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Identification, molecular characterization and relative incidence of begomoviruses infecting bean crops in northwestern Argentina: an update

Gonzalo Varela[1] · Vera Ávalos[2] · Pablo Reyna[3,4] · Irma G. Laguna[4] · Patricia Rodriguez Pardina[3]

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Abstract
Three begomoviruses were identified in bean crops in northwestern Argentina: *Bean golden mosaic virus* (BGMV), *Tomato yellow spot virus* (ToYSV) and *Soybean blistering mosaic virus* (SbBMV). Nucleic acid hybridization probes specific for these three begomoviruses were developed, and used to assay field samples. As some of the analysed begomovirus-infected samples did not react with any of the specific probes, we suspected that other viruses were present. DNA of two of these samples P160, and PRCA4, was amplified by rolling circle amplification (RCA), and cloned. Four clones were sequenced by primer walking and nucleotide comparisons established that clones P160*Sac*I and P160*Apa*I shared 93 and 94% nucleotide identity with DNA-A and DNA-B of *Sida golden mosaic Brazil virus* (SiGMBRV), respectively. Clone PRCA4.6*Pst*I had 94% sequence identity with DNA-A of *Tomato mottle wrinkle virus* (ToMoWV), and clone PRCA4.17*Pst*I exhibited 95% nucleotide sequence identity with the DNA-A of *Tomato yellow vein streak virus* (ToYVS), indicating a mixed infection. Specific probes were designed using part of the common region of the viral genome of the newly identified begomoviruses and were used to test bean samples during the 2014–2016 growing seasons. ToMoWV was the most frequently detected virus. The incidence of the three previously characterized begomoviruses (BGMV, SbBMV and ToYSV) was also assessed, and BGMV was found to exhibit the highest incidence. The diversity of begomoviruses found in bean in our country is remarkable, since, six different species have been detected up to the present.

Keywords *Phaseolus vulgaris* · *Tomato mottle wrinkle virus* · *Tomato yellow vein streak virus* · *Sida golden mosaic Brazil virus* · ,

Introduction

Geminiviruses comprise a family of plant viruses (*Geminiviridae*) with a single stranded DNA (ssDNA) genome, which can have one or two components ranging between 2.5 and 3 kb in size. Each component is encapsidated in a geminated (twinned) incomplete icosahedral particle (Brown et al. 2012). This family is actually divided into nine genera, according to the organization of their genome, insect vector, host range and phylogeny: *Becurtovirus, Begomovirus*, *Capulovirus, Curtovirus, Eragrovirus, Grablovirus, Mastrevirus, Topocuvirus* and *Turncurtovirus*. With more than 322 species, *Begomovirus* is the largest genus of plant viruses (Zerbini et al. 2017). Begomoviruses are transmitted by whitefly (*Bemisia tabaci*) (*Hemiptera Aleyrodidae*) and infect a vast variety of dicotyledonous species, mostly in tropical and subtropical regions of the world, often causing severe damage to economically important crops, such as tomato, cassava, cotton and bean (Navas-Castillo et al. 2011; Brown et al. 2012; Inoue-Nagata et al. 2016). Most begomoviruses have a bipartite genome, consisting of two ssDNA, molecules of 2.5–2.7 kb, known as DNA-A and DNA-B. In contrast, there are some monopartite begomoviruses containing only one genome component that is homologous to the DNA-A of bipartite begomoviruses. Most of the begomoviruses found in the Old World (OW-Europe, Africa, Asia, and Oceania) are monopartite, whereas

✉ Patricia Rodriguez Pardina
rodriguez.patricia@inta.gob.ar

[1] INICSA-CONICET Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, UNC, Córdoba, Argentina

[2] Laboratorio Regional de Investigación Forense-Zona Sur Poder Judicial de Santa Cruz Pellegrini, 415 (9400) Río Gallegos, Santa Cruz, Argentina

[3] Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Camino 60 cuadras Km 5,5, X5020ICA Córdoba, Argentina

[4] Concejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290 (1425 FBQ) CABA, Buenos Aires, Argentina

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those from the New World (NW-America) are bipartite. Genes in the DNA-A of bipartite begomoviruses encode proteins involved in the replication of viral genome, regulation of gene expression, particle encapsidation and suppression of host defences, whereas genes in the DNA-B encode two proteins: the nuclear shuttle protein (NSP), responsible for carrying out the DNA from the nucleus, and the movement protein (MP), involved in transporting the viral genome from one cell to another through plasmosdesmata. The NSP also acts as a suppressor of host defences, while both of them define the viral host range. All geminiviruses have a region of approximately 200 nucleotides known as the intergenic region for monopartite viruses and as common region (CR) in those with bipartite genomes. This region contains *cis*-acting signals required for DNA replication and transcription (Jeske 2009; Hanley-Bowdoin et al. 2013).

The common dry bean (*Phaseolus vulgaris* L.) is the second crop in importance within legumes after soybean. World bean production has grown in the last 50 years. In 1960, 11 million tons were produced while at present the production is approximately 30 million metric tons. The Indian subcontinent (Bangladesh, India and Pakistan) harvests the fourth part of the world's bean production being India, the world's leading producer. On the other hand almost one fifth of the world's bean is produced in South America (18–19%), in this region the main producer is Brazil that harvested 3,294,586 tons in 2014, making this country the second largest producer on a global scale. In Argentina, beans are produced mainly in the subtropical northwestern region (provinces of Tucumán, Salta, Jujuy, Santiago de Estero and Catamarca). During the 2014 growing season, 350,150 ha were harvested in the country, with a production of 429,832 tons (De Bernardis 2014; FAOSTAT 2014).

Several begomovirus species have been identified from naturally infecting beans. *Bean golden mosaic virus* (BGMV) and *Bean golden yellow mosaic virus* (BGYMV) cause bean golden mosaic, one of the most important diseases of bean crops in South America and Central America/Caribbean Basin (Karkashian et al. 2010; Wyant et al. 2012). *Bean dwarf mosaic virus* (BDMV) has been detected throughout Latin America, but although it is currently found to have a low incidence, this virus should consider as a potential risk (Morales and Anderson 2001). Other bipartite or even monopartite begomoviruses, such as bean yellow chlorosis virus (BYCV), bean white chlorosis mosaic virus (BWCMV), *Macroptilium mosaic Puerto Rico virus* (MacMPRV), *Macroptilium yellow mosaic Florida virus* (MacYMFV), pepper leafroll virus, *Tomato chlorotic spot virus*, *Tomato yellow leaf curl virus*, *Tomato yellow leaf curl Malaga virus* and *French bean leaf curl virus* have also been reported infecting bean crops (Idris et al. 2003; Monci et al. 2005; Fiallo-Olivé et al. 2013; Kamaal et al. 2013; Martínez-Ayala et al. 2014; Shahid and Natsuaki 2014; González-Alvarez et al. 2017).

So far, three begomoviruses have been described infecting bean crops in Argentina: BGMV, *Soybean blistering mosaic virus* (SbBMV) and *Tomato yellow spot virus* (ToYSV) (Rodríguez Pardina et al. 2006.; Rodríguez Pardina et al. 2011), but due to previous studies (Alemandri et al. 2012), the presence of other viral species is suspected. Here we report the detection of three begomoviruses, previously unreported in bean and the survey of bean crops of the northwestern region of Argentina, using specific probes developed for each of the detected viruses.

Materials and methods

Plant samples

One hundred sixty seven symptomatic bean samples were collected from different localities of Salta and Jujuy provinces during the 2010 growing season. During the 2013 growing season, a trial of different cultivars located in Leales (Tucumán) was evaluated and 330 samples of the different cultivars were collected. In both cases, apical young leaves were maintained at −20 °C, until analysis.

Sample analysis

All samples were tested with a general probe that detects all begomovirus species, according to the protocol previously described (Rodríguez Pardina et al. 2011). Dot-blot hybridization analysis was conducted under low stringency conditions (hybridization at 65 °C, followed by two washing steps for 5 min at room temperature with 5× SSC and 0.1% SDS, and two for 15 min at 65 °C with 2× SSC and 0.1% SDS). The samples that reacted positively with the general probe were later analysed with the specific probes for the detection of BGMV, SbBMV and ToYSV. This analysis was conducted under high stringency conditions: hybridization at 65 °C followed by two washing steps for 5 min at room temperature with 2.5× SSC and 0.1% SDS, and two for 15 min at 65 °C with 1× SSC and 0.1% SDS (Rodríguez Pardina et al. 2011).

Rolling circle amplification (RCA), cloning, sequence comparisons and phylogenetic analysis

Nine samples (seven from 2010 and two from 2013) that tested positive for the general probe but negative for the specific ones were selected for further analysis. Total DNA was extracted by the CTAB method (Doyle and Doyle 1987) and amplified by RCA using TempliPhi kit (GE Healthcare), (Inoue-Nagata et al. 2004). Unit length genomes were excised with *Pst*I, *Apa*I, *Xba*I, *Bam*HI, *Cla*I, *Eco*RV, *Hind*III or *Cla*I restriction enzymes. Linearized DNA was further cloned in pBluescript SK+ plasmid vector, digested with the same

restriction enzyme and transformed into *Escherichia coli* DH5α. Viral inserts were sequenced at the Genomic Unit of the Biotechnology Institute-INTA (Argentina).

Sequences were compared with those of other begomoviruses available in the GenBank (www.ncbi.nlm.nih.gov). Database searches were performed using blastn algorithm (Altschul et al. 1990). Pairwise sequence comparison was carried out using Sequence Demarcation Tools version 1.2 (SDT v1.2) (Muhire et al. 2014), with the MUSCLE alignment option (Edgar 2004). Maximum Likelihood (ML) phylogenetic trees were constructed with MEGA 5.2 program (Tamura et al. 2011), using the GTR + I + G nt substitution model and 3000 bootstrap iterations.

Development of DNA probes for specific virus detection by molecular hybridization

Since the CR is the least conserved region among begomovirus genomes, probes specific for the newly identified viruses were prepared by polymerase chain reaction (PCR) based on their common region. PCR reactions were done in a final volume of 25 μl with 1× Taq DNA polymerase buffer, 0.25 mM $MgCl_2$, 0.25 mM of dNTP mix, 2.5 μM of each primer, 2.5 μl (150 to 500 ng) of template DNA and 0.2 units of *Taq* DNA polymerase (Promega Corporation, Madison, Wisconsin, USA). Amplification conditions were as follows: an initial denaturation at 94 °C for 2 min, and 35/40 cycles of denaturation at 94 °C for 30 s, annealing for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. The annealing temperature was adjusted for each pair of primers.

The probes were prepared with the DIG-Labelling and detection kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer's instructions. In all cases, the previously described high stringency conditions were used (Rodríguez Pardina et al. 2011).

Survey of bean crops in the northwestern region of Argentina

During 2014, 2015 and 2016 growing seasons, bean samples with viral symptoms were collected in different localities of Salta, Santiago del Estero and Tucumán provinces. All samples were analysed by Dot-blot hybridization using the general probe under low stringency conditions. The samples that tested positively with the general probe were later analysed with the specific probes, which were designed to detect BGMV, ToYSV, SbMV and the newly identified viruses. The results were statistically analysed using a generalized linear model, with a binomial distribution (Diggle et al. 2002).

Results

Sample analysis

Of the 147 symptomatic bean samples assessed during the 2010 growing season, 48.9% were infected with begomovirus, according to the results obtained with the general probe (Table 1). Fifty seven percent of the samples were infected with BGMV, whereas 0.6% showed to be infected with ToYSV, 17.7% with SbBMV and 24.7% tested negative for all the three evaluated viruses. Of the 330 samples from the cultivar trial, 28% tested positive for begomoviruses when analysed with the general probe. Of these samples, 8.6% were positive for BGMV, 14.6% for ToYSV and 35.5% for SbBMV. Thus, 41.3% of the begomovirus-positive samples from the 2013 cultivar trial tested negative for the three evaluated viruses. Nine samples (seven from 2010 and two from 2013) that tested positive for the general probe but negative for the specific ones were selected for further analysis.

Rolling circle amplification, cloning, sequence comparisons and phylogenetic analysis

Nineteen clones were obtained from the nine analysed samples and were partially sequenced. Surprisingly, although all the plant samples tested negative with the specific probe, eight of the clones showed more than 90% of homology with the BGMV (GenBank accession number M88686), indicating the presence of this virus. Clones P1608C, P1607C, linearized with *Sac*I, and P1615A, linearized with *Hind*III, showed 93% of homology with DNA-A of *Sida golden mosaic Brazil virus* (SiGMBRV) (GenBank accession number FN436001), whereas clone P16034 (*Apa*I) had 94% homology with DNA-B of the same virus (FN436002). Furthermore, clone P98 7B (EcoRV) showed 93% homology with DNA B of *Solanum mosaic Bolivia virus* (SoMBoV) (GenBank accession number NC_024305).

Table 1 Detection of begomoviruses by molecular hybridization using general ("begomovirus") probes in bean crops in northwestern Argentina during the 2010 growing season

Locality	Province	Positive samples/ analysed samples	%
Las Varas	Salta	27/32	84.38
Tartagal	Salta	32/64	50
Güemes	Salta	0/3	0
Rosario de Lerma	Salta	1/1	100
Joaquin V. González	Salta	0/5	0
San Pedro	Jujuy	1/10	10
Fte. Santa de Murcia	Jujuy	11/32	34.37

Finally, clones PCRCA4.6, PRCA4.11, PRCA4.18, PRCA4.17, PRCA2.7 and PRCA2.11 from samples of the cultivar trial of Tucumán, showed more than 90% of homology with DNA-A of *Tomato mottle wrinkle virus* (ToMoWV) (accession number KM243020) or of *Tomato yellow vein streak virus* (ToYVSV) (accession number KC136337) (Table 2). Clones P1608C, P16034, PRCA4.6 and PRCA 4.17 were completely sequenced.

Clones P1608C, PRCA4.6 and PRCA4.17 exhibited the typical genome organization of DNA-A of the Western Hemisphere bipartite begomoviruses with five open reading frames (ORFs). One ORF, encoding the coat protein (CP), was located on the positive or virion sense, whereas ORFs encoding Rep, TrAP, REn and AC4 proteins were located on the complementary sense strand. Size of the DNA-A is 2664 nt for P1608C (GenBank accession number KY555798), 2565 nt for PRCA4.6 (ToMoWV) (accession number KY555800) and 2567 nt for PRCA4.17 (ToYVSV) (GenBank accession number KY555801). Clone P16034 was determined to be 2603 nt long (GenBank accession number KY555799) and showed, as in all DNA-B components, two ORFs, one on the viral sense strand (BV1/NSP) and the other on the complementary sense strand (BC1/MP).

The phylogenetic trees of DNA-A nucleotide sequences showed that each of the three isolates clustered with other previously sequenced isolates of the respective species: SiGMBRV (P1608C) ToMoWV (PRCA4.6) and ToYVSV (PRCA4.17). The three bean infecting begomoviruses were placed in two distinct monophyletic clusters, one including SiGMBRV and BGMV, and the other including SbBMV, ToMoWV and ToYVSV (Fig. 1).

Development of DNA probes for specific virus detection by molecular hybridization

Three pairs of primers were designed to amplify part of the common region of the SiGMBRV, ToMoWV and ToYVSV bean isolates (Table 3). PCR reactions were performed as described above, at the following annealing temperatures: 50 °C for SiGMBRV isolate, 55 °C for ToYVSV, and 54 °C for ToMoWV. All the three specific probes reacted only with their homologous viral targets and did not yield a noticeable hybridization signal from asymptomatic bean samples.

Survey of bean crops in northwestern Argentina

A total of 279 bean samples showing virus-like symptoms were analysed using molecular hybridization. The analysis using the general probe yielded 42% (119) positive samples. ToMoWV was the most frequently detected virus, with 37 (31%) of the samples being infected with this virus, whereas 23 (19.3%) were infected with BGMV, 19 (15.9%) with ToYSV, 12 (10.1%) with SiGMBRV, 7 (5.8%) with SbBMV, and 7 (5.8%) with ToYVSV (Table 4). The analysis of the results focused on the year showed statistically significant differences for SiGMBRV and ToMoWV (*p*-values 0.0056 and 0.0001, respectively), with the relative incidence of SiGMBRV being higher during 2014 and for

Table 2 Description of the sequenced clones, origin of the samples and detected virus

Sample	Clone	Province	Locality	Virus
P49	P 49 (1 ClaI)	,Salta	Tartagal	BGMV (DNA-A)
	P51 (5A EcoRV)	Salta	Tartagal	BGMV (DNA-A
P51	P51 (9A EcoRV)	Salta	Tartagal	BGMV (DNA-A)
	P51(11 A EcoRV)	Salta	Tartagal	BGMV (DNA-A)
P54	P54 (26 BglII)	Salta	Tartagal	BGMV (DNA-A)
P98	P98 (4B EcoRV)	Salta	Tartagal	BGMV (DNA-A)
	P98 (7B EcoRV)	Salta	Tartagal	SoMBoV (DNA-B)
P147	P147 (7B HindIII)	Jujuy	El Chaguaral	BGMV (DNA-A)
	P160 (34 ApaI)	Jujuy	Fuente Santa de Murcia,	SiGMBRV (DNA-B)
P160	P160 (7C SacI)	Jujuy	Fuente Santa de Murcia,	SiGMBRV (DNA-A)
	P160 (8C SacI)	Jujuy	Fuente Santa de Murcia,	SiGMBRV (DNA-A)
P161	P161 (5A HindIII)	Jujuy	Fuente Santa de Murcia,	SiGMBRV (DNA-A)
	P161 (7AHindIII)	Jujuy	Fuente Santa de Murcia,	BGMV (DNA-A)
	PRCA4.6 (PstI)	Tucumán	Leales	ToMoWV (DNA-A)
PRCA4	PRCA4.11 (PstI)	Tucumán	Leales	ToMoWV (DNA-A)
	PRCA4.18 (PstI)	Tucumán	Leales	ToMoWV (DNA-A)
	PRCA4.17 (PstI)	Tucumán	Leales	ToYVSV (DNA-A)
PRCA2	PRCA2.7 (XbaI)	Tucumán	Leales	ToYVSV (DNA-A)
	PRCA2.11 (XbaI)	Tucumán	Leales	ToYVSV (DNA-A)

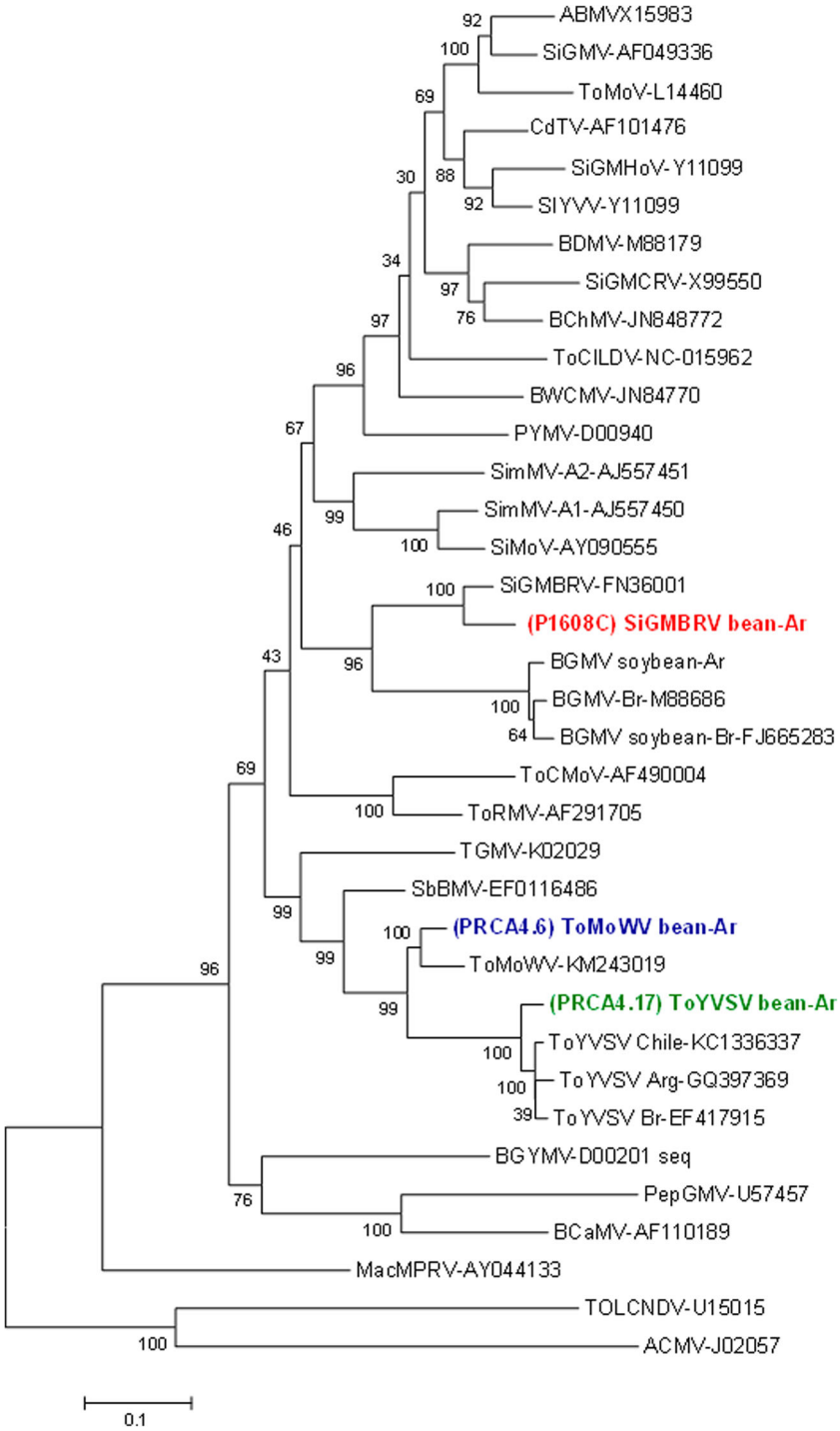

Fig. 1 Phylogenetic tree based on the complete DNA-A nucleotide sequences of the ToYVSV, ToMoWV and SiGMBRV bean isolates and other selected begomoviruses. Bootstrap values (3000 replicates) are indicated at nodes. AbMV, *Abutilon mosaic virus*; ACMV, *African cassava mosaic virus*; BDMV, *Bean dwarf mosaic virus*; BGMV, *Bean golden mosaic virus;* BGYMV, *Bean golden yellow mosaic virus*; BCaMV, *Bean calico mosaic virus*; BChMV, *Bean chlorotic mosaic virus*; BWCMV, *Bean white chlorosis mosaic virus*; CdTV, *Chino del tomate virus*; MacMPRV, *Macroptilium mosaic Puerto Rico virus*; PepGMV, *Pepper golden mosaic virus*; PHYVV, *Pepper huasteco yellow vein virus*; PYMV, *Potato yellow mosaic virus*; PYMTV, *Potato yellow mosaic Trinidad virus;* SbBMV, *Soybean blistering mosaic virus*; SiGMBRV, *Sida golden mosaic Brazil virus*; SiGMV, *Sida Golden mosaic virus;* SiGMHoV, *Sida golden mosaic Honduras virus*; SiGMCRV, *Sida Golden mosaic Costa Rica virus*; SiMMV, *Sida micrantha mosaic virus*; SiMoV, *Sida mottle virus*; SiYVV, *Sida yellow vein virus*; SLCuV, *Squash leaf curl virus*; TGMV, *Tomato golden mosaic virus*; ToClLDV *Tomato chlorotic leaf distortion virus* ToCMoV, *Tomato chlorotic mottle virus*; ToLCNDV, *Tomato leaf curl New Delhi virus*; ToMoV, *Tomato mottle virus;* ToMoWV, *Tomato mottle wrinkle virus*; ToRMV, *Tomato rugose mosaic virus;* ToYVSV, *Tomato yellow vein streak virus*

Table 3 Primers designed to amplify by PCR part of the common region of bean isolates of *Sida golden mosaic Brazil virus, Tomato mottle wrinkle virus* and *Tomato yellow vein streak virus*

Virus	Primer	Primer sequence	Fragment length
SiGMBRV	Sonda 7cFw	5' GCGCGATCTTCTTTTCC 3′	172 pb
	Sonda 7c Rv	5' CCGCTTTGGCATTCTGAA 3'	
ToMoWV	ToMoWV Fw	5'GGCTCAAATAGACACGTGG 3′	177 pb
	ToMoWV Rv	5'CGACCACTAACGCATGTCC 3′	
ToYVSV	ToYVSV Fw	5'GATGGCCGCGCGATTTT 3'	171 pb
	ToYVSV Rv	5'GGGGSATCGCSCTTAGGCA 3′	

Table 4 Detection of begomoviruses in bean crops from 2014 to 2016 growing seasons by molecular hybridization using general (Begomovirus) and virus-specific (SiGMBRV, ToYVSV, ToMoWV, BGMV, SbBMV and ToYSV) probes

Year	Province	Locality	begomovirus		SiGMBRV		ToYVSV		ToMoWV		BGMV		SbBMV	ToYSV		
			Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
2014	Salta	Padre Lozano	11/12	91.6	7/11	63.6	n/d	n/d	n/d	n/d	0/11	0	2/11	18.2	3/11	27.3
		Las Varas	7/7	100	0/7	0	1/7	14	6/7	86	6/11	86	1/11	14.3	4/11	57
		Grl Ballivian	0/3	0	0	0	0	0	0	0	0	0	0	0	0	0
		Grl Ballivian	0/4	0	0	0	0	0	0	0	0	0	0	0	0	0
		Grl Ballivian	2/14	14.3	1/2	50	1/2	50	1/2	50	1/2	50	2/2	100	0/2	0
		Embarcación	5/8	62.5	1/5	20	2/5	40	4/5	80	0/5	0	0/5	0	0/5	0
		Embarcación	1/5	20	0/1	0	0/1	0	0/1	0	0/1	0	0/1	0	0/1	0
	Tucumán	Trancas	1/10	10	0	0	n/d	n/d	n/d	n/d	1/1	100	1/1	100	1/1	100
		Trancas	3/18	16.7	1/3	33.3	0/3	0	3/3	100	1/3	33	1/3	33	2/3	66
		Zárate	1/12	8.3	0/1	0	0/1	0	1/1	100	1/1	100	0/1	0	1/1	100
2015	Salta	La Candelaria	4/8	50	0/4	0	0/4	0	3/4	75	1/4	25	0/4	0	1/4	25
		Los Mogotes	0/4	0	0	0	0	0	0	0	0	0	0	0	0	0
		Tartagal	8/10	80	1/8	12.5	0/8	0	2/8	25	0/8	0	0/8	0	1/8	12.5
		Tartagal	1/5	20	0/1	0	0/1	0	1	100	0/1	0	0/1	0	0/1	0
		Las Varas	7/8	87.5	0/7	0	1/7	14.3	5/7	71.4	7/7	100	0/7	0	2/7	28.6
	Tucumán	Trancas	1/8	12.5	0/1	0	1	100	1	100	0/1	0	0/1	0	1/1	100
		Trancas	1/6	16.6	0/1	0	1	100	1	100	1/1	100	0/1	0	1/1	100
		Zárate	0/8	0	0	0	0	0	0	0	0	0	0	0	0	0
		Zárate	2/8	25	0/2	0	0/2	0	2/2	100	0/2	0	0/2	0	2/2	100
2016	Salta	Rosario de la Frontera	3/4	75	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0
		Grl Ballivian	1/5	20	0/1	0	0/1	0	0/1	0	0/1	0	0/1	0	01/	0
		Coronel Cornejo	7/12	58.3	0/7	0	0/7	0	1/7	13.3	4/7	57.1	0/7	0	0/7	0
	Tucumán	Taco Ralo	7/12	58.3	0/7	0	0/7	0	0/7	0	0/7	0	0/7	0	0/7	0
		n/d	4/4	100	0/4	0	0/4	0	0/4	0	0/4	0	0/4	0	0/4	0
		Trancas	14/15	93.3	1/14	7.1	0/14	0	1/14	7.1	0/14	0	0/14	0	0/14	0
		Trancas	4/8	50	0/4	0	0/4	0	1/4	25	0/4	0	0/4	0	0/4	0
		Zárate	9/10	90	0/9	0	0/9	0	2/9	22.2	0/9	0	0/9	0	0/9	0
		San Fernando	7/11	63.6	0/7	0	0/7	0	0/7	0	0/7	0	0/7	0	0/7	0
	Santiago del Estero	Charco	8/40	20	0/8	0	0/8	0	2/8	25	0/8	0	0/8	0	0/8	0

Reference: n/d = No data

ToMoWV in 2014 and 2015 growing seasons (Fig. 2). When the data were analysed by provinces, a significantly higher relative incidence was found for ToMoWV (p-value 0.0325) and BGMV (p-value 0.0185) in Salta province (Fig. 3).

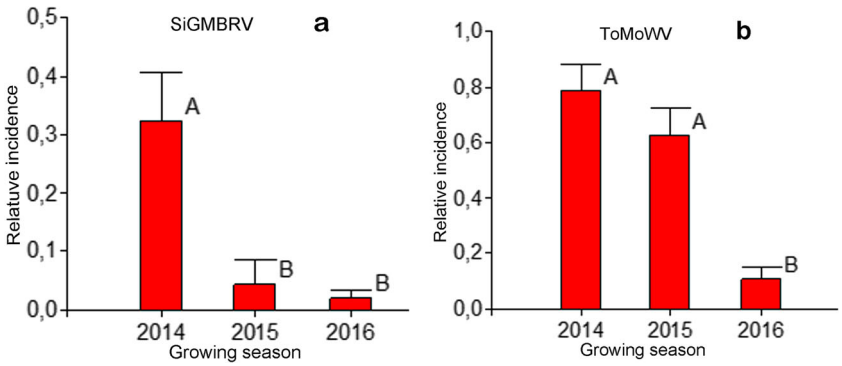

Fig. 2 Average incidence of (a) *Sida golden mosaic Brazil virus* (SiGMBRV) and (b) *Tomato mottle winkle virus* (ToMoWV) during the 2014–2016 growing seasons

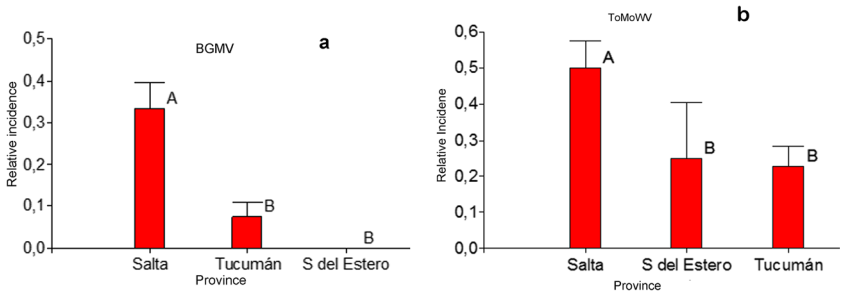

Fig. 3 Average incidence of (a) *Bean golden mosaic virus* (BGMV) and (b) *Tomato mottle winkle virus* (ToMoWV) in Salta, Santiago del Estero and Tucumán provinces during the 2014–2016 growing seasons

Discussion

Begomoviruses are a threat to crop production worldwide; in Latin America, these viruses cause severe damage to major crops. Several begomoviruses can naturally infect bean crops and cause significant yield losses (Inoue-Nagata et al. 2016). In Argentina, three species were previously reported to infect this crop: BGMV, SbBMV, and ToYSV (Rodríguez Pardina et al. 2011). In this work, we describe the presence of three other begomoviruses naturally infecting beans: SiGMBRV, ToMoWV and ToYVSV. The diversity of begomoviruses found in bean in our country is remarkable, since six different species have been detected up to the present. *Sida golden mosaic Brazil virus* was first reported infecting *Sida* plants in Mato Grosso du Sul, Brazil (Paprotka et al. 2010). The fact that it was found in bean crops supports previous findings indicating that weeds belonging to the *Sida* genus are the most abundant natural reservoirs of begomoviruses infecting wild and cultivated plants in Latin America (Morales and Anderson 2001; Jovel et al. 2004; Fiallo-Olivé et al. 2012; Mauricio-Castillo et al. 2014; Stewart et al. 2014; Ferro et al. 2017). On the other hand, ToYVSV is widely distributed in South America; it has been reported in tomato in Brazil, Uruguay, Argentina (Colariccio et al. 2007; Arruabarrena et al. 2015) and Chile (GenBank accession number KC136336), and in potato in Brazil (Albuquerque et al. 2010), whereas ToMoWV is a newly described virus that seems to have arisen by recombination between SbBMV and ToYVSV (Vaghi Medina et al. 2015). To our knowledge, this is the first time that these three viruses are reported in bean. In addition, the DNA-B of SoMoBV was found in a sample from Tartagal (Salta), suggesting that this virus may also be present in bean crops.

Of the symptomatic bean samples analysed during 2014–2016 growing seasons, only 42% were infected with begomoviruses, but according to our knowledge the other symptomatic samples could be infected with other viruses, previously described in Argentina (Rodríguez Pardina et al. 2003; Rodríguez Pardina et al. 2004), suffer damage by herbicides or nutritional deficiency, among other pathologies. When the begomovirus-infected samples were tested with the specific probes, ToMoWV was the most frequent virus, especially during 2014 and 2015 growing seasons, when it was found with an incidence of 79 and 62.5%, respectively. For SiGMBRV the highest incidence (32%) was found in 2014, whereas ToYVSV had low incidence during the three years. On the other hand, BGMV, the most widespread begomovirus of bean crops, was detected with an incidence that did not exceed 35% during the three years; however, as ToMoWV, its incidence was highest in Salta, the major bean producer province of Argentina. Finally, it should be noted that still some samples that tested positive when analysed with the general probe did not react with any of the six specific probes; therefore, the presence of other uncharacterized geminivirus species is suspected.

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