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SHORT COMMUNICATION

High incidence of *Tomato chlorosis virus* alone and in mixed infection with begomoviruses in two tomato fields in the Federal District and Goiás state, Brazil

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ABSTRACT

Tomato chlorosis virus (ToCV), a species in the *Crinivirus* genus, was first reported in tomatoes in Brazil (state of São Paulo) in 2008. This was followed by reports in several other Brazilian states. Tomato plants with chlorotic spots and leaf roll symptoms are frequently observed in tomato fields with high whitefly populations in Central Brazil. These plants could be infected with a begomovirus, a crinivirus, or with both viruses. A survey of two selected tomato fields in the Federal District and Goiás State was conducted in 2012 and 2013 specifically to determine the occurrence of begomoviruses and criniviruses. A total of 150 samples were collected and subjected to RT-PCR for ToCV detection and PCR for begomovirus detection. About 48% of the tested plants were infected with both viruses, 32% were infected with ToCV alone and 20% were only infected with the begomovirus ToSRV. The increasing incidence of ToCV associated with high whitefly populations in the field highlights the need for studying this virus disease to clarify its impact on tomato crops and minimize its potential damage.

Key words: *Solanum lycopersicum*, *Tomato severe rugose virus*, begomovirus, crinivirus.

After the first report of the occurrence of *Tomato chlorosis virus* (ToCV) in samples collected during 2006 to 2007 at São Paulo state, Brazil (Barbosa et al., 2008), this crinivirus was subsequently reported in the states of Bahia, Espírito Santo, Goiás, Minas Gerais and Rio de Janeiro (Barbosa et al., 2011). A second tomato-infecting crinivirus, *Tomato infectious chlorosis virus*, has not been reported from Brazil yet. Plants infected with ToCV display symptoms of interveinal chlorosis, leaf curling and chlorotic spots, which usually appear first in the older leaves. These symptoms are similar to those induced by begomoviruses, hence diagnosis of begomovirus and crinivirus diseases cannot be performed based on symptoms. In addition, viruses from both genera (*Begomovirus* and *Crinivirus*) are transmitted by the same insect vector (the whitefly *Bemisia tabaci*). Many begomovirus species have been reported in Brazil (Ambrozevicius et al., 2002; Ribeiro et al., 2003; Fernandes et al., 2006; Ribeiro et al., 2007; Fernandes et al., 2008), but *Tomato severe rugose virus* (ToSRV) seems to be the predominant species (Fernandes et al., 2008).

In 2012 and 2013, a high incidence of plants showing chlorosis and leaf curling was observed in the Federal District and Goiás state. These symptoms resembled those caused by ToCV, but interveinal chlorosis, yellow mosaic and stunting symptoms, more typical of begomovirus infection, were also observed. The aim of this study was to determine the incidence of criniviruses and begomoviruses in tomato plants grown in the Federal District and Goiás State.

To test for the presence of these viruses, two fields were visited for sampling. In October 2012, 123 samples of symptomatic tomato plants (cv. Dominador, undetermined growth habit) were collected in a field at the municipality of Goianápolis, Goiás state. At the time of the visit this field consisted of approximately 70 day-old plants, nearly 80% of which showing symptoms of interveinal chlorosis, leaf curling and rugosity, suspected to be caused by begomovirus infection (Figure 1A) or leaf rolling and interveinal chlorotic areas mainly in basal and median leaves of the plant, suspected to involve infection with ToCV (Figure 1B). Only plants with these symptoms were collected. Plants with did not display virus-like symptoms were not collected. In February 2013, a total of 27 tomato samples (cv. AP 533, determined growth habit) displaying similar symptoms to those previously described were collected from a processing tomato field in the region of the Programa de Assentamento Dirigido, Federal District (PAD-DF). In this field, of approximately 40 day-old plants, nearly all plants showed virus-like symptoms (Figure 1D, E).

To test for the presence of ToCV, total RNA was extracted with Trizol (Invitrogen) from leaf tissue of each sample and subjected to RT-PCR with the ToCV-specific primer pair ToC-5/ToC-6 (Dovas et al., 2002), which amplifies a 463 bp fragment. Five randomly selected PCR products

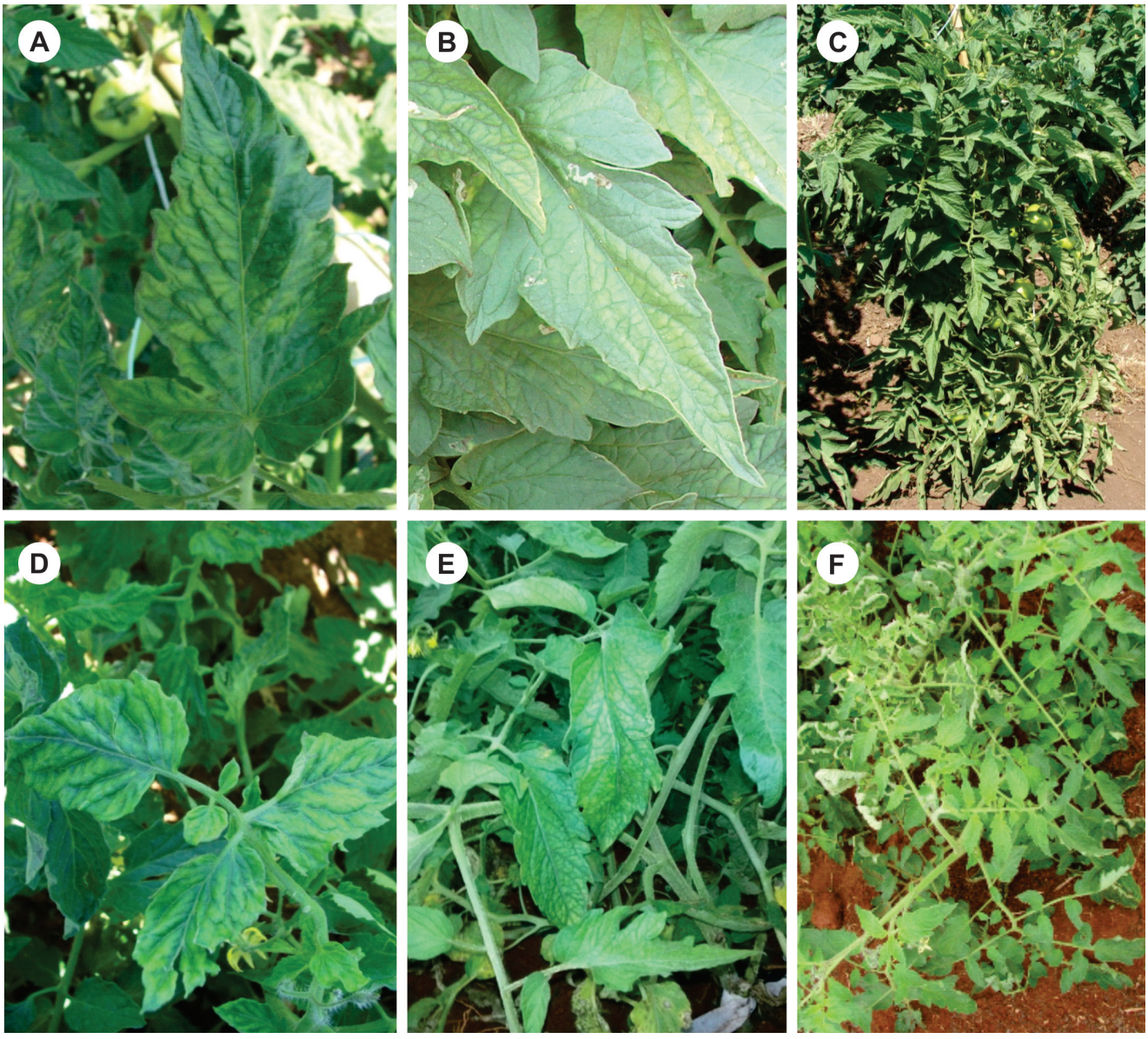

FIGURE 1 - Plants of the hybrid Dominador collected at Goianápolis, Goiás state, infected with a begomovirus (**A**), a crinivirus (**B**) and both viruses (**C**). Plants of hybrid AP533 collected at PAD-DF infected with a begomovirus (**D**), a crinivirus (**E**) and with both viruses (**F**).

were directly sequenced to confirm the amplification of ToCV-derived fragments. Additionally, from the same leaf samples, total DNA was extracted (Dellaporta et al. 1983) and tested for begomovirus infection by PCR using the universal primer pair PAR1c496/PAL1v1978 (Rojas et al., 1993), which yields a fragment of ca. 1.1 kbp. All positive samples for begomovirus were tested by PCR with specific primers for ToSRV (ToSRV1f-ToSRV2r), which amplifies a fragment of about 820 bp (Fernandes et al., 2010). Five PCR products, randomly selected, were directly sequenced to confirm the results.

A high incidence of both viruses was confirmed in the two fields (Table 1). All 123 samples from the Goianápolis field tested positive: 23 for begomovirus only, 44 for crinivirus only, and 56 samples for both viruses. In this field, 79 plants (64%) were infected with a begomovirus and 100 (81%) with ToCV. Likewise, all 27 samples from the PAD-DF field also tested positive: seven for begomovirus only, three for ToCV only and 17 for both viruses. Therefore, 24 plants (89%) were infected with a begomovirus and 20 (74%) with ToCV.

All begomovirus positive samples tested positive with ToSRV-specific primers. Five of them were randomly selected for direct sequencing of PCR products, and it was confirmed that ToSRV was present in all samples. This is in agreement with previous reports indicating that ToSRV

TABLE 1 - Detection of crinivirus and begomovirus in tomato samples collected from tomato fields in central Brazil in 2012 and 2013.

Location	Hybrid	No. infected plants/no. collected plants (%)		
		crinivirus only	begomovirus only	mixed infection
PAD-DF	AP 533	3/27 (11)	7/27 (26)	17/27 (63)
Goianápolis	Dominador	44/123 (36)	23/123 (19)	56/123 (46)
Total		47/150 (31)	30/150 (20)	73/150 (49)

is the prevalent tomato-infecting begomovirus in Goiás and the Federal District (Fernandes et al., 2008). Direct sequencing of RT-PCR products confirmed the presence of ToCV.

The tomato hybrid cultivar Dominador is tolerant to begomovirus infection and susceptible to crinivirus, but AP 533 is susceptible to both viruses. This implies that begomovirus replication and systemic movement may occur in Dominador plants, and these infected plants show milder symptoms compared to susceptible plants. This may explain the higher incidence of ToSRV in AP533 (89%, compared to 74% for ToCV) and ToCV in Dominador (81%, compared to 64% for ToSRV). Despite this difference in susceptibility between the cultivars, these results demonstrated that in the tomato growing areas of Federal District and Goianápolis, begomoviruses and criniviruses may occur in high incidence in tomato fields with both determined and undetermined growth habits.

The symptoms of begomovirus infection in Dominador plants included interveinal chlorosis, leaf distortion and leaf rolling in the younger leaves (Figure 1A). On the other hand, ToCV infection in this cultivar caused curling and chlorosis in the older leaves, and mild mosaic in intermediate leaves (Figure 1B). Young leaves showed no symptoms. When both viruses were present in the same plant, begomovirus symptoms were observed on the top portion of the plant, and crinivirus-like symptoms on the bottom portion (Figure 1C). When a plant was infected with both viruses, its leaves showed stronger chlorosis and rugosity symptoms than when infected by ToSRV only. Begomovirus symptoms in AP 533 plants appeared as vein clearing, interveinal chlorosis and leaf curling on the top leaves (Figure 1D). Crinivirus symptoms were not clearly seen because they appear later in the season and older leaves are hidden below younger leaves. Nevertheless, occasionally, it was possible to see leaf curling and chlorosis, mainly in older leaves (Figure 1E). In some mixed infected plants, begomovirus-like symptoms were observed on the top leaves and crinivirus-like symptoms on the bottom leaves (Figure 1F).

During 2006 and 2007 the incidence of ToCV in tomato fields in São Paulo state was between 0.25 to 3.42% (Barbosa et al., 2008). Our results show that ToCV incidence can be equivalent to that of begomoviruses in regions with high infestation of whiteflies, as observed in the two sampled areas of Goiás state and the Federal District. The cultivation system of tomato in these regions may contribute for the ToCV spread (Lourenção & Nagai, 1994), such as the high transmission efficiency and the presence of alternate hosts (Wintermantel & Wisler, 2006). Additionally, these data demonstrate the high susceptibility of two widely planted hybrids to ToCV infection. The high incidence of ToCV in the fields combined or not with begomoviruses is cause for concern to tomato growers. Nevertheless, losses to tomato production by ToCV, alone or in combination with begomoviruses, have not been estimated yet.

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