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Evaluation of the pathogenicity of three isolates of Tomato brown rugose fruit virus in tomato plants (*Solanum lycopersicum* L.) from Coahuila, Mexico

Evaluación de la patogenicidad de tres aislados del Tomato brown rugose fruit virus en plantas de tomate (*Solanum lycopersicum* L.) de Coahuila, México

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ABSTRACT

Tomato brown rugose fruit virus (ToBRFV) poses an emerging threat and represents a significant economic risk in tomato production. This study aimed to evaluate three ToBRFV isolates obtained from greenhouses in Coahuila, Mexico, to assess their pathogenicity in tomato plants of the variety 172-300 and establish a diagrammatic scale for accurately visualizing the severity of the symptoms in leaflets and fruits. Three ToBRFV isolates (TB1, TQ2, and FQ3) were obtained from greenhouse tomatoes and used for pathogenicity tests on the hybrid 172-300 under greenhouse conditions. The FQ3 isolate exhibited a greater impact on the agronomic variables of tomato plants compared to the other isolates, resulting in a 53.9% decrease in plant height and a 38.9% reduction in the dry weight of the aerial plant part. Regarding fruit quality variables, the presence of FQ3 led to a reduction of 43.1% in equatorial diameter and a 43.2% decrease in yield. Furthermore, FQ3 caused an incidence ranging from 33.9% to 50% and a severity ranging from 41% to 87.5% compared to the other isolates. These findings underscore the importance of understanding and managing the variability in the response of tomato plants to different ToBRFV isolates. In this context, the use of hybrids emerges as an effective option for mitigating the severity levels of the virus.

KEY WORDS: Incidence, Mexico, Agronomic parameters, Severity, ToBRFV.

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RESUMEN

El virus de la fruta rugosa marrón del tomate (ToBRFV) es una amenaza emergente ya que representa un riesgo económico en la producción de tomate. El objetivo fue evaluar tres aislados del ToBRFV provenientes de invernaderos de Coahuila, México, para evaluar su patogenicidad en plantas de tomate variedad 172-300, y establecer una escala diagramática que permita una visualización precisa de la severidad de los síntomas en folíolos y frutos. Se obtuvieron tres aislados del ToBRFV (TB1, TQ2 y FQ3) procedentes de invernaderos de tomate, y se han empleado para realizar pruebas de patogenicidad en el híbrido 172-300 en el invernadero. El aislado denominado FQ3 tuvo un impacto mayor en las variables agronómicas de las plantas de tomate en comparación con los demás aislados, se obtuvo una disminución del 53.9 % en la altura de la planta y del 38.9 % en el peso seco de la parte aérea de la planta. En las variables de calidad del fruto, se observó una reducción del 43.1 % en el diámetro ecuatorial y del 43.2 % en el rendimiento, debido a la presencia de FQ3. Además, FQ3 generó una incidencia que osciló entre el 33.9 al 50 % y una severidad entre el 41 al 87.5 % en comparación con los otros aislados. Estos descubrimientos demuestran la importancia de comprender y gestionar la variabilidad en la respuesta de las plantas de tomate a diversos aislados de ToBRFV. En este contexto, el uso de híbridos se presenta como una opción eficaz para preservar niveles de severidad reducidos del virus.

KEY WORDS: Incidencia, México, Parámetros agronómicos, Severidad, ToBRFV.

Introduction

Tomato (*Solanum lycopersicum* L.) plays an essential role in the human diet due to its nutritional contribution, positioning it as a vegetable of national and international importance (Ruíz-Aguilar *et al*., 2023). In Mexico, the areas of greatest production are located in the Bajío and northwestern regions of the country; in 2019, approximately 90% of exports went to the United States (Sandoval-Cabrera & Borja-Rodríguez, 2023).

The northern region of Coahuila state has been characterized as the main melon producer (Espinoza-Arellano *et al*., 2023). However, rising costs and the absence of financing from the state government caused a reduction in credits to producers and, consequently, a decrease in cultivated

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hectares (Espinoza-Arellano *et al*., 2019). This situation led to the transition to tomato production systems in protected agriculture (Orona-Castillo *et al*., 2022). In 2020 alone, in the northern region of Coahuila, 718 Ha were cultivated with a production of 112,180.25 tons and an average yield of 156.24 kg ha^{-1}, highlighting the production in the protected agriculture system (SIAP, 2020). The Servicio de Información Agroalimentaria y Pesquera (SIAP) reported that during 2022 there was a 15.7% increase in tomato production yields in Coahuila state (SIAP, 2023).

According to an economic analysis by Orona-Castillo *et al*. (2022), it is observed that three out of five agricultural units export between 74% and 85% of their production to the United States. Likewise, the average production cost per kilogram of tomato is estimated to be 4.02 MXN, and it is suggested that as the cultivated area increases, costs decrease. These factors highlight the importance of tomato cultivation in protected agriculture in the producing regions of Coahuila state.

Among the restrictions affecting the production of this crop are systemic pathogens, especially seed-borne and mechanically transmitted viruses (González-Concha *et al*., 2023; Salem *et al*., 2022). Tobamoviruses are the most important pathogens for agriculture given their genetic diversity, transmission mechanisms, adaptation, interaction, and host range (Aiewsakun & Katzourakis, 2016). Most viruses of the Tobamovirus genus affect the Solanaceae family, resulting in significant crop losses (Caruso *et al*., 2022). Tomato brown rugose fruit virus (ToBRFV) was first detected in Jordan in 2015 and is currently considered a global threat to tomato production (Zhang *et al*., 2022). Due to its epidemiology, it rapidly spread worldwide (Van de Vossenberg *et al*., 2020). In Mexico, it was first identified in tomato plants located in Ensenada, Baja California state, and later in Yurécuaro, Michoacán state (Camacho-Beltrán *et al*., 2019; Cambrón-Crisantos *et al*., 2019). ToBRFV has been dispersed in various regions of Mexico since its detection, showing variations in incidence and severity depending on the isolates collected both in greenhouses and in the field (Cambrón-Crisantos *et al*., 2019; Vásquez-Gutiérrez *et al*., 2023[a]).

The effort to develop materials with superior phenotypic attributes, such as yield and fruit quality, may increase vulnerability to emerging viral diseases, such as ToBRFV (Ashkenazi *et al*., 2018). The study of genotypes with tolerance and resistance denotes an option for genetic control against ToBRFV. Recent research suggests that natural resistance genes in tomato varieties are more effective alternatives to prevent viral diseases (Shi *et al*., 2011). Kabas *et al*. (2022) evaluated resistance to ToBRFV in 11 interspecific hybrids, finding that all showed high susceptibility, with severity ranging from 54.2% to 100%. This phenomenon is possibly due to the loss of ToBRFV-resistant genes during genetic exchange, resulting in increased vulnerability to the virus (Jewehan *et al*., 2022[a]).

The pathogenicity of ToBRFV isolates varies depending on the environmental conditions of the crop (Davino *et al*., 2020). Under appropriate conditions, noticeable symptoms such as severe interveinal yellowing, deformations, mosaics, and necrosis occur; in fruits, it causes mottled spots, incomplete ripening, and, in advanced stages, brown roughness (Zhang *et al*., 2022). Therefore, this study aimed to isolate ToBRFV from three tomato varieties of Coahuila state, Mexico. To assess the viral pathogenicity in tomato plants, a diagrammatic scale was developed to allow accurate visualization of the severity of symptoms in leaflets and fruits. Finally, the study was

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addressed to corroborate that the variety 172-300 (Yüksel) has genetic potential for cultivation in greenhouses in Coahuila state.

Material and Methods

Sampling and identification of Tomato brown rugose fruit virus

Collections were made in commercial greenhouses, encompassing three tomato varieties. Sample TB1 was obtained from Nunhems' Blindon F1 variety, sample TQ2 from Enza Zaiden's Azores variety, and sample FQ3 from Enza Zaiden's Quiroga variety. The samples were collected from the General Cepeda municipality, Coahuila, Mexico (25°19'04.0" N 101°24'10.6" W). The collection was conducted in a four-hectare area, with 200 leaflets per hectare (one leaflet per plant). To identify ToBRFV symptoms, the pictorial keys proposed by Luria *et al*. (2017) and Cambrón-Crisantos *et al*. (2019) were used.

Tomato brown rugose fruit virus detection

As a first step for detection, a serological assay was conducted at the sampling sites using the ImmunoStrip test (Agdia). Subsequently, positive samples were transported to the Phytopathology Laboratory of the Agricultural Parasitology Department and stored in a refrigerator at a controlled temperature of 5°C for five days until experimental evaluation (Chanda *et al.,* 2021).

Molecular identification was carried out following the protocol outlined by Ortiz-Martínez *et al*. (2022), using 100 mg of infected tissue to extract RNA from samples TB1, TQ2, and FQ3, along with a healthy control (-). Additionally, an infected control with ToBRFV (+), provided by a phytosanitary inspection unit certified by the Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA) of Coahuila state, was included. The RNA Isolation System kit (Promega) was employed for RNA extraction and quality quantification by spectrophotometry using a NanoDrop 2000 (Thermo Scientific). To assess the RNA integrity of samples, 1% agarose gel electrophoresis (Invitrogen) was performed.

cDNA from the endogenous gene was utilized in the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method, employing the oligonucleotides ToBRFV-F (5'-AACCAGAGTCTTCCCTATACTCGGAA-3') and ToBRFV-R (5'-CTCACCATCTCTCTTAATAATCTCCT-3'), designed to amplify a 475 bp fragment (Rodriguez-Mendoza *et al.,* 2019). The reaction conditions comprised 12.5 µL Master mix (Platinum), 1.25 µL of both oligonucleotides (ToBRFV-F and ToBRFV-R), 5 µL SuperFi GC (Thermo Fisher), 2 µL cDNA, and 3 µL molecular grade water (Invitrogen), in a total volume of 25 µL. The following program was executed on the thermal cycler: an initial cycle at 98 °C for 90 s, followed by 35 cycles at 98 °C for 10 s, 55 °C for 20 s, 72 °C for 40 s, and finally one cycle at 72 °C for 5 min. The resulting amplification products were visualized on a 2% agarose gel. Additionally, an analysis was conducted on the obtained samples (TB1, TQ2, and FQ3) through a certified laboratory to rule out the presence of other viruses and confirm specific ToBRFV infection (Table 1).

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Table 1. External evaluation of infection and confirmation of ToBRFV in samples.

Virus	Analyzed samples			Detection methods	
	TB1	TQ2	FQ3	ELISA	RT-PCR
Tomato brown rugose fruit virus (ToBRFV)	Positive	Positive	Positive	NR	R
Cucumber mosaic virus (PepMV)	Negative	Negative	Negative	NR	R
Tobacco mosaic virus (TMV)	Negative	Negative	Negative	R	NR
Tomato spotted wilt virus (TSWV)	Negative	Negative	Negative	R	NR
Cucumber mosaic virus (CMV)	Negative	Negative	Negative	R	NR

The analysis was performed at Labfrusco, S.A. de C.V., a certified laboratory following the quality management system accredited to Mexican standard NMX-EC-17025-IMNC: 2017 (ISO/IEC 17025:2017). The R notation indicates that the analysis was performed, while NR indicates that it was not performed.

ToBRFV pathogenicity assay in tomato plants

To achieve an inoculum at a concentration of $1x10^1$ (w/v) suitable for treatment application, the method outlined by Vásquez-Gutiérrez *et al*. (2024) with modifications, was followed. Symptomatic tissue from samples TB1, TQ2, FQ3, and the positive control were macerated in a cold-sterilized mortar. Phosphate buffer solution (PBS) at pH 8 (0.01 M) was used, and Celite (Sigma Aldrich) was added as an abrasive.

In a greenhouse of the Agricultural Parasitology Department, tomato plants of variety 172-300 (Yüksel), 30 days post-emergence (dpe), were transplanted into polyethylene bags (25x25 cm) with peat moss as a substrate. The treatments included an absolute control (AC), a positive control (PC) infected with ToBRFV, and the isolates (TB1, TQ2, and FQ3) at a concentration of $1x10^1$ (p/v). Inoculation was performed 10 days after transplanting (dpt) on the first three true leaves using a swab impregnated with an inoculum of TB1, TQ2, FQ3, and PC, while AC alone was treated with sterile tap water (STW). For nutrient supply, Steiner's 50% nutrient solution (Steiner, 1961) was used. Symptom observation was conducted nine days after inoculation (dai) to determine the incubation period and to evaluate the incidence and severity of infected plants. Agronomic variables were recorded at 30 dai, including plant height (PH) in cm with a flexometer, stem diameter (SD) in mm with a vernier caliper, dry root weight (DRW), and aerial part dry weight (PDW) in grams with an analytical balance (Ohaus). Fruit quality variables were assessed at 40 dai, including polar diameter (FPD) and equatorial diameter of the fruit (FED) in mm with a Vernier caliper, as well as the yield of each treatment (Y) in grams with an analytical balance (Ohaus). Plants infected with ToBRFV (PC) showed a 100% mortality rate, resulting in no yield. For fruit uniformity, the fruit index (FI) was calculated using the formula proposed by Sanchez (2019):

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$$FI = \left(\frac{Equatorial\ diameter}{Polar\ diameter}\right)$$

Severity scale design

A diagrammatic severity scale (Table 3) was developed to evaluate the percentage of damage in the leaf area, following the methodology outlined by Ortiz-Martínez *et al*. (2022) with modifications. Observations began at nine dai, and sampling was conducted every 15 days from the detection of the first symptoms until plant death. In each sampling and treatment, five leaflets with varying degrees of severity were selected. The leaflets were digitized using an MP C2003 PCL6 printer (RICOH), and ImageJ 1.53t software (NIH, USA) was employed to calculate the total area of each leaflet as well as the affected area.

Regarding the percentage of fruit severity, the data were transformed into arcsine for normalization, and eight classes were designed using the statistical program InfoStat, version 9.0. The evaluation of the fruit damage scale covered the period from the beginning of the harvest to the end of the trial. Fruit collection was conducted at 40 dai, with three weekly collections over 30 days. In each sampling, 10 fruits per treatment were selected, each representing a different level of viral damage. These levels were classified according to severity and represented in the diagrammatic scale previously used for leaflets. The severity was calculated using the formula proposed by Vásquez-Gutiérrez *et al*., (2023[b]).

$$Severity = \left(\frac{Affected\ area}{Total\ area}\right) x100$$

Data analysis

The experiment was established using a completely randomized design with 10 replicates per treatment, and each experimental unit consisted of one plant per bag. All evaluated variables underwent an analysis of variance. A Tukey test ($p \leq 0.05$) was employed for means comparisons using InfoStat 9.0 software.

Results and Discussion

Tomato and bell pepper seeds are suspected to be primarily responsible for the worldwide spread of ToBRFV disease (Salem *et al*., 2022). This may be explained by the susceptibility of seeds from foreign commercial houses to the pathogen (Jewehan *et al*., 2022[b]), which also serves as a reservoir of viral particles, acting as a source of inoculum for virus spread in producing areas of Mexico (González-Concha *et al*., 2019). Currently, resistant genotypes have been identified, such as the Tolerant VC532 and Resistant VC554 genotypes, but they are not yet commercially

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available in our country (Zinger *et al*., 2021). The relevance of ToBRFV lies in its ability to eliminate the acquired genetic resistance by tomato plants against tobamovirus attack, by suppressing R-type genes Tm-1, Tm-2, and Tm-2^2 (Zhang *et al*., 2022). Therefore, the selection of a tomato variety tolerant to ToBRFV infection could be employed for tomato production in Coahuila regions.

Figure 1 below shows the results of the ImmunoStrip test, and the RT-PCR products obtained from the performed assays, compared with the molecular weight marker (1 kb, Plus Invitrogen™), confirming the ToBRFV presence.

Strategies for ToBRFV identification that require reduced time and higher specificity contribute to preventing high infection levels in tomato crops (Rodriguez-Mendoza *et al*., 2019). Likewise, the analytical sensitivity of serological tests such as ImmunoStrip (ranging from 64 to 320 pg/mL) provides high specificity in the diagnosis of various ToBRFV isolates of up to 100 % (Eads *et al*., 2023). Hence, the importance of using ImmunoStrip for both field monitoring and rapid diagnosis followed by its confirmation by RT-PCR technique is highlighted, as detailed in Figure 1. The use of the immunoStrip test not only prevents but also accelerates detection at the early stages of the crop, which offers significant benefits to growers. This is explained as they prefer the cost of a rapid test for virus detection with a cost of $191 per unit, compared to RT-PCR diagnosis, which ranges from $1000 to $3000 of cost per sample. In addition, it is essential to identify ToBRFV-infected parts, as these represent a source of inoculum that can spread the disease throughout the production area. The implementation of this measure will contribute to strengthening hygiene practices in the growing areas, thus reducing the risks of dissemination to other tomato-growing regions (Klap *et al*., 2020).

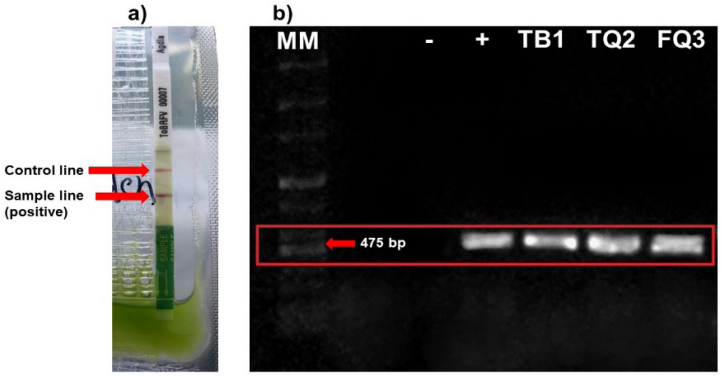

Figure 1. Diagnostic methods for ToBRFV infection in tomato plants.

a) ImmunoStrip test (Agdia) and b) RT-PCR, where MM=Molecular Marker 1 kb (Plus Invitrogen), (-) = negative healthy control, (+) = positive ToBRFV control, and TB1, TQ2, and FQ3 represent the results of the isolates.

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ToBRFV pathogenicity assay in tomato plants

In the evaluation of agronomic parameters (Table 2), it was evident that FQ3 exhibited the most significant reduction in PH (49.48 cm), SD (14.08 mm), DRW (59 g), and PDW (280 g). Meanwhile, TB1 showed decreases to 57.87 cm in PH, 15.53 mm in SD, and 71 g in DRW. This situation can be attributed to the presence of avirulence genes in FQ3 and TB1 isolates, as the seeds were obtained from different commercial companies (Avni *et al.,* 2019). Additionally, it was observed that FQ3 exhibited higher incidence and severity compared to TB1 and TQ2 in the greenhouses before the study.

Table 2. Physiological parameters of tomato plant varieties 172-300 infected with different ToBRFV samples 30 days post-inoculation.

Treatments	Evaluated variables			
	PH (cm)	SD (mm)	DRW (g)	PDW (g)
TB1	57.87±1.36^{b}	15.53±2.14^{abc}	71±3.89^{bc}	310±3.53^{b}
TQ2	51.28±3.18^{c}	14.98±1.98^{cd}	70±3.45^{bc}	311±3.65^{b}
FQ3	49.48±1.23^{cd}	14.08±1.36^{cd}	59±3.37^{d}	280±3.19^{bc}
AC	107.38±1.39^{a}	17.47±2.95^{a}	88±4.18^{a}	457±4.38^{a}
P-value		0.0001		

Means with the same letter in each column show no significant differences according to Tukey's test ($p \leq 0.05$).
PH: plant height; SD: stem diameter; DRW: dry root weight; PDW: plant dry weight.

An inherent challenge of tobamoviruses lies in their ability to persist in soil or irrigation water, facilitating their rapid spread and prolonged periods of infection (Ortiz-Martínez *et al.,* 2022). These viruses can spread through leaf wounds, root contact, contaminated seeds, and nutrient solutions. Currently, these factors represent critical points that contribute to rapid infection by ToBRFV (Panno *et al.,* 2020), resulting in a decrease in agronomic variables and fruit quality, as shown in Tables 2 and 3.

PH experienced remarkable changes in all three treatments, especially with FQ3, which decreased by 53.9% compared to AC. This finding aligns with the research of Takács *et al*. (2001), in which TMV infection reduced the growth of tomato plants. Regarding SD, it was observed that the isolate FQ3 showed greater affectation, followed by TQ2, due to the severity caused in tomato plants. This phenomenon is consistent with the results of Bhat and Rao (2020), who reported that virus-infected plants exhibited reductions in plant physiological variables. While no statistically significant differences were observed among isolates for DRW and PDW, it has been found that FQ3 exhibits a more significant effect on these variables. This is in agreement with

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the findings of Rys *et al*. (2014), who reported that the *obuda pepper virus* (OPV) affects the photosynthetic apparatus of plants, reducing light uptake through leaves and causing a reduction in leaf bud proliferation in plants. The detailed analysis of agronomic variables is essential for effective disease management, preventive measures implementation, loss quantification, and the ToBRFV-resistant tomato varieties development (Eads *et al.,* 2023; Caruso *et al.,* 2022; Zhang *et al.,* 2022). Additionally, the reduction in plant biomass due to viral attacks serves as an infection severity indicator and the damage incurred by plants (Pagán *et al.,* 2007).

Regarding fruit quality variables (Table 3), it is observed that the FQ3 treatment is associated with a statistically significant difference, exhibiting the lowest values in FED and Y, registering 3.31 mm and 85.6 g, respectively. Additionally, this treatment also affects FPD (3.22 mm) and FI (1.03 mm) compared to other treatments.

Table 3. Quality parameters of tomato fruit variety 172-300 infected with different ToBRFV samples 40 days after inoculation.

Treatments	Variables evaluated			
	FPD (mm)	FED (mm)	FI (mm)	Y (g)
TB1	4.17±0.94^{b}	5.12±1.41^{bc}	1.23±0.89^{b}	131.2±4.39^{bc}
TQ2	3.52±0.86^{bc}	4.12±0.98^{cd}	1.17±0.83^{bc}	125.4±4.17^{cd}
FQ3	3.22±0.85^{cd}	3.31±0.87^{of}	1.03±0.81^{c}	85.6±3.43^{of}
AC	4.36±0.98^{a}	5.82±1.67a	1.33±0.96^{a}	150.8±5.13^{a}
P-value	0.0001	0.0002	0.0001	0.0001

Means with the same letter in each column show no significant difference according to Tukey's test ($p < 0.05$).
FPD: fruit polar diameter; FED: fruit equatorial diameter; FI: fruit index; Y: yield of each treatment.

The adverse effects of ToBRFV on various organs of the tomato plant, including male reproductive organs, facilitate infection and decrease fruit quality (Avni *et al.,* 2022). Furthermore, ToBRFV disrupts homogeneity in fruit size and color (Menzel *et al.,* 2019). This explains why FQ3, followed by TQ2 and TB1, significantly reduced polar and equatorial fruit diameter, notably affecting quality and size (FI). Additionally, studies have documented yield losses in tomatoes due to ToBRFV ranging from 40% (González-Concha *et al.,* 2021) to as high as 70% (Ortiz-Martínez *et al.,* 2022). Avni *et al*. (2019) revealed, through fluorescence in situ hybridization (FISH), that tomato genotypes carrying the resistance gene Tm-2^2 experienced a yield decrease of 55%, whereas tomatoes of the TOP-2299 cultivar (with unknown genes) exhibited a decrease of 40% (González-Concha *et al.,* 2023). According to the results presented in Table 3, the present study indicates that the yield decrease ranged from 12.9% (TB1) to 43.1% (FQ3) depending on the inoculated isolate. These findings are consistent with the aforementioned reports.

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Severity Scale

A diagrammatic EDS-ToBRFV severity scale was developed based on various ToBRFV isolates (Table 4), covering a severity range from 0 to 80.54. The isolates exhibited different levels of pathogenicity, resulting in eight classes to quantify the degree of damage. Specifically, classes 1 to 3 correspond to isolate TB1, which presented the lowest severity in leaflets and fruit. Classes 3 to 5 are associated with TQ2, while classes 4 to 8 pertain to isolating FQ3, which caused greater severity.

Table 4. The severity scale for leaflets and fruits of tomato 172-300 variety infected with ToBRFV.

Class	Severity[a]			**ToBRFV reaction**
0	0			Healthy
1	1.81 - 10.47			Very slight
2	10.47 - 28.86			Minimum severity
3	28.86 - 34.57			Moderate severity
4	34.57 - 43.33			Severe

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Continuation

Table 4. The severity scale for leaflets and fruits of tomato 172-300 variety infected with ToBRFV.

Class	Severity[a]			ToBRFV reaction
5	43.33 -51.30			Very severe
6	51.30 - 64.23			Moderately aggressive
7	64.23 - 72.05			Aggressive
8	72.05 - 80.54			Very aggressive

[a] arcsine.

The development of a diagrammatic severity scale provides an important standardized tool for accurately assessing and diagnosing ToBRFV infection in tomato cultivars (González-Concha *et al*., 2023). Additionally, this scale enables continuous observation of infection evolution, facilitating the implementation of control measures such as selecting resistant genotypes and applying effective management strategies (Luria *et al*., 2017). In the study by Kabas *et al*. (2022), 44 accessions of wild and commercial tomato species were utilized to establish a scale of symptom severity and disease reaction to ToBRFV infection at 30 dai, resulting in the classification of symptoms into three severity categories. Similarly, Jewehan *et al*. (2022[a]) designed a scale with five severity categories starting at 15 dai using 173 wild tomato accessions. However, their scale does not include specific ranges for symptom severity caused by ToBRFV. González-Concha *et al*. (2023) developed a scale using leaflets of TOP-2299 and MACIZO cultivars, establishing five categories based exclusively on qualitative features. The scale designed in this study for evaluating the severity caused by ToBRFV is fundamental in phytopathological research since it

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provides a more accurate diagnosis of the disease and allows quantitative monitoring, facilitating effective decision-making.

The different isolates of ToBRFV were inoculated on tomato plants (Table 5), revealing that FQ3 exhibited a higher incidence (90%) and severity (63.43%) than other isolates. Additionally, the incubation period (IP) was shorter, with nine dai in FQ3.

Table 5. Incidence and severity of the different isolates of ToBRFV in tomato plants variety 172-300.

Isolated	Incidence	Class	IP (dai)	Severity[a]
PC	90	4-8	9	90
TB1	60	1-3	12	33.83
TQ2	67.21	3-5	11	45
FQ3	90	4-8	9	63.43
AC	0	0	0	0
P-value	0.0003			0.0001

IP: incubation period; dai: days after inoculation. [a]arcsine.

Ortiz-Martínez *et al*. (2022) reported that the ToBRFV inoculation of eight different chili bell pepper varieties produced an IP between 6 and 11 dai, as well as severity ranging from 20 to 57%. These results highlight the connection between the chili bell pepper variety and the infection severity, together with its influence on the affected leaf area. Remarkably, our findings revealed similarities in the incubation period, albeit with higher severity levels. Gaafar and Ziebell (2020) indicated that plants respond to biotic stress by activating an RNA silencing mechanism in the cytoplasm, involving microRNAs of 21 to 24 nucleotides that suppress tobamovirus RNA transcription. In tomato plants, ToBRFV inoculation activates a hypersensitive response (HR), similar to other tobamoviruses (Luria *et al.,* 2017). As depicted in Tables 4 and 5, the three isolates exhibited distinct behaviors, despite TQ2 and FQ3 originating from plants grown in the same area and supplied by the same company. This divergence may be attributed to avirulence (Avr) genes, particularly the 50 kDa helicase domain (TMV P50), involved in interactions among P50 domains, N receptors, and the NRIP1 protein (Avni *et al.,* 2019).

However, ToBRFV has demonstrated the ability to evade resistance conferred by dominant R-type genes such as *Tm-1*, *Tm-2*, and *Tm2*^{2} in tomato plants (Zhang *et al.,* 2022), potentially explaining the significant variations in incidence and severity observed among the isolates in this experiment. Moreover, climatic factors also influence the ToBRFV pathogenicity. Klap *et al*. (2020) showed how temperature affects ToBRFV infection, with lower incidence occurring at daytime temperatures of 16 to 20 °C, and symptoms appearing at 35 dai. Recently, Nolasco-García *et al*. (2023) analyzed 22 bioclimatic variables as predictors for ToBRFV incidence and severity, finding that rainfall (27.7%) and ambient relative humidity (26.4%) contribute significantly to virus spread.

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These findings align with our study, where the studied isolates were exposed to temperatures ranging from 26 °C at the beginning of the crop to 38 °C during the fruiting stage, showing a significant increase in virus severity with rising temperatures.

In leaves of *Nicotiana tabacum*, ToBRFV dissemination is attributed to movement proteins (MP), which facilitate movement through leaf plasmodesmata. These proteins, belonging to plasmodesmata-located protein 5 (PDLP5), are closely associated with the cell wall (Kutsher *et al.,* 2021). This infection mechanism by ToBRFV leads to increased infection severity (Table 5) and accumulation of viral load, affecting plant leaflets (Table 4).

In the study by Jewehan *et al*. (2022[a]), it was observed that different isolates of ToBRFV from Jordan had varying effects on disease expression in tomato plants. Some isolates led to mild symptoms, including blockage in root development, necrosis, and stem deformations, as depicted in Figure 2a.

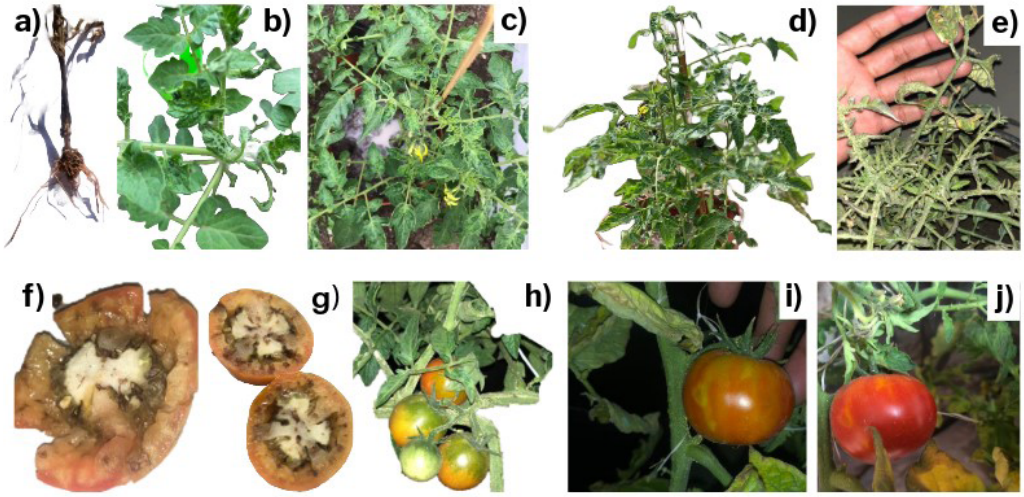

Figure 2. Symptoms produced by the different isolates of ToBRFV in tomato plants variety 172-300.

a) root damage, b-e) leaflet damage, f-g) necrosis in fruit locules, and h-j) irregular ripening and slight brown spots in fruits. Source: own, taken by Ubilfrido Vásquez-Gutiérrez

In *N. benthamiana* and tomato plants infected with ToBRFV, specific key amino acids (A^{134}, I^{147}, H^{67}, N^{125}, and K^{129}) present in ToBRFV have been identified to play a crucial role in overcoming *Tm2*^{2} gene resistance (Yan *et al*., 2021). Additionally, Jewehan *et al*. (2022[a]) observed that symptomatic leaves in tomato and *N. glutinosa* plants inoculated with ToBRFV exhibited higher

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levels of viral nucleic acid compared to infected plants without visible symptoms. This resulted in visual symptoms such as leaf curling, slight yellowing, mosaic patterns, and blistering (Figure 2 b, c, and d). As the infection progressed, symptoms intensified, including mottling and more severe leaf damage such as cordate malformation (Table 4 and Figure 2 e). Fruit infected with ToBRFV exhibit brown necrotic spots on the epicarp, mesocarp, and locules (Figure 2 f-g), irregular spots on the external surface, along with brown spots and irregular ripening (Figure 2 h-j). It has been suggested that ToBRFV might utilize lysosomes to facilitate viral infection and manipulate host proteins, including post-translationally modified proteins such as TOM1-TOM2, to increase viral RNA content (Van Damme *et al.,* 2023). Silencing of SITOM1a and SITOM3 genes in tomato plants leads to increased ToBRFV accumulation in the exocarp (Kravchik *et al.,* 2022), contributing to the observed damage in Figure 2 and a reduction in fruit quality, as indicated in Table 3. Managing virus infections in tomato crops requires specific attention to genetic inheritance and climatic circumstances. However, it's crucial to note that tomato exhibits limited genetic heterogeneity due to its domestication process and intensive selection (Panno *et al.,* 2021). Nearly half of emerging diseases in tomato production are attributed to viruses, with ToBRFV being a prime example, spreading through various means including transmission through external teguments and seed endosperm, as well as mechanical transmission via microlesions induced during agronomic practices (Caruso *et al.,* 2022).

To produce ToBRFV-free tomatoes and control their spread in protected agriculture systems, it is imperative to identify and implement a combination of phytosanitary practices throughout the tomato production process (Panno *et al.,* 2020). An innovative strategy to achieve this goal involves selecting an F1 tomato hybrid tolerant to tobamoviruses, coupled with ToBRFV-free seeds. Additionally, it is beneficial if the plant can produce high levels of total soluble proteins, phenolic compounds, and significant catalase and peroxide dismutase enzyme activity (Nadeem *et al.,* 2022; Panno *et al.,* 2021). The results from this analysis underscore the significance of the ToBRFV inoculum source and its role in the selection process of tomato varieties, even in scenarios where the virus has already been detected in the same locality.

Conclusions

The Tomato brown rugose fruit virus (ToBRFV) poses a significant economic threat to tomato production in Coahuila state, Mexico. Our study highlights the substantial impact of ToBRFV isolate FQ3 on the agronomic parameters and fruit quality of the tomato variety 172-300. FQ3 exhibited notably higher incidence and severity compared to other isolates, underscoring its virulence. Moreover, variety 172-300 (Yüksel) emerges as a promising genetic material for commercial tomato production under greenhouse conditions in Coahuila state. These findings deepen our understanding of ToBRFV pathogenesis and provide valuable insights for developing integrated management systems to combat emerging diseases in tomato production.

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Authors' contribution

UVG and GAFT were responsible for the conception, design, and execution of the research. JCDO, LAAU, and HLL conducted data analysis and interpretation. UVG drafted the manuscript with input and support from GAFT, AFO, and HLL.

All authors participated in the discussion, revision, and approval of the final manuscript version.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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