



Occurrence of *Tomato chlorosis virus* in tomato crops in five Brazilian states

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ABSTRACT

Tomato chlorosis virus (ToCV) is a species in the genus *Crinivirus*, transmitted by *Bemisia tabaci* biotype B, first detected in Brazil in 2006 in the county of Sumaré, State of São Paulo. During the period from 2007 to 2010, RT-PCR analyses followed by nested-PCR with specific primers for ToCV detection, and sequencing of the amplicons, confirmed the presence of this virus in samples of symptomatic tomato plants (*Solanum lycopersicum*) from crops in counties in the states of Bahia (BA), Espírito Santo (ES), Goiás (GO), Minas Gerais (MG), and Rio de Janeiro (RJ). This is the first ToCV report for the states of BA, ES, GO, MG, and RJ, suggesting that this virus is widely disseminated in Brazil.

Key words: *Solanum lycopersicum*, *Crinivirus*, diagnosis.

RESUMO

Ocorrência de *Tomato chlorosis virus* em tomateiro em cinco estados Brasileiros

Tomato chlorosis virus (ToCV) é uma espécie do gênero *Crinivirus*, transmitida por *Bemisia tabaci* biótipo B e que foi primeiramente detectada no Brasil em 2006 no município de Sumaré, Estado de São Paulo. No período de 2007 a 2010, análises de RT-PCR seguida de "nested-PCR", com oligonucleotídeos iniciadores específicos para a detecção do ToCV e o sequenciamento dos fragmentos amplificados confirmaram a presença desse vírus em amostras de plantas sintomáticas de tomate (*Solanum lycopersicum*) provenientes de plantações em municípios dos estados da Bahia (BA), Espírito Santo (ES), Goiás (GO), Minas Gerais (MG) e Rio de Janeiro (RJ). Este é o primeiro relato do ToCV nos Estados da BA, ES, GO, MG e RJ, sugerindo uma ampla disseminação desse vírus no Brasil.

Palavras-chave: *Solanum lycopersicum*, *Crinivirus*, diagnose.

Tomato (*Solanum lycopersicum*) is one of the most important vegetables worldwide. In 2008, the world tomato production reached the level of approximately 136 million tons (t) of fresh fruits, in an estimated area of 4.8 million hectares (ha) (FAO, 2011). In Brazil it was cultivated in an area of nearly 66,964 ha in 2009, with a production of 4,204,638 tons. The states of São Paulo and Goiás were the largest producers, with planted areas of approximately 10,160 ha and 18,109 ha, respectively (IBGE, 2010).

Several phytosanitary problems affect the tomato crop in Brazil, including those of viral nature. Recently, Barbosa et al. (2008) observed the presence of *Tomato chlorosis virus* (ToCV) in the region of Sumaré, State of São Paulo, with incidence values ranging from 0.25% to 3.42%. They also experimentally demonstrated that it was transmitted by *Bemisia tabaci* biotype B. This virus belongs to the genus *Crinivirus*, family *Closteroviridae*, with long flexuous particles of approximately 800 to 850 nm in length. A bipartite genome is present, consisting of two single-stranded, positive-sense RNA molecules. Infected tomato plants display interveinal chlorosis symptoms especially

in the lower leaves, which become brittle and may show necrotic spots.

ToCV was first isolated and described in the United States (Wisler et al., 1998). After that the virus was detected on tomato crops in Portugal (Louro et al., 2000), Spain (Navas-Castillo, et al., 2000), Puerto Rico (Wintermantel et al., 2001), Italy (Accotto et al., 2001), Greece (Dovas et al., 2002), Taiwan (Tsai et al., 2004), Israel (Segev et al., 2004), Mexico (Alvarez-Ruiz et al., 2007), Cuba (Martínez-Zubiaur et al., 2008), Brazil (Barbosa et al., 2008), and most recently in Costa Rica (Castro et al., 2011) and Hungary (Bese et al., 2011). Particularly in the U.S.A. and Spain, ToCV has been identified as a serious problem for tomato production (Wintermantel & Wisler, 2006; OEPP/EPPO, 2005).

During the period from 2007 to 2010, the Plant Virology Laboratory, ESALQ/USP, received 14 leaf samples of tomato showing interveinal chlorosis symptoms (Figure 1), characteristic of those caused by ToCV. The samples were obtained from crops in 10 counties located in the states of Bahia - BA (Barreiras and Ibicuará), Espírito Santo



FIGURE 1 - *Tomato chlorosis virus* on plant showing leaf symptom of interveinal chlorosis. Afonso Cláudio ES. 2007.

- ES (Afonso Claudio and Domingos Martins), Goiás - GO (Corumbá de Goiás, Goiânia, and Silvânia), Minas Gerais - MG (Araguari and Coimbra), and Rio de Janeiro - RJ (Paty de Alferes). Five additional samples of symptomatic tomato plants were also received from crops in the State of São Paulo - SP (Avaré, Barão de Antonina, São Pedro do Turvo, and Sumaré), where the virus was first detected in Brazil.

Total RNA was extracted with Trizol[®] (Invitrogen) from symptomatic leaf tissue of each sample. Next, it was submitted to RT-PCR with the HS-11/HS-12 pair of primers (Dovas et al., 2002), which amplifies a 587 bp fragment in the homologous and highly conserved region of the heat shock protein (HSP-70) reported for ToCV and *Tomato infectious chlorosis virus* (TICV), another crinivirus that infects tomato, which has not yet been reported in Brazil. The RT-PCR product was later submitted to a nested-PCR to separately detect TICV and ToCV, using the specific primer pairs TIC-3/TIC-4 and ToC-5/ToC-6, respectively (Dovas et al., 2002). Only the amplification of a single fragment of approximately 463-bp with the pair of primers specific for ToCV was observed in several symptomatic samples from all six Brazilian States. ToCV was not detected only in tomato samples from the counties of Ibicuara (BA), and Coimbra (MG). TICV was not detected in the analyzed samples, as illustrated in Figure 2. All RT-PCR

products were directly sequenced using the primers ToC-5/ToC-6. Nucleotide sequences ranging from 281 to 463 nt were compared to the corresponding sequence of the first reported Brazilian isolates of ToCV (GenBank Accession EU868927) and showed nucleotide identity ranging from 95 to 99%, confirming the presence of ToCV in the samples.

All samples analyzed for the presence of ToCV and TICV were also analyzed for potential infection with other viruses, such as begomovirus, tospovirus, *Tomato mosaic virus* (ToMV), and *Pepper yellow mosaic virus* (PepYMV). Infection by begomovirus was tested by PCR using total DNA extracted separately from each sample (Dellaporta et al., 1983). The universal begomovirus pair of primers PAR1c496/PAL1v1978 was used (Rojas et al., 1993). The detection of potential infections by the other viruses was achieved by means of mechanical inoculations into the following indicator plants: *Chenopodium amaranticolor*, *C. quinoa*, *Cucurbita pepo* 'Caserta', *Datura stramonium*, *Gomphrenaglobosa*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. tabacum* 'TNN', and 'Havana', *Physalis* sp., and *S. lycopersicum*. All PCR-analyzed samples for the presence of begomoviruses proved negative. No test plants inoculated with extracts from all samples showed symptoms typical of viral infection.

The presence of ToCV in the states of BA, ES, GO, MG, and RJ is a strong indication that this crinivirus can be present in other states as well, if not in all tomato producing regions of Brazil, since *B. tabaci* biotype B, which is an effective vector for this virus, can be found throughout the country. The possible unnoticed occurrence of this virus in tomato crops of other Brazilian states may be associated with the fact that its symptoms are seen rather late and are similar to those related to nutritional disorders caused by magnesium deficiency.

In addition to *B. tabaci* biotype B, this crinivirus is also transmitted by *B. tabaci* biotype A, *Trialeurodes abutilonea*, and *T. vaporariorum*, with *B. tabaci* biotype B and *T. abutilonea* as the most efficient vectors (Wintermantel & Wisler, 2006). There are no reports on the occurrence of *T. abutilonea* in Brazil. However, *T. vaporariorum*, which seemed to be limited to protected cultivations (greenhouses), has been recently reported in tomato and bean plants (*Phaseolus vulgaris*) under open field conditions in São Paulo (Lourenção et al., 2008). Experimental transmission tests carried out with *T. vaporariorum* collected in zucchini squash plants grown under greenhouse conditions in Itatiba County, State of São Paulo, showed that this aleyrodid was able to transmit the tomato isolate of ToCV collected by Barbosa et al. (2008) (Freitas et al., 2011). If this is verified in other regions of the country, this aleyrodid species may also contribute to a larger dissemination of this virus. Studies to better evaluate the distribution of this crinivirus in Brazil, as well as the damage caused to tomato yield, are required to identify which management strategies should be established for the disease.

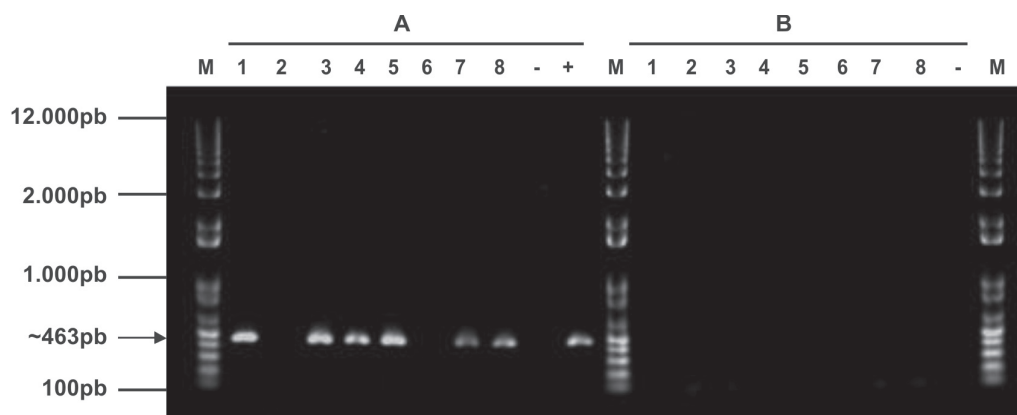


FIGURE 2 - Representative gel electrophoresis of nested-PCR reaction products with specific primers for ToCV (**A**) and TICV (**B**). M: 1Kb marker; 1 to 8: Samples from Araguari MG, Coimbra MG, Paty do Alferes RJ, Domingos Martins ES, Corumbá de Goiás GO, Ibicuará BA, Avaré SP, and São Pedro do Turvo SP; -: healthy tomato sample; +: ToCV isolate, Sumaré (SP) (Barbosa et al., 2008). The arrow indicates the amplified fragment.

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