




Survey of begomoviruses and the crinivirus, tomato chlorosis virus, in solanaceous in Southeast/Midwest of Brazil

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Abstract

The golden mosaic (begomovirus) and the yellowing (crinivirus) diseases are among the main viral diseases occurring in solanaceous crops in Brazil. A survey of viruses associated with both diseases was conducted on cultivated solanaceous plants from 2013 to 2017 to study their diversity and distribution in the Southeast/Midwest regions of Brazil. Samples from potato, eggplant, sweet pepper and tomato plants were collected in fields of seven Brazilian states (Bahia, Espírito Santo, Goiás, Minas Gerais, Paraná, Rio de Janeiro and São Paulo) and in the Federal District. Total RNA/DNA was extracted and tested by RT-PCR/PCR to detect the crinivirus tomato chlorosis virus (ToCV) and begomoviruses, respectively. Representative amplicons were directly sequenced for virus identification. Out of 343 samples, 54 were positive for ToCV: 38.6% in potato, 0.9% in sweet pepper, and 20.9% in tomato. For begomovirus detection, 234 samples were positive. In potato and sweet pepper plants, only tomato severe rugose virus (ToSRV) was detected, while four begomoviruses were detected in tomato plants. ToSRV was detected in 80.1% of the tomato samples, and was the predominant begomovirus. These results indicate a low diversity of crinivirus and begomovirus species infecting cultivated solanaceous crops in Brazil during the survey period.

Keywords *Bemisia tabaci* · Tomato chlorosis virus · Tomato severe rugose virus

Solanaceous crops such as potato (*Solanum tuberosum*), tomato (*S. lycopersicum*) and sweet pepper (*Capsicum annuum*) are among the most important vegetables crops worldwide, with high socioeconomic value. Several diseases worldwide can cause significant economic losses to these crops. Golden mosaic and yellowing diseases, caused respectively by tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV), are presently among the main viral diseases in tomato crops in Brazil. These viruses are transmitted by whiteflies (*Bemisia tabaci*), in a persistent-circulative and semi-

persistent manner, respectively (Inoue-Nagata et al. 2016b; Navas-Castillo et al. 2011).

ToSRV is classified in the genus *Begomovirus* (family *Geminiviridae*), with a genome of circular single-stranded (ss) DNA encapsidated in twinned quasi-icosahedral virions (ca. 18 × 30 nm) (Rojas et al. 2005). Begomoviruses are either monopartite, with a single genomic DNA of ~ 2.8 kb, or bipartite, with two DNA components of ~ 2.6–2.8 kb (DNA-A and DNA-B). In Brazil, the vast majority of begomoviruses reported infecting tomato are bipartite (e.g., Matyis et al. 1975; Faria et al. 1997; Fernandes et al. 2006; Calegario et al. 2007; Castillo-Urquiza et al. 2008). However, monopartite begomoviruses have been recently reported in tomato (Macedo et al. 2018). In tomato crops, as well as in other solanaceous crops in the Southeast region of the country, ToSRV is the predominant begomovirus (Fernandes et al. 2008; Macedo et al. 2014). ToSRV-infected tomato plants exhibit vein clearing, chlorosis, mosaic, foliar wrinkling, and reduced growth. In addition to tomato, ToSRV has been reported infecting other cultivated crops, such as pepper (Nozaki et al. 2010), potato (Souza-Dias et al. 2008), eggplant (Moura et al. 2018) and a number of weeds (Barbosa et al. 2009, 2011a, b).

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ToCV belongs to the genus *Crinivirus* (family *Closteroviridae*), has filamentous particles, and a genome composed of two molecules of positive sense, ssRNA. Plants infected with ToCV exhibit symptoms of interveinal chlorosis, leaf curling and chlorotic spots, which usually appear first in older leaves (Wisler and Duffus 2001). In Brazil, ToCV was first reported in tomato in 2006 in the Sumaré municipality, state of São Paulo (Barbosa et al. 2008). Subsequently, this virus was reported in tomato plants in the states of Bahia, Espírito Santo, Goiás, Minas Gerais and Rio de Janeiro (Barbosa et al. 2011b) and in the Federal District (Macedo et al. 2014). ToCV was also detected in sweet pepper plants in São Paulo state in 2010 (Barbosa et al. 2010) and in potato in Goiás state in 2012 (Freitas et al. 2012). Recently, this virus was described naturally infecting eggplant (*Solanum melongena*), scarlet eggplant (*S. aethiopicum*) (Fonseca et al. 2016), wild radish (*Raphanus* spp.), garden rocket (*Eruca sativa*) (Boiteux et al. 2016), and other *Solanum* species within the subgenus *Leptostemonum* (Boiteux et al. 2018).

The objective of this study was to investigate the distribution and prevalence of the most common crinivirus, ToCV, and diversity and distribution of begomoviruses on cultivated solanaceous plants (eggplant, potato, sweet pepper and tomato) in some regions of the country. From 2013 to 2017, samples of potato, eggplant, sweet pepper and tomato plants were collected in the states of Bahia (Barra de Estiva and Ibicoara), Espírito Santo (Afonso Cláudio, Brejetuba, Castelo, Cachoeiro de Itapemirim, Domingos Martins, Santa Maria de Jetibá, Santa Teresa and Muniz Freire), Goiás (Cristianópolis, Itaberaí, Luziânia and Morrinhos), Minas Gerais (Cambuí, Itacarambi, Jaíba, Pará de Minas, Patos de Minas, Perdizes, Pimenta and Sacramento), Rio de Janeiro (Vassouras), São Paulo (Cardeal, Elias Fausto, Guaíra, Itápolis, Lins, Monte Mor, Pardinho, Sumaré and Vitoriana), and the Federal District (Brazlândia and Taquara).

Three-hundred and seventy-five solanaceous samples exhibiting ToCV- and/or begomovirus-like symptoms were evaluated for the presence of viruses. Total RNA was extracted from leaf tissue with Trizol reagent (LS, Invitrogen) and examined by RT-PCR with a ToCV-specific primer pair (ToC-5 and ToC-6), which amplifies a 463 bp fragment of the HSP70 gene (Dovas et al. 2002). To detect begomoviruses, total DNA was extracted from leaf tissue (Dellaporta et al. 1983) and examined by PCR with a degenerate primer pair (PAL1v1978/PARc496) which amplifies a 1.1 kb fragment of the DNA-A (Rojas et al. 1993). PCR-positive products were directly sequenced with ToC-5 and PAL1c496 primers for ToCV and begomovirus, respectively. The sequences obtained were compared with sequences available in public databases using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/blast>).

ToCV detection by RT-PCR revealed that out of 343 samples, 54 (15.7%) were infected with this crinivirus. The

positive samples were from potato (38.6%), tomato (20.9%) and sweet pepper (0.9%), whereas all eggplant samples were ToCV-negative (Table 1). Most of the samples analyzed for begomovirus were PCR-positive (62.4%), 38 from potato (47.5%), 60 from sweet pepper (56.6%) and 136 from tomato (72.0%) (Table 2). ToSRV was the only begomovirus detected in potato and sweet pepper plants. In tomato samples, four begomoviruses were identified: ToSRV (80.1%), tomato common mosaic virus (ToCmMV, 8.3%), tomato mottle leaf curl virus (ToMoLCV, 7.1%), and tomato yellow vein streak virus (ToYVSV, 1.3%). ToSRV was detected in tomato plants collected in the states of Espírito Santo, Goiás, Minas Gerais, Rio de Janeiro, and São Paulo (Fig. 1). The distribution of other begomoviruses was restricted to specific locations: ToMoLCV was detected in Goiás, Minas Gerais and Bahia, ToCmMV only in Espírito Santo, and ToYVSV only in São Paulo state (Fig. 1).

The incidence of golden mosaic and yellowing diseases in tomato crops in Brazil has increased in recent years (Inoue-Nagata et al. 2016a, b). The first report of tomato-infecting begomoviruses in Brazil was in 1960's (Costa 1976). The causal agent of that disease, named golden mosaic, was later characterized as tomato golden mosaic virus (TGMV, Matyis et al. 1975). TGMV has never become an economic problem in tomato crops in Brazil. From the 1960's to the 1990's, only a few reports of begomoviruses infecting tomato were published (Inoue-Nagata et al. 2016a, b). In the 1990's, an exotic *B. tabaci* species, *B. tabaci* Middle East Asia Minor 1 (MEAM1) was reported. This new species is more polyphagous than the indigenous *B. tabaci* New World, considering that it feeds on several cultivated and noncultivated plant species. *B. tabaci* MEAM1 might have acted as an important bridge, allowing movement of begomoviruses from wild to

Table 1 Detection of tomato chlorosis virus (ToCV) in potato, eggplant, sweet pepper and tomato plants collected in the states of Bahia (BA), Espírito Santo (ES), Goiás (GO), Minas Gerais (MG), Paraná (PR), Rio de Janeiro (RJ), São Paulo (SP) and the Federal District (DF)

Host	Location	Positive samples ^a
Potato	MG	16/41
	PR	0/1
	SP	1/3
Eggplant	SP	0/20
Sweet-pepper	SP	1/106
Tomato	ES	9/69
	SP	8/48
	MG	11/31
	DF	8/18
	BA	0/6
Total		54/343 (15.7%)

^a Number of positive samples/number of collected samples

Table 2 Occurrence and identification of begomoviruses in potato, eggplant, sweet pepper and tomato plants collected in the states of Bahia (BA), Espírito Santo (ES), Goiás (GO), Minas Gerais (MG), Paraná (PR), Rio de Janeiro (RJ) and São Paulo (SP) and the Federal District (DF)

Host	Location	Positive samples ^a	Identification of begomovirus species ^b			
			ToSRV	ToCmMV	ToMoLCV	ToYVSV
Potato	DF	10/25	4/4	0/4	0/4	0/4
	GO	26/45	5/5	0/5	0/5	0/5
	MG	2/6	2/2	0/2	0/2	0/2
	PR	0/1	–	–	–	–
	SP	0/3	–	–	–	–
Sweet-pepper	SP	60/106	9/9	0/9	0/9	0/9
Tomato	ES	52/69	36/52	13/52	0/52	0/52
	GO	34/34	30/34	0/34	6/34	0/34
	MG	16/31	12/16	0/16	4/16	0/16
	RJ	2/2	2/2	0/2	0/2	0/2
	SP	31/47	25/31	0/31	0/31	2/31
	BA	1/6	0/1	0/1	1/1	0/1
Total		234/375 (62.4%)	125/156 (80.1%)	13/156 (8.3%)	11/156 (7.1%)	2/156 (1.3%)

ToSRV tomato severe rugose virus, *ToCmMV* tomato common mosaic virus, *ToMoLCV* tomato mottle leaf curl virus, *ToYVSV* tomato yellow vein streak virus

^a Number of positive samples/number of collected samples. ^b Number of positive samples/ number of sequenced samples

cultivated plants that were previously not fed upon by *B. tabaci* NW. Therefore, this invasive species may have driven the emergence of several new species of tomato-infecting begomoviruses in Brazil and worldwide (Gilbertson et al. 2015). Furthermore, these begomoviruses occurring on tomatoes in Brazil are unique and possibly emerged from the

natural begomovirus diversity present in multiple wild reservoir plants (Rocha et al. 2013).

To date, at least 16 tomato-infecting begomoviruses have been described in Brazil (Ribeiro et al. 2003; Fernandes et al. 2006; Castillo-Urquiza et al. 2008; Barbosa et al. 2011a; Albuquerque et al. 2012; Rocha et al. 2013; Inoue-Nagata et al. 2016a, b). The present survey, however, confirms a previous assessments which indicated that few begomoviruses predominate in tomato and other solanaceous crops in the country. ToSRV is the predominant species occurring in the Southeast and Central-West regions, while ToMoLCV was found in the states of Bahia, Goiás and Minas Gerais. ToCmMV, which was previously reported infecting tomato in the states of Minas Gerais, Rio de Janeiro and Espírito Santo (Castillo-Urquiza et al. 2008; Rocha et al. 2013; Barbosa et al. 2016), seems to be now restricted to Espírito Santo. ToYVSV was only found in São Paulo state.

So far, only ToSRV and ToYVSV were reported in potato and pepper plants in Brazil. In addition, tomato golden vein virus (TGVV) was reported on pepper plants (Inoue-Nagata et al. 2016a, b). Overall, the incidence of the begomovirus disease in potato and peppers is usually low (Inoue-Nagata et al. 2016a, b). In the present study, only ToSRV was found infecting these plants. Although there are no reports of significant economical damage caused by begomoviruses in potato and pepper crops, these hosts may play an important role as sources of inoculum in the epidemics of ToSRV in tomato and other solanaceous crops. For ToCV, recent epidemiological studies under greenhouse conditions revealed that ToCV-

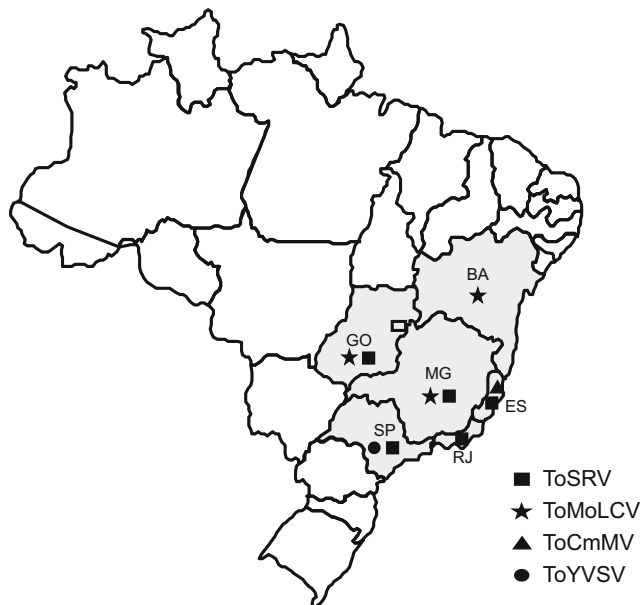


Fig. 1 Distribution of begomoviruses infecting tomato samples in six Brazilian states. ToSRV: tomato severe rugose virus, ToMoLCV: tomato mottle leaf curl virus, ToCmMV: tomato common mosaic virus, ToYVSV: tomato yellowing vein streak virus

infected tomato and potato plants were efficient sources of inoculum for virus acquisition by *B. tabaci* MEAM1 and for transmission to tomato and potato plants. However, sweet pepper was not efficient as inoculum source of ToCV for virus acquisition by the whitefly and might not be important in the epidemiology of the disease caused by this crinivirus (Mituti et al. 2018).

Several factors likely contribute to the dynamics of virus populations in the field. ToSRV and ToCV are efficiently transmitted by *B. tabaci* MEAM1, currently the predominant whitefly in Brazil. Among the begomoviruses, transmission efficiency by *B. tabaci* MEAM1 can also vary, as was experimentally demonstrated for ToSRV and TGVV in single and mixed infections: ToSRV was approximately five times more efficiently transmitted by this vector than TGVV (Macedo et al. 2015). The recent detection of *B. tabaci* Mediterranean (MED) in protected ornamental crops in the country (Moraes et al. 2017) may alter the incidence/predominance of begomoviruses and of ToCV in solanaceous crops, as the movement of this insect into the open field represents a real threat. *B. tabaci* MED is an efficient vector of ToCV, retaining the virus for six days, whereas *B. tabaci* MEAM1 retains the virus for three days (Wintermantel and Wisler 2006; Orfanidou et al. 2016). *B. tabaci* MED also transmits ToSRV efficiently to tomatoes, but is not well adapted to colonize this host, whereas sweet pepper is a very suitable host for this whitefly species (Sun et al. 2013). Consequently, sweet pepper crops might suffer a negative impact from the presence of this exotic whitefly. Furthermore, *B. tabaci* MED has lower susceptibility to several insecticides and is more competitive than *B. tabaci* MEAM1 when insecticides are used (Horowitz et al. 2005; Horowitz and Ishaaya 2014; Sun et al. 2013).

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