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CROP PROTECTION

Incidence of *Begomovirus* and *Crinivirus* in tomato and potato crops in Paraná State, Brazil

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ABSTRACT: In the last decades, the high incidence of viruses transmitted by whiteflies has become a problem in the tomato fields, threatening, more recently, the potato crops. The present study carried out a survey of begomoviruses and criniviruses in tomato and potato crops, from 2015 to 2018, in the municipalities of Araucária, Campo do Tenente, Campo Largo, Contenda, Lapa, Faxinal, Morretes, Reserva, Castro, Palmeira and São Mateus do Sul, in Paraná State, Brazil. Total DNA and RNA from leaves were extracted and used as templates to detect, respectively, begomoviruses by PCR and criniviruses by RT-PCR. Out of 215 tomato samples, 14 from Faxinal were infected by crinivirus. The other tomato samples and 243 potato samples showed negative results for begomovirus and crinivirus. Results indicated a low incidence (6.5%) of crinivirus infecting tomato crops in Paraná State, and the nucleotide sequence of one amplified fragment shared 99.71% identity with tomato chlorotic virus (ToCV).
Key words: tomato chlorosis virus, tomato severe rugose virus, whitefly.

Incidência de *Begomovirus* e *Crinivirus* nas culturas do tomateiro e batateira no Estado do Paraná, Brasil

RESUMO: Nas últimas décadas, a alta incidência de vírus transmitidos por mosca-branca tornou-se um problema nos campos de tomateiros, ameaçando, mais recentemente, a cultura da batateira. O presente trabalho teve como objetivo realizar um levantamento de begomovírus e crinivírus em lavouras de tomateiro e batateira nos municípios de Araucária, Campo do Tenente, Campo Largo, Contenda, Lapa, Faxinal, Morretes, Reserva, Castro, Palmeira e São Mateus do Sul, no Estado do Paraná, Brasil, de 2015 à 2018. DNA e RNA totais de folhas foram extraídos e utilizados como molde para detectar begomovírus por PCR e crinivírus por RT-PCR. Das 215 amostras de tomateiros coletadas, 14 provenientes de Faxinal estavam infectadas por crinivírus. As demais amostras de tomateiro e as 243 amostras de batateira analisadas apresentaram resultados negativo para begomovírus e crinivírus. Os resultados indicaram baixa incidência (6,5%) de crinivírus infectando lavouras de tomateiros no Estado do Paraná e a sequência de nucleotídeos de um amplicon apresentou 99,71% de identidade com o crinivírus tomato chlorotic virus (ToCV).
Palavras-chave: tomato chlorosis virus, tomato severe rugose virus, whitefly.

Tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.) are subjected to various phytosanitary problems, including those caused by viruses transmitted by whitefly (NAVAS-CASTILLO et al., 2011). Among the primary viral diseases associated with whiteflies in the tomato crops in Brazil are the begomoviruses, with the most prevalent being the tomato severe rugose virus (ToSRV) and the crinivirus, tomato chlorosis virus (ToCV) (INOUE-NAGATA et al., 2016). These viruses are also present in potato fields representing a significant threat to this crop (INOUE-NAGATA et al., 2016; LIMA, 2016; MITUTI et al., 2019).

ToSRV is classified in the genus *Begomovirus* (*Geminiviridae*), and it is persistent/circulative transmitted by the complex of cryptic species of *Bemisia tabaci* (Gennadius) (ROSEN et al., 2015). Tomato plants infected with ToSRV exhibit vein clearing, chlorosis, mosaic, foliar wrinkling, and reduced growth (MITUTI et al., 2019). Potato plants show symptoms of mosaic and leaf deformation (LIMA, 2016) which can be confused in the field by those caused by other viruses that frequently affects the crop, as potato leafrool virus (PLRV), that also causes rolled leaves, or even the potato virus Y (PVY) (SOUZA-DIAS et al., 2008), which causes mosaic, chlorosis, and leaf deformation (KREUZE et al., 2020).

ToCV belongs to the genus *Crinivirus* (*Closteroviridae*), and it is semipersistently transmitted by whiteflies, including the cryptic

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species of *B. tabaci* MEAM1, MED, *Trialeurodes vaporariorum*, and *T. abutilonea* (WISLER et al., 1998; WINTERMANTEL & WISLER, 2006). However, the latter has not been reported in Brazil yet. Tomato plants infected with ToCV show symptoms of interveinal chlorosis, especially in the lower leaves, which become brittle and may show necrotic spots causing loss of photosynthetic area and reducing production (WISLER et al., 1998). Potato plants infected with ToCV exhibit leaf roll symptoms and interveinal chlorosis, especially on older leaves and, as begomovirus, ToCV symptoms also can be confused by those caused by PLRV and PVY (FREITAS et al., 2012).

ToSRV and ToCV share the same vector and infect important solanaceous cultivated crops, such as pepper (NOZAKI et al., 2010), sweet pepper (BARBOSA et al., 2010), eggplant (MOURA et al., 2018, FONSECA et al., 2016), potato, and tomato, which are frequently planted in nearby areas, besides cucumber (BELLO et al., 2019). Moreover, a numerous of weeds, that occur in and surrounding the fields, can serve as a reservoir of the ToRSV (ROJAS et al., 2018) and ToCV (SOUZA et al., 2020) and as a host to the whitefly. With this scenario, the flux of virus between these plants mediated by the whitefly transmission is favored. Ever since, several studies involving mixed infections, epidemiology, and survey of begomoviruses and criniviruses in tomato, potato, and solanaceous have been conducted in the country to better understand the diseases (LIMA, 2016; MACEDO et al., 2019, MITUTI et al., 2019).

A survey of diseases on tomatoes in Brazil from 2008 to 2011, including those of viral etiology, revealed the presence of begomovirus and crinivirus infection in samples from Faxinal, State of Paraná, with the predominance of ToRSV and tendency of increasing the importance of ToCV (QUEZADO-DUVAL et al., 2013). From 2016 to 2017, 380 samples of symptomatic tomato plants collected in the States of Goiás, Rio de Janeiro, and Paraná indicated the presence of ToCV in 66 samples. Out of 66 samples infected with ToCV, 28 were from the municipality of Faxinal, Paraná (COELHO et al., 2019).

So far, all reports in Paraná are concentrated in the municipality of Faxinal and nearby areas, the main tomato-producing region of the State. As ToSRV and ToCV can migrate from tomato to potato crops and vice-versa, it is necessary to expand the surveys of these pathogens to other regions of the State of Paraná. Therefore, this study investigates the occurrence of begomoviruses and criniviruses in tomato and potato crops in 11 municipalities in the State of Paraná.

From 2015 to 2018, plants with symptoms suspected of viral infection (Figure 1) were collected obtaining a total of 215 leaf samples of tomato plants exhibiting symptoms of mosaic, reduced growth or interveinal chlorosis at the municipalities of Araucária (8), Campo do Tenente (20), Campo Largo (16), Contenda (6), Faxinal (113), Morretes (3), Lapa (35) and Reserva (14). A total of 243 leaf samples of potato plants showing symptoms of interveinal chlorosis, leaf roll and mosaic were collected in Araucária (92), Campo do Tenente (5), Campo Largo (37), Castro (16), Contenda (50), Lapa (25), Palmeira (13) and São Mateus do Sul (5). The collected leaves were stored at -80 ºC.

To detect begomoviruses, the total DNA was extracted from symptomatic leaves (DELLAPORTA et al., 1983), and submitted to PCR using the universal primer pair PAL1v1978/PAR1c496, which amplifies a 1.2 Kb amplicon within DNA A (ROJAS et al., 1993). For the crinivirus detection, total RNA was extracted with Trizol (LS, Invitrogen) from each symptomatic leaf tissue and submitted to RT-PCR with degenerated primers HS-11/HS-12, which amplify a 587 bp fragment of the highly conserved region of the heat shock protein 70 family homolog gene (Hsp70h), reported for ToCV and tomato infectious chlorosis virus (TICV) (DOVAS et al., 2002). Positive controls consisted in tomato plants infected by ToSRV and ToCV isolates, kindly provided from Laboratory of Virology from ESALQ/USP and maintained separately into cages with anti-insect nets in a greenhouse. Healthy tomato plants, maintained at the same conditions were used as negative controls. The results were analyzed by agarose gel electrophoresis (1.0%) to check for the presence of amplicons.

The amplicons were purified and directly sequenced using the primers HS-11 and HS-12 (EPPO, 2013). The nucleotide sequence identities were analyzed and compared with others available in GenBank using BLASTn (ALTSCHUL et al., 1990). Phylogenetic analysis was performed by the maximum likelihood method implemented in RAxML-HPC v.8 on XSEDE (STAMATAKIS, 2014) using the Cipres Science Gateway web server, and the appropriate model for the analysis was selected with jModelTest2 on XSEDE (2.1.6) (DARRIBA et al., 2012) using the Akaike information criterion (AIC). The statistical significance of tree branching was assessed by performing 1000 bootstrap replications.

All PCR-analyzed samples of tomatoes and potatoes for the presence of begomoviruses proved

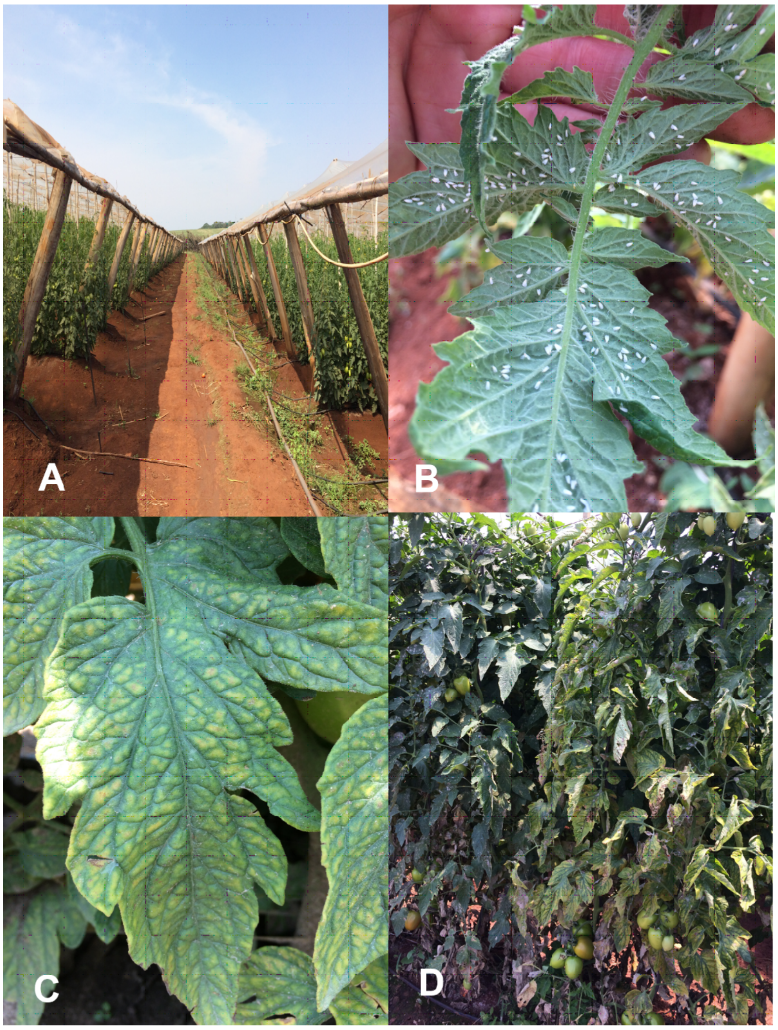

Figure 1 - Tomato plants (PaiPai variety) from municipality of Faxinal, the main tomato-producing region of the Paraná State A. Commercial area of tomato crop produced in a greenhouse, Londrina model; B. Tomato plant showing high whitefly infestation; C. Commercial area of greenhouse-cultivated tomato crops showing symptoms of interveinal chlorosis and thickening on lower leaves; D. the symptoms advance towards the upper part of the plant.

negative. However, RT-PCR results revealed the amplification of a single fragment of approximately 580 bp in 14 out of 215 samples of tomatoes from Faxinal (6.5%) with the pair of primers universal for crinivirus - ToCV and TICV. One of the 14 samples was characterized at the species level and the nucleotide sequence (GenBank accession number OL449950) shared 99.71 to 99.41% identity with corresponding nucleotide sequences of ToCV, including those reported in Brazil (accession numbers: KP713347, KP713345, EU069363, KT727953, KT727959 and MK930366). The phylogenetic analysis revealed that ToCV isolate from Faxinal grouped with ToCV isolates from Brazil, Turkey, Cyprus and Greece (Figure 2).

In this survey, tomato samples showed negative results for begomovirus and observed a low incidence (6.5%) of *Crinivirus* infecting tomato crops in Paraná, being identified ToCV in Faxinal. No detection of begomovirus and crinivirus in potato crops was observed. These results are essential to assist in managing these diseases in tomato and potato crops, since outbreaks of whiteflies are frequent in production fields.

Currently, the control of begomoviruses in tomatoes is based on resistant cultivars and frequent

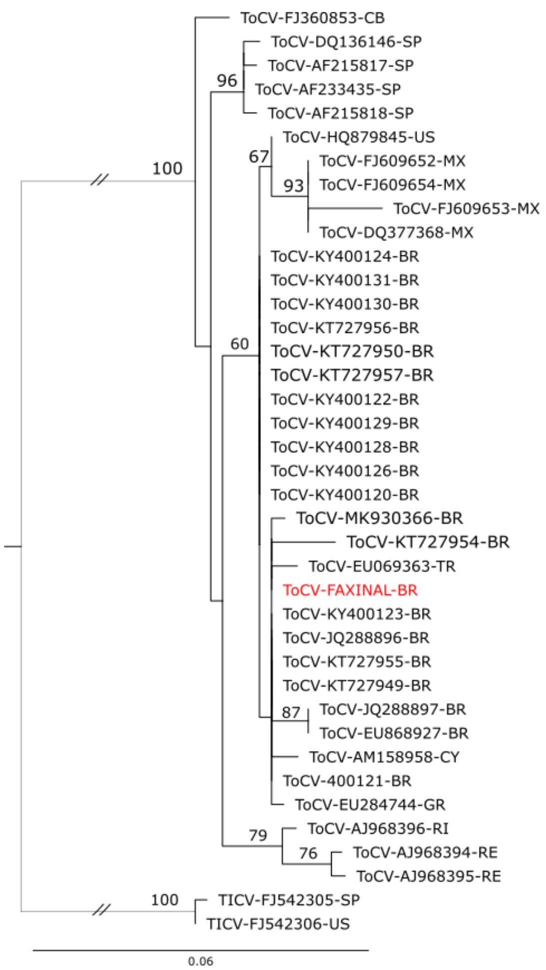

Figure 2 - Maximum-likelihood tree of 472-base alignment of partial heat shock protein - 70 sequences of tomato chlorosis virus from Paraná, Brazil (highlighted in red), and 38 sequences from GenBank, conducted with evolutive model HKY+G and 1000 bootstrap replicates. The outgroup is *Tomato infections virus* (TiCV). Taxon labels are GenBank accession numbers followed by two-letter country codes: CB, CUBA; SP, SPAIN; US, UNITED STATES; MX, MEXICO; BR, BRAZIL; TR, TURKEY; CY, CYPRUS; GR, GREECE; RE, REUNION ISLAND.

insecticide applications for vector control (INOUE-NAGATA et al., 2016). Considering the mechanisms of dispersion of begomovirus appears to occur through primary dispersion by the constant influx of viruliferous whiteflies from the external sources of inoculum (MACEDO et al., 2019), one hypothesis to explain the absence of begomovirus in the tomato crop is the absence of inoculum source in the vicinity. Thus, the recommended control measures to prevent the arrival of viruliferous whiteflies in production areas are to avoid planting near other solanaceous such as potato, sweet pepper, and eggplant, besides cucumber which are excellent hosts for the virus, in addition to cucurbits, okra, soybean, bean, and cotton plants, that are hosts of the insect, and some of them, also host the virus. Avoiding successive cultivation of tomatoes in the same area also can be helpful as a strategy for implementing whitefly host-free period (INOUE-NAGATA et al., 2016). Additionally, it is indicated the management of weeds around fields, which can host the virus, and carry out regular inspections of whitefly in the production fields (ROJAS et al., 2018).

Considering the previous occurrence of ToCV in tomato fields from Faxinal (QUEZADO-DUVAL et al., 2013), after the present survey, ToCV still occurring in this region. It was noticed high populations of whiteflies and overlapping crops of tomatoes that should be avoided once infected plants in established fields are important inoculum sources for newly planted fields in addition to maintaining a high vector population. Faxinal is the second largest tomato producer municipality of Paraná State, and due to the absence of resistant cultivars to ToCV (MANSILLA-CORDOVA et al., 2018), monitoring the whitefly population is crucial, since the major disease control method is based on chemical spraying.

Crinivirus was not detected in Araucária, Campo do Tenente, Campo Largo, Contenda, Morretes and Lapa. These municipalities are characterized by small and isolated tomato fields, areas with no history of tomato cultivation, and, despite that, high populations of whiteflies were observed in many of them. Thus, as the vector has a wide host range and it is already known that the virus naturally infects other solanaceous such as eggplant, scarlet eggplant (*S. aethiopicum*) (FONSECA et al., 2016), wild radish (*Raphanus* spp.), garden rocket (*Eruca sativa*) (BOITEUX et al., 2016), and other eight *Solanum* species (with long vegetative cycle) in the subgenus *Leptostemonum* (BOITEUX et al., 2018), it is recommended to avoid planting tomato crops near these solanaceous and other plants that can serve to host the virus. Therefore, as mentioned, the recommendations to the whitefly control also include regular inspections of the insect (MICHEREFF-FILHO & INOUE-NAGATA, 2015).

This research showed that begomovirus and crinivirus were not detected in potato crops. Some factors may have contributed to the sanity of the fields. The first is the use of seed potato of good sanitary quality, once potato is vegetatively propagated, the risk of virus accumulation to the next generation is minor when healthy tubers are used (KRAUSE et al, 2020). Potato plants were tested by double-antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) for the presence of PVY, PLRV, potato virus X (PVX) and potato virus S (PVS), using appropriate polyclonal antibodies (Agdia), to elucidate the symptoms of viral infection. The plants were infected with potyvirus PVY (7.2%) and with polerovirus PLRV (10.5%), both viruses transmitted by aphids. In the municipality of Araucária only PVY was detected in the samples, while Campo do Tenente the samples reacted positively to PLRV. Both PVY and PLRV were present at the municipalities of Campo Largo, Contenda, and Lapa. Mixed infection by both viruses was detected in 3.7% of potato plants. No sample showed positive reaction for PVX and PVS.

The potato fields had plants predominantly in the same vegetative stage, and they were not surrounded by other solanaceous, reducing the number of vector and hosts to the begomovirus and crinivirus. Some fields showed high populations of whiteflies and were intensively sprayed with insecticide since the appearance of the vector. In these areas, despite the high infestation by the vector, the collected samples did not show positive results for the presence of both genus analyzed. Therefore, the supposed absence of primary inoculum in potato fields, confirmed by the negative results of molecular tests, suggested no occurrence of primary infections, which plays a fundamental role in the epidemic. However, as recent outbreaks of whiteflies have been observed in potato crops (SALAS et al., 2017), careful monitoring of begomoviruses and ToCV should occur, especially in regions where the problem is not well known. Thus, periodic surveys of these viruses are indicated, as potato crops is vegetatively propagated, and attention should be paid to the production of virus-free seed-potato once begomoviruses, ToCV, PVX and PVY can be perpetuated in the tubers (FORTES & NAVAS-CASTILLO, 2012; LIMA, 2016).

As conclusion, we can state that, in general, the potato and tomato fields from Paraná State showed low incidence of viruses. Despite the high populations of whiteflies observed in the visited potato and tomato fields, the presence of begomoviruses was not detected in both crops, whereas ToCV/Criniviruses were detected only in tomato fields from one municipality (Faxinal). Furthermore, only PVY and PLRV were detected in potato plants, but in lower incidence. However, as ToSRV and ToCV have been reported in potato fields and the high populations of the whitefly were observed in the crop, future collections should be carried out at different growing seasons to evaluate the sanity of plants once these viruses have the same vector. Additionally, in Faxinal, it is fundamental to understand the ToCV incidence combined with tomatoes' tolerance to begomovirus incidence to evaluate the production losses once the data have never been estimated in Paraná State, being a concern of tomato growers.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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