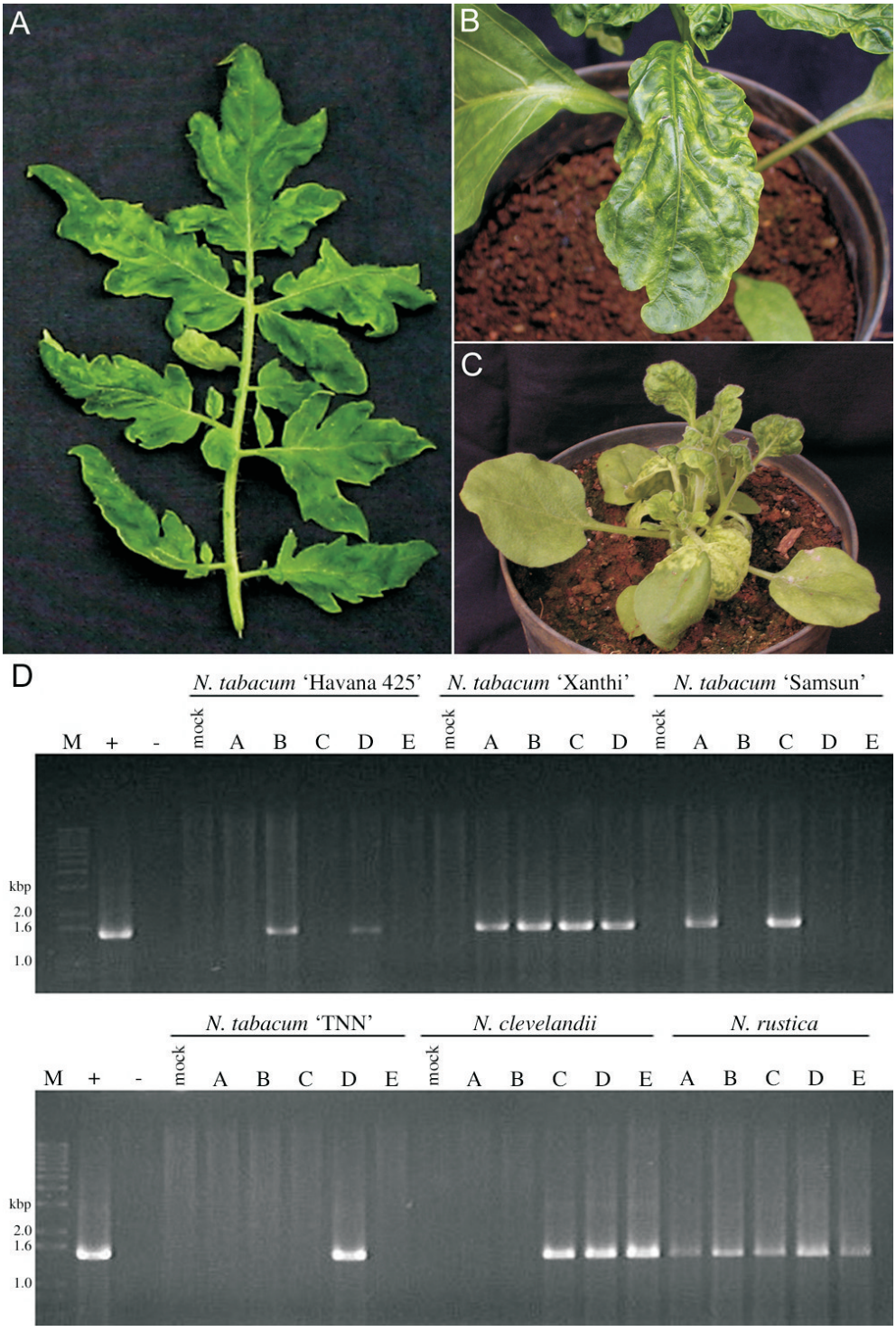

Figure 1. Symptoms induced in tomato (A), sweet pepper (B) and *Nicotiana benthamiana* (C), after biolistic inoculation with Tomato yellow spot virus-[Bi2] (ToYSV-[Bi2]). D: PCR-amplification of a DNA-A-specific fragment from plants sap-inoculated with ToYSV-[Bi2]. M, DNA ladder; **+**, amplification from a *N. benthamiana* plant infected with ToYSV-[Bi2]; **-**, amplification from a mock-inoculated tomato plant.

When plants were biolistically inoculated, a systemic infection was established in *N. benthamiana*, *N. glutinosa*, *N. tabacum*, sweet pepper, tomato and *G. globosa*. In contrast, plants of *D. stramonium*, *N. clevelandi*, *N. rustica* and *N. silvestris* were systemically infected only when sap-inoculated.

It is noteworthy that sweet pepper is a host for TGV-[Bi2] and displays severe symptoms upon infection. Furthermore, the common weed *D. stramonium* was also infected by TGV-[Bi2] and could serve as a virus reservoir in the field.

In summary, the host range of TGV-[Bi2] is similar to that of ToRMV and ToCMoV in which it is restricted to the Solanaceae. ToCMoV also infects important crop species such as sweet pepper and tobacco, as well as *D. stramonium* (Ambrozevicius et al., 2002; Ribeiro et al., 2007). ToRMV infects potato, tobacco, *D. stramonium* and *Nicandra physaloides*, but does not infect sweet pepper (Fernandes et al., 2006).

The complete genome of TGV-[Bi2] was amplified using the DNA polymerase of phage φ29 (Figure 2 A). Restriction analysis indicated that *Sac* I and *Xba* I cleaved both DNA components at a single site (Figure 2 B), and therefore these enzymes were used for cloning. Recombinant plasmids containing inserts of approximately 2,600 nt, corresponding to full-length DNA-A and DNA-B, were obtained (Figure 2 C).

The complete nucleotide sequences of the DNA-A and DNA-B of TGV-[Bi2] are 2,674 and 2,626 nt long (GenBank accession numbers DQ336350 and DQ336351, respectively). Both components have all the typical features of bipartite, New World begomoviruses, including six open reading frames (four in DNA-A, two in DNA-B) and a common region with approximately 200 nt, containing the origin of replication and the conserved elements (iterons) required for origin recognition by the Rep protein.

Sequence comparisons with other begomoviruses indicated a maximum sequence identity for the complete nucleotide sequence of the DNA-A of 87% with SiMoV, 83% with SiYMV and 81% with SimMV (Table 3). The DNA-B has a maximum nucleotide sequence identity of 79% with SiMoV and 66% with SimMV. Amino acid sequence identities calculated for each viral protein indicated a maximum identity with SiMoV for all proteins, ranging from 86% for the Rep and Ren proteins to 96% for the capsid protein (Table 3). According to current ICTV criteria for the demarcation of begomovirus species (Stanley et al., 2005), these results indicate that TGV-[Bi2] constitutes a novel begomovirus species, hereby named Tomato yellow spot virus (ToYSV-[Bi2]), since it displayed less than 89% nucleotide sequence identity for the DNA-A with other viruses.

Phylogenetic trees based on the complete nucleotide sequences of the DNA-A and DNA-B are presented in Figure 3. Both trees clearly separate begomoviruses on

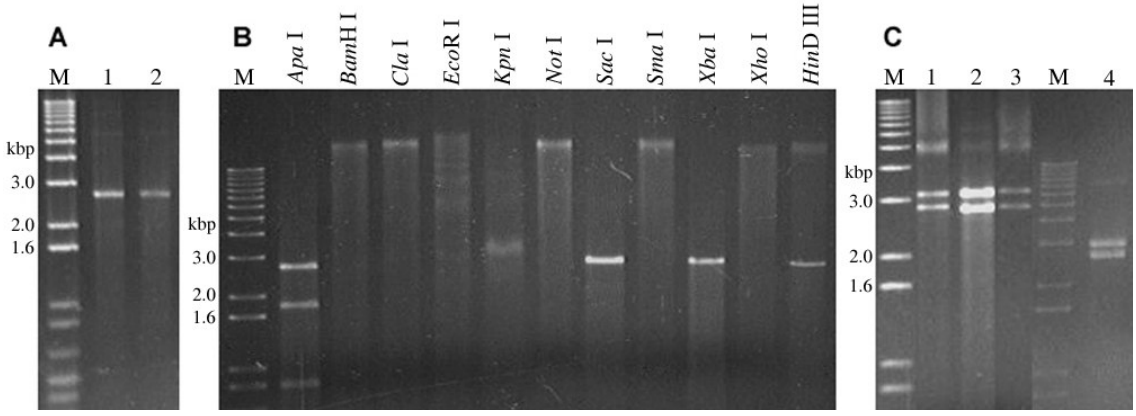

Figure 2. A: amplification of the full-length DNA components of Tomato yellow spot virus-[Bi2] (ToYSV-[Bi2]), using the DNA polymerase of phage φ29; M: DNA ladder; 1–2: amplification of full-length DNA components (~2.6 kbp) from an infected *Nicotiana glutinosa* plant. B: restriction analysis of amplified full-length DNA components. Bands migrating slower than 2.6 kbp correspond to noncleaved DNA components. Bands migrating at approximately 2.6 kbp correspond to DNA components cleaved at a single site. Bands migrating faster than 2.6 kbp correspond to fragments of DNA components cleaved at least twice. The enzymes *Sac* I and *Xba* I linearized both DNA components. M: DNA ladder. C: recombinant plasmids containing inserts corresponding to the complete DNA-A and DNA-B. M: DNA ladder; 1–3, recombinant plasmids cleaved with *Sac* I; 4, recombinant plasmid cleaved with *Xba* I.

Table 3. Nucleotide and amino acid sequence identities between Tomato yellow spot virus-[Bi2] (ToYSV-[Bi2]) and some closely related begomoviruses[1].

Viral species	Percent identities with ToYSV-[Bi2][2]							
	DNA-A	DNA-B	CP	Rep	Trap	Ren	MP	NSP
Sida mottle virus (SiMoV-[A1B3])	87	79	96	86	88	86	94	87
Sida yellow mosaic virus (SiYMV)	83	n.a.[3]	89	75	73	72	n.a.	n.a.
Sida micrantha mosaic virus (SimMV-[A2B2])	81	66	95	84	76	76	80	78
Tomato severe rugose virus (ToSRV-[Ub2)	78	n.a.	90	79	71	78	n.a.	n.a.
Tomato rugose mosaic virus (ToRMV)	77	63	90	80	71	78	78	73
Tomato golden mosaic virus (TGMV)	76	62	89	80	69	80	84	72
Tomato chlorotic mottle virus ToCMoV-[BA-Se1])	75	60	90	74	68	77	77	70
Bean golden mosaic virus (BGMV)	75	62	92	77	74	77	79	73

[1]CP, coat protein; Rep, replication-associated protein; Trap, trans-activating protein; Ren, replication enhancer protein; MP, movement protein; NSP, nuclear shuttle protein. [2]Nucleotide sequence identities for DNA-A and DNA-B, deduced amino acid sequence identities for the viral proteins. [3]Sequence not available.

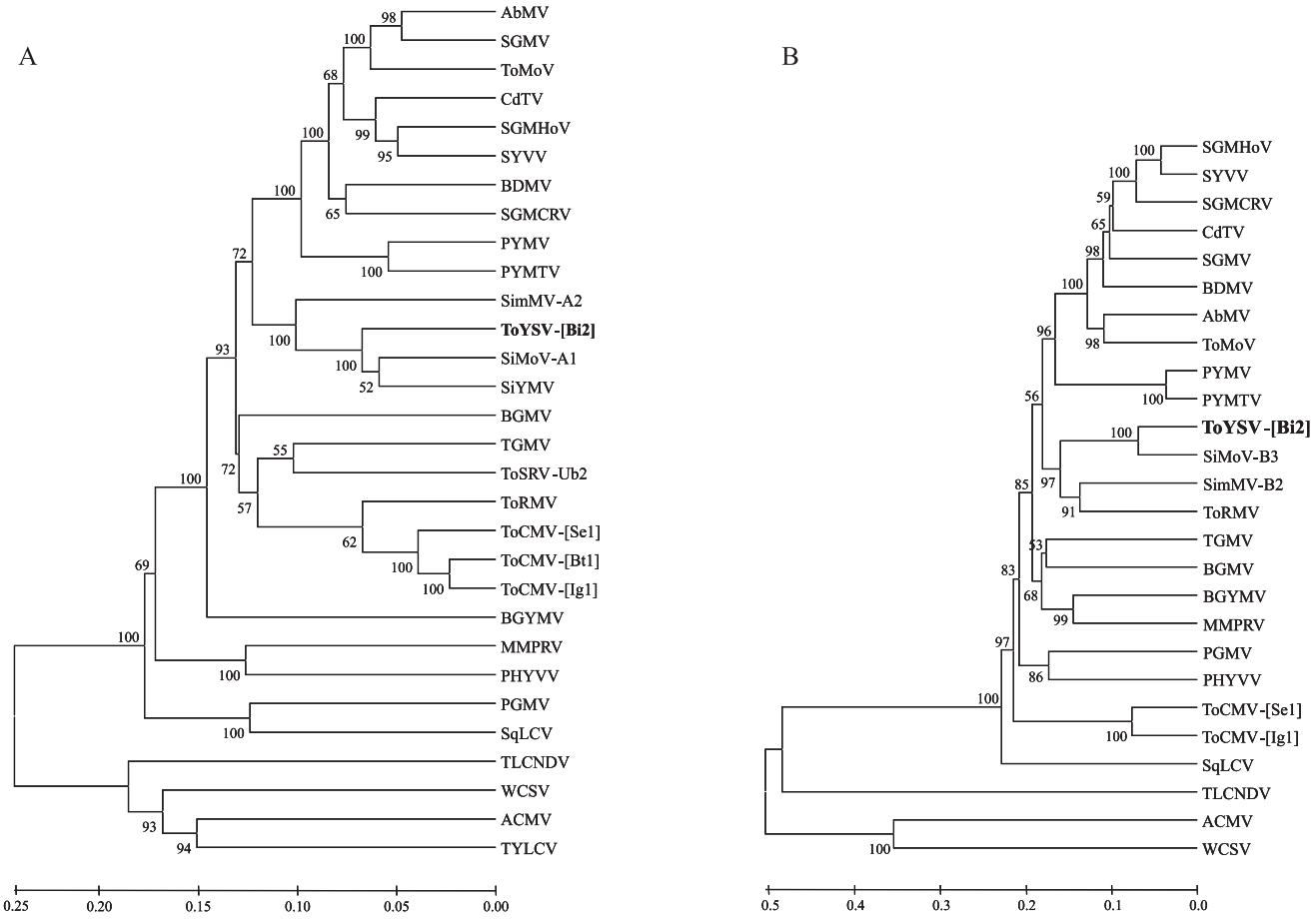

Figure 3. Phylogenetic tree based on a multiple sequence alignment of the complete DNA-A (A) and DNA-B (B) of selected New World begomoviruses, using the UPGMA method. Branches were bootstrapped with 1,000 replications. Acronyms and accession numbers are in Table 1. The scale indicates the number of substitutions per site.

the basis of geographical location, with Brazilian viruses clustered together. In the tree based on DNA-A, ToYSV-[Bi2] is located in a monophyletic branch with 100% bootstrap value including the three *Sida* begomoviruses from Brazil, SimMV, SiMoV and SiYMV. In the tree based on DNA-B, ToYSV-[Bi2] is located in a monophyletic branch with 97% bootstrap value including SimMV, SiMoV and ToRMV (there is no DNA-B sequence available for SiYMV). Together, these results reinforce those of sequence comparisons, indicating a close relationship between ToYSV and the Brazilian *Sida*-infecting begomoviruses.

The hypothesis to explain the sudden and rapid emergence of tomato-infecting begomoviruses in Brazil assumes that indigenous viruses were transferred from wild hosts to tomatoes after the introduction and dissemination of the B biotype of *B. tabaci*. The new vector allowed these indigenous viruses to reach a new host, where mechanisms of recombination and pseudorecombination (reassortment of genomic components) generated novel species with greater fitness to the new host (Inoue-Nagata et al., 2006; Ribeiro et al., 2007). On the basis of this close relationship with *Sida*-infecting viruses, its severe phenotype in tomatoes and its low relative incidence in the field, it is tempting to speculate that ToYSV could be a "missing link" between an indigenous *Sida*-infecting virus, such as SiMoV (which has never been detected in tomatoes), and the more widespread (and therefore better adapted) tomato-infecting begomoviruses, such as ToRMV and ToCMoV. Alternatively, it could have arisen by recombination involving one of the *Sida*-infecting viruses. Recombination has been proposed to play a major role in the emergence and evolution of tomato-infecting begomoviruses in Brazil (Ribeiro et al., 2007). Initial attempts to verify the role of recombination in the origin of ToYSV led to inconclusive results. The identification and molecular characterization of additional begomovirus species may be necessary before such analysis yields more definitive results.

Conclusions

1. The host range of ToYSV includes several solanaceous plants, with economically important crop plants as sweet pepper, and common weeds such as *Datura stramonium*.

2. Unlike other tomato-infecting begomoviruses from Brazil, ToYSV is readily sap-transmitted to some hosts such as *Nicotiana benthamiana*, but not to tomato.

3. Based on sequence comparisons and phylogenetic analysis, and in accordance with current ICTV criteria, ToYSV is a distinct begomovirus species.

4. ToYSV is phylogenetically closer to viruses infecting the weed *Sida* sp. than to viruses infecting tomato.

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