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MOLECULAR CHARACTERIZATION OF THE TOMATO CHLOROSIS VIRUS (ToCV) IN UZBEKISTAN

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SUMMARY

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop worldwide. The Tomato chlorosis virus (ToCV), transmitted by the whitefly crinivirus (family Closteroviridae), causes severe interveinal chlorosis, yellowing of leaves, and reduced tomato yields. This study took place to identify and molecularly characterize the ToCV affecting greenhouse tomato varieties in the Tashkent Region of Uzbekistan. Field studies commenced in September 2024 at three greenhouse farms located in the Gazalkent, Kibray, and Chinoz districts. Leaf samples with disease symptoms totaled 90 (30 samples each from the Cherry, Buran F1, and Alamina F1 varieties), as collected from a total cultivation area of 0.6 hectares. Symptoms included interveinal chlorosis, leaf curling, brittleness, and stunted growth. Symptoms detection appeared on the lower leaves of tomatoes. Monitoring showed 10%–36% of tomatoes sustained infection with a viral disease caused by ToCV. The application of mechanical inoculation confirmed viral infectivity. Molecular identification via RT-PCR used specific primers ToCV_F1 (5'-GAAGAGGAGTTCGAGAAGATACTC-3') and ToCV_R1 (5'-GCCGGTACCAACCATGGCT-3'), targeting a 378 bp fragment of the coat protein gene. Electrophoresis analysis of PCR products has succeeded on 1.5% agarose gel. This study provides the first molecular evidence of the presence of ToCV in Uzbekistan, laying the foundation for future epidemiological studies and breeding programs.

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Keywords: Tomato (*S. lycopersicum* L.), cultivars and hybrids, tomato chlorosis virus (ToCV), virus identification, molecular characterization, RT-PCR, RNA

Key findings: Molecular characterization confirmed the ToCV in the studied virus samples of the tomato cultivars and hybrids in Uzbekistan.

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are greatly important both in agriculture and human nutrition. The tomato is one of the most widely grown vegetable crops in the world. However, tomato production faces threats from the highest prevalence of biotic and abiotic stresses, as well as increased postharvest losses and poor agronomic practices, resulting in massive importation of tomatoes and their products to meet local demands (Nkansah *et al.*, 2021). Despite the increased annual production of tomatoes over the past five years, the tomato yield and quality gain considerable effects from an array of pests and diseases in the field and postharvest periods. According to past research reports, around 136 viral species have infected the tomato crop (Hanssen *et al.*, 2010).

Viral diseases, such as the tobacco mosaic virus (TMV) and tomato bronzing virus (TBV), can significantly affect crop yield and quality. The TMV causes leaf variegation, deformation, and reduced yields. The TBV causes bronze leaves, ring spots on fruits, and generally stunted growth. One of the new viruses impacting tomato and other Solanaceae family plants is the tomato chlorosis virus (ToCV). The virus family Closteroviridae includes almost 60 plant virus species grouped in the genera *Ampelovirus*, *Closterovirus*, *Crinivirus*, and *Velarivirus*, with also numerous unassigned species (Fuchs *et al.*, 2020). Criniviruses have segmented genomes encapsulated in separate, long, flexuous virions that occur only in the phloem and companion cells (Kiss *et al.*, 2013). Criniviruses also reach transmission via whiteflies (Hemiptera: Aleyrodidae) in a semi-persistent manner in nature (Tzanetakis *et al.*, 2013).

The identification of the tomato chlorosis virus (ToCV) first transpired in the mid-1980s on greenhouse tomato plants with the 'yellow leaf drop' syndrome in Florida, USA (Navas-Hermosilla *et al.*, 2021). Since ToCV's first reporting in 1989 in Florida, USA, it has now reached worldwide distribution, infecting multiple host plant species belonging to several plant families. These include Asteraceae, Amaranthaceae, Apocynaceae, Aizoaceae, Chenopodiaceae, Araliaceae, Malvaceae, and Portulacaceae (Shakeel *et al.*, 2017). This syndrome has been evident since 1989, but its manifestation has shown an initial association with physiological and nutritional disorders (Fiallo-Olivé and Navas-Castillo, 2019). ToCV is the typical member of the genus *Crinivirus* (family Closteroviridae) with a bipartite genome of single-stranded RNA of positive polarity (Albuquerque *et al.*, 2013). Nowadays, ToCV is an emerging virus that threatens tomato production worldwide.

Tomato plants with virus symptoms of interstitial chlorosis, collected in autumn of 1998 and in summer and autumn of 1999 in Algarve, Southern Portugal, showed positive results by testing with RT-PCR using specific primers for ToCV (Louro *et al.*, 2000). In the Mediterranean countries, the first report of ToCV was in 2000 in Spain (Navas-Castillo *et al.*, 2000). Later, ToCV also entailed identification in Greece (Dovas *et al.*, 2002), Italy (Accotto *et al.*, 2001), Israel (Segev *et al.*, 2004), France (Dalmon *et al.*, 2005), Cyprus (Papayiannis *et al.*, 2006), Lebanon (Abou-Jawdah *et al.*, 2006), Tunisia (Gharsallah *et al.*, 2015), and Turkey (Çevik and Erkiş, 2008) (Table 1).

Past studies conducted in 2006 and 2007 on the viral diseases of tomato (*S. lycopersicum* L.) occurred in three different crops grown in the Sumare Region of São Paulo State, Brazil. Their results showed a low

Table 1. ToCV isolates found worldwide.

No.	Isolate Name	Country	Year of Isolation	Host	GenBank ID
1	ToCV-ES	Spain	2004	<i>Solanum lycopersicum</i>	AY903447
2	ToCV-FL	USA (Florida)	2005	<i>Solanum lycopersicum</i>	AY903448
3	ToCV-BR	Brazil	2006	<i>Solanum lycopersicum</i>	DQ377111
4	ToCV-KR	South Korea	2010	<i>Solanum lycopersicum</i>	HQ593617
5	ToCV-IL	Israel	2009	<i>Solanum lycopersicum</i>	GU396991
6	ToCV- CN	China	2013	<i>Solanum lycopersicum</i>	KF730345
7	ToCV-GR	Greece	2008	<i>Solanum lycopersicum</i>	EU583834
8	ToCV-MA	Morocco	2012	<i>Solanum lycopersicum</i>	JQ710150
9	ToCV-TN	Tunisia	2014	<i>Solanum lycopersicum</i>	KM093245
10	ToCV-IT	Italy	2011	<i>Solanum lycopersicum</i>	JX912371

incidence of the disease (0.25% to 3.42%) in randomly distributed plants (Barbosa *et al.*, 2008). During January 2014, surveys on open field and greenhouse tomato crops in the peripheral areas of Riyadh Region, Saudi Arabia (Al-Aflaj, Al-Kharj, Al-Waseel, and Al-Dalam), were successful. In all surveyed tomato crops, yellowing symptoms were visible on the lower leaves, which had the possible infection of a whitefly-transmitted *Crinivirus* (family Closteroviridae), such as the tomato chlorosis virus (ToCV) and tomato infectious chlorosis virus (TICV) (Al-Saleh *et al.*, 2014). The ToCV is a whitefly-transmitted and phloem-limited *Crinivirus*, and in 2013, severe interveinal chlorosis and bronzing on tomato leaves, known as ToCV infection, were notable in greenhouses in Korea (Lee *et al.*, 2018).

Transmission

ToCV transmission is not through seeds, and more than 36 species of weeds have reached reporting as natural reservoirs that act as unique sources of the virus and its vectors when harvesting susceptible crops (Chrysoula *et al.*, 2016). ToCV transmission is semi-persistent by at least five different whitefly species and biotypes: *Bemisia tabaci* biotypes A, B (Wintermantel and Wisler 2006), and Q (Navas-Castillo *et al.*, 2000). However, ToCV transmission efficiency may vary through *Bemisia* species, which can refer to factors such as the number of individuals and the host plant (Ng and Falk, 2006).

The spread of the ToCV virus is only through the phloem, as transmitted by whiteflies (*Bemisia tabaci* and *Trialeurodes*

vaporariorum) in a semi-persistent manner (Fiallo-Olive and Navas-Castillo, 2019; Lee *et al.*, 2018). In addition to *B. tabaci* biotype B, the greenhouse whitefly *Trialeurodes vaporariorum* has also had reports as a vector for ToCV, although it appeared less effective than the *B. tabaci* biotype B in transmitting the virus (Barbosa *et al.*, 2008; Wisler *et al.*, 1998). ToCV has rapidly spread to tomato-growing regions; therefore, its timely diagnosis is crucial to prevent further spread and economic losses. Given the similarity in symptoms with other viral diseases, ToCV identification requires molecular techniques.

Symptoms

In tomato (*S. lycopersicum* L.) plants, ToCV symptoms initially comprise chlorotic areas that are frequently polygonal and limited by the main veins, which turn into interveinal bright yellowing. These virus symptoms mostly begin on the lower leaves and progress to the upper parts of the tomato plant (Fiallo-Olivé and Navas-Castillo, 2019). ToCV also affects a wide range of plants, such as tomatoes, peppers, and potatoes (Fiallo-Olivé and Navas-Castillo, 2019). In tomatoes, the ToCV symptoms include leaf chlorosis (yellowing), interveinal chlorosis, upward leaf curling, and growth retardation.

In the initial stage of infection, chlorotic areas often have a polygonal shape as limited by the main veins. In later stages, yellow interveinal areas may form reddish-brown necrotic spots. The lower leaves appear curled, thickened, and brittle, with a crispy texture. Although there are no obvious

symptoms on the fruit, a significant yield decrease occurs due to loss of photosynthesis area. Symptomatic plants emerged less vigorous and showed a yield loss due to reduced fruit growth and delayed ripening. In tomato plants, the ToCV symptoms were indistinguishable from those caused by the related crinivirus, such as tomato infectious chlorosis virus (TICV) (Duffus *et al.*, 1996). Plant growth slows down, causing dwarfism due to the decrease in the plant's photosynthetic area because plant growth usually acquires sufficient nutrients (Sobirova *et al.*, 2024, 2025a, and 2025b).

In ToCV severe cases, the affected tomato plants may show a significant decrease in yield and fruit quality. The ToCV spread poses a serious threat to tomato production worldwide, especially in warm-climate regions where whiteflies are actively breeding. The virus' main symptoms on the leaves were severe yellowing, curling, and brittleness (Dovas *et al.*, 2002). Plants with disease symptoms showed yellowing between the veins on older leaves, followed by general yellowing. However, disease symptoms were not evident on younger plants and fruits. In spring, *Trialeurodes vaporariorum* populations were generally abundant, while *Bemisia tabaci* became prevalent in summer and autumn (Dalmon *et al.*, 2005).

The affected tomato plants were less vigorous and produced lower yields due to slower fruit growth and delayed ripening (Navas-Castillo *et al.*, 2000). The ToCV is a typical member of the genus *Crinivirus* (family Closteroviridae), with a bipartite genome of single-stranded RNA of positive polarity (Lozano *et al.*, 2006).

MATERIALS AND METHODS

Field research and sample collection

The Cherry and F1 hybrids, Buran and Alamina, are tomato (*S. lycopersicum* L.) cultivars widely grown under greenhouse conditions in Uzbekistan. The presented study sought to accurately diagnose ToCV under

greenhouse conditions using molecular-genetic analysis based on the prevalence and specificity of disease symptoms and the CP gene of the said virus in the Tashkent Region, Uzbekistan. In this experiment, the Cherry, Buran F1, and Alamina F1 tomato cultivars served as the study objects. These cultivars reached planting in greenhouse farms in the District Chinoz, Tashkent Region.

In September 2024, the sampling conducted continued in three greenhouse farms in the District of Chinoz of the Tashkent Region, where planted tomatoes exist. The tomato plants in each greenhouse received visual inspection for symptoms specific to ToCV. For molecular-genetic analysis, the typical tomato samples with symptoms of viral infection in the 90 available plants (30 from each cultivar) underwent packing in sterile plastic bags and placing in a portable refrigerator before transporting to the laboratory.

Biological identification of the virus

Mechanical transmission of the virus proceeded using conventional methods (Vlasov *et al.*, 2016). In doing this, 100 mg of the infected tomato (*S. lycopersicum* L.) plant received 0.1 M phosphate buffer (pH 7.2) in a 1:1 ratio before its grinding in a porcelain mortar. The liquid part of the homogenate, which had reached the same mass, underwent filtration through a sterile gauze. The supernatant received centrifugation at 7,000–8,000 rpm for 15 min, with the supernatant separated after. The separated supernatant attained sprinkling with carborundum powder on the leaves of the target tomato plants, introducing the virus into the leaf cells by mechanical microtrauma through rubbing the inoculum. After an hour, the excess carbide sustained water washing. Mechanical inoculation of the plant usually occurred in the late afternoon, as the infected plants should remain in a dark place. The development of the virus symptoms on the infected plant then gained visual observations for 15–20 days, depending on the plant cultivar (Vlasov *et al.*, 2016).

Molecular diagnostics of the ToCV

Primer selection

Diagnostic ToCV primers, specific for the CP gene, succeeded in developing based on the viral genomic sequence of the ToCV-sh-SNU rna2 (mw490608.1) isolation from the GenBank database using the Primer3 software (Rozen and Skaletsky, 2000). The diagnostic primer sequences were as follows:

ToCV_F1 (GAAGAGGAGTTCGAGAAGATACTC);
ToCV_R1 (GCCGGTACCAACCATGGCT).

Isolation of total RNA from samples

The 200 mg of the collected samples underwent extraction and homogenization using liquid nitrogen. Total RNA extraction was according to the manufacturer's protocol employing the Invitrogen™ Purelink™ RNA mini kit (Thermo Fisher Scientific, USA). The total amount and quality of RNA entailed measurement with a NanoDrop Eight spectrophotometer (Thermo Fisher Scientific, USA), with the RNA samples then stored at -80 °C until performing the reverse transcription reactions.

Performing RT-PCR on isolated RNA samples

The cDNA synthesis from the isolated RNA took place by adding 5 µl of RNA to a mixture of ToCV_R1 primer, SuperScript IV reverse transcriptase (Thermo Fisher Scientific, USA), and 20 µl of final volume buffer. The T960 PCR amplifier in 25 µl PCR tubes sustained incubation at 37 °C for one hour and then heated at 70 °C for 10 min to stop the synthesis of cDNA.

The PCR performed for the CP ToCV gene used specific diagnostic primers (ToCV_F1 and ToCV_R1), as well as the Platinum™ II Hot-Start PCR Master Mix (2×) reagent kit (Thermo Fisher Scientific, USA), for amplifying the CP gene in the Miniamp Tmplus DNA amplifier (Thermo Fisher Scientific, USA). The amplifier then reached programming as

follows: initial denaturation at 94 °C for 2 min; 45 cycles of 30 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C for 5 min. The expected product size after PCR was 378 bp.

RESULTS AND DISCUSSION

Field surveys conducted in September 2024 revealed widespread ToCV symptoms in three greenhouse farms across Gazalkent, Kibray, and Chinoz districts of Tashkent Region. The virus incidence was evident in all surveyed greenhouses covering 0.6 hectare of total area planted with Cherry, Buran F1, and Alamina F1 tomato cultivars. Disease symptoms were initially visible on lower leaves as polygonal chlorotic areas limited by main veins, progressing to interveinal bright yellowing and upward curling. In advanced stages, leaves developed reddish-brown necrotic spots and became thickened and brittle with a crispy texture. Young leaves showed mosaic chlorosis and blistering. Symptomatic plants exhibited reduced vigor, delayed fruit ripening, and significant yield decrease due to reduced photosynthetic area. No fruit symptoms were apparent. Mechanical inoculation successfully transmitted the virus to healthy tomato plants, with symptoms appearing 15–20 days post-inoculation depending on cultivar susceptibility.

The frequent occurrence of such symptoms on the leaves is important because it revealed the tomato plant was relatively immune to ToCV infection. Young plant leaves appeared highly susceptible to viral infection, which leads to the development of noticeable mosaic chlorosis and blisters on the leaf surface. It has been noticeable that the symptoms worsen as the leaves grow and spread throughout the tomato plants (Figure 1).

Determining the disease type comprised samples taken from infected tomato plants and an infectious suspension prepared in the laboratory being mechanically grafted onto healthy samples of a laboratory-grown tomato cultivar (Figure 2). In identifying ToCV in the collected samples, RNA extraction came from those tomato leaves showing symptoms of



Figure 1. Symptoms of ToCV-specific disease in Alamina F1 tomatoes. a-c) Complete chlorosis of the leaves in infected tomato plants and b) ToCV disease symptoms on the top of leaves of tomato plants.



Figure 2. ToCV-specific disease symptoms caused by artificial infection.

chlorosis, as described in the 'Material and Methods' section, with the virus genome amplified using RT-PCR. The obtained PCR products proceeded to analysis by gel electrophoresis in a 1.5% agarose gel with a 1xTBE buffer (pH = 8.3), with the gel stained with a solution of 0.5 $\mu\text{g/ml}$ of ethidium bromide (EtBr). Visualization and photographic documentation of PCR results ensued using a UVT365 transilluminator and the microdoc compact gel documentation system (Clever Scientific Ltd., UK) (Figure 3).

The molecular weight symbol '2000 bp Plus DNA ladder' (Thermo Fisher Scientific, SSHA) served to determine the length of PCR

products. The collected samples, as tested by PCR for the presence of the CP ToCV gene with 380 bp, showed a positive result, which confirms the presence of ToCV (Figure 3). This study also examines the spread of the ToCV virus in the various regions of Uzbekistan, which poses a serious threat to tomato production. The presented results will provide valuable insights into the global dynamics of the virus's spread and can contribute to the development of future strategies to combat this disease. In previous years, this virus has become a serious threat for tomato production and its export quality.

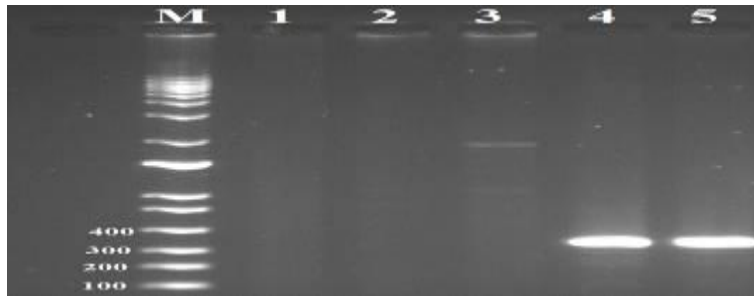


Figure 3. Electropherogram of the PCR product obtained using the special primers ToCV_F1 and ToCV_R1 (380 bp). M-2000 BP DNA Ladder Plus. 1) control (sample with water instead of DNA), 2) Cherry cultivar, 3) Buran F1, and 4 and 5) Alamin F1.

ToCV has rapidly extended its geographical as well as host range within the last decade, and its association with *B. tabaci* has inflicted substantial loss of crop yields all over the world (Barbosa *et al.*, 2008; Çevik and Erkis, 2008). The primary symptoms of ToCV infection include interveinal chlorosis, leaf curling, and yellowing, which progressively lead to leaf senescence and diminished photosynthetic capacity (Tzanetakis *et al.*, 2010). These symptoms often resemble those caused by other viral pathogens, complicating field diagnosis and necessitating reliable molecular methods for accurate identification and assessment (Kucharek *et al.*, 2000).

The ToCV symptoms were similar to those associated with magnesium deficiency in tomato plants. However, the chlorosis displayed a tendency to move up, affecting medial and apical leaves, and prevailed even after supplementary magnesium application to the soil, indicating potential infection by either Tomato chlorosis virus (ToCV) or Tomato infectious chlorosis virus (TICV) (Arruabarrena *et al.*, 2014). Molecular diagnosis and characterization have become the cornerstone of ToCV identification, with RT-PCR and real-time quantitative PCR (qPCR) methods providing sensitive and specific detection of viral RNA in infected plant tissues (Wintermantel and Wisler, 2006). Recent advances include the development of multiplex PCR assays capable of simultaneously detecting ToCV and other co-infecting viruses,

thus improving diagnostic efficiency (Velázquez *et al.*, 2014).

Serological methods, such as ELISA, also entailed employment; however, their sensitivity was often lower than the molecular techniques, especially in early infection stages when viral titers were low (Marais *et al.*, 2008). The identification of ToCV vectors, primarily whiteflies (*B. tabaci* and *T. vaporariorum*) has further informed epidemiological studies and control strategies. Phylogenetic analyses based on coat protein gene sequences revealed genetic variability among the ToCV isolates worldwide, highlighting that molecular tools are not only crucial for detection but also for understanding virus evolution and various spread patterns (Navas-Castillo *et al.*, 2011). This genetic diversity poses challenges for developing universal diagnostic assays and resistant tomato cultivars.

The primary means of ToCV transmission, whiteflies (*B. tabaci* and *T. vaporariorum*) are widespread and capable of efficiently spreading the virus over long distances. Consequently, vector control remains a key component in ToCV management. Chemical insecticides have shown some effectiveness; however, their overuse contributes to the development of resistance in whiteflies and environmental concerns (Navas-Castillo *et al.*, 2011). In contrast, integrated pest management (IPM) strategies—including biological control, use of reflective mulches, and cultural methods, such

as crop rotation—have demonstrated promise in reducing whitefly populations in a more sustainable manner (Diaz-Pendon *et al.*, 2010). Host plant resistance remains a critical long-term solution. However, as of now, no commercially available tomato cultivars exist with strong and stable resistance to ToCV. Breeding programs are actively exploring sources of tolerance and resistance genes, often using wild *Solanum* species as donors (López *et al.*, 2012). Molecular breeding and CRISPR/Cas9-based genome editing offer new opportunities to accelerate the development of resistant tomato lines, though these approaches are still in early stages for ToCV.

Despite these strategies, ToCV continues to persist due to its complex epidemiology, the polyphagous nature of its vectors, and the absence of considerable natural resistance in cultivated tomatoes. The results further underscore the need for an integrated approach that combines diagnostic innovation, ecological vector suppression, and breeding of resilient tomato cultivars. Early and accurate detection is also essential for limiting ToCV spread. Molecular diagnostics, particularly RT-PCR and qPCR, have proven to be vital for early-stage identification, especially when plants are asymptomatic (Wintermantel and Wisler, 2006). Their use supports effective quarantine and eradication measures and prevents widespread outbreaks. These study results demonstrated the importance of recognizing the symptoms of viral diseases, as well as the role of molecular diagnostics in viral diseases.

CONCLUSIONS

Based on virological observations, the tomato (*S. lycopersicum* L.) plants infected with ToCV exhibited symptoms characteristic of ToCV, such as chlorosis, yellowing of leaves, and general growth retardation. The genetic material of the virus succeeded in its identification using the RT-PCR method, confirming the highest accuracy. The results showed ToCV poses a serious threat to tomato production in some regions of Uzbekistan. We recommend strengthening phytosanitary

measures, such as early detection of ToCV, the use of healthy planting material, and the control of the whitefly vector.

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