**International Journal of Horticulture, Agriculture and Food Science (IJHAF)**
**ISSN: 2456-8635**
**[Vol-7, Issue-2, Mar-Apr, 2023]**
**Issue DOI: https://dx.doi.org/10.22161/ijhaf.7.2**
**Peer-Reviewed Journal**

# Severity of *Tomato brown rugose fruit virus* in tomato (*Solanum lycopersicum* L.) from a region of Coahuila, México

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Received: 19 Feb 2023; Received in revised form: 15 Mar 2023; Accepted: 21 Mar 2023; Available online: 31 Mar 2023


***Abstract***

*The purpose of this research was to describe the Tomato brown rugose fruit virus, from three isolates collected in the field and also to use a diagrammatic scale of severity for its evaluation. The isolation was carried out with the collection of 200 ha-1 leaflets, according to a statistical method, from commercial greenhouses. Agdia® immunological strips were used to confirm the symptoms and the virus was identified by RT-PCR. A completely randomized experimental design was established in tomato plants var. Río Grande®, with three treatments or isolates and five repetitions: Blindom F1® tissue, Quiroga® Enza zaiden tissue and Quiroga® Enza zaiden fruit; Controls with phosphate buffer and buffer+celite were used as negative control. The trial began with the inoculation of 45-day-old tomato plants, where only the primary leaves were inoculated. Fertilization was carried out twice a week using macro and microelements from commercial companies. To quantify the percentage of damage to foliage and fruit, a diagrammatic scale of severity was used. The three isolates evaluated differed in the symptoms produced by ToBRFV, where; The Fruto Quiroga® Enza zaiden isolate stood out, with a higher incidence, severity and shorter incubation period compared to the two isolates evaluated.*

***Keywords*— Solation, damage, diagrammatic scale, incubation period and inoculation.**

## I. INTRODUCTION

Tomato is one of the most important vegetables in the world, due to its high yield, it plays a main role in people's lives and in the economic development of producing countries. Mexico ranks tenth worldwide as a tomato producer, with Coahuila standing out in 16th place nationally. ToBRFV is considered one of the main diseases causing yield decline, it is a stable virus with the ability to be found in seeds and in the tomato exocarp, causing systemic infections and producing symptoms in different parts of the plant (Klap *et al*. ,2020). ToBRFV has become an emerging threat, which has affected several tomato production greenhouses. The infection and distribution of ToBRFV in commercial greenhouses was first reported in Jordan (Salem *et al*., 2016). Subsequently, the presence of the disease was discovered in Israel (Luria *et al*., 2017) and later in the state of Baja California. In the US, the disease was recorded in greenhouses located south of California. In Europe, sightings were reported in Germany, the United Kingdom (Skelton *et al*., 2019), Italy (Panno & Davino, 2019), Greece, On the other hand, in the Mediterranean, the epidemic was reported in Palestine (Alkowni *et al*., 2019), Turkey and Egypt. The presence of the disease was also demonstrated in China, currently it prevails in 38 countries. In our country, it was detected for the first time in 2018 in tomato and pepper nurseries in the municipality of Yurécuaro, Michoacán, according to the

National Phytosanitary Epidemiological Surveillance System in Mexico (Cambrón *et al*., 2018), it is currently distributed in 79 municipalities of 23 states of the country. Regarding the Phytosanitary status, it is categorized as a Regulated Non-Quarantine Pest (PNCR), whose presence in plants for planting causes economic and phytosanitary problems, therefore it is regulated in the territory of the importing contracting party. The main hosts are solanaceae, specifically tomato, chili and some weeds, the virus dispersal mechanism occurs from seed and vegetable remains as a source of inoculum, later it is disseminated through cultural practices, clothing, tools, mechanical transfer and the participation of pollinators (Bombus terrestris) during the flowering and fruiting stage. Given the potential importance of the pathogen, the virulence produced in greenhouse tomato plants and the lack of knowledge of the causative agent in greenhouse buildings. It was proposed to describe by isolating the virus in tomato plants under greenhouse conditions and to evaluate its behavior using a diagrammatic scale of severity.

## II. MATERIALES Y MÉTODOS

### 1. Establishment of the experiment.

Commercial tomato greenhouses were visited in General Cepeda, Coahuila, México., with a high presence of ToBRFV, the collection of symptomatic leaflets and fruits was carried out randomly in 4 hectares of surface, where there were 2 varieties of tomato var. Quiroga ® and Blindom F1 ®. The establishment of the culture was carried out by means of a randomized complete block experimental design with three treatments corresponding to the isolates carried out in the field, and five repetitions. The observed symptoms are consistent with, (Davino *et al*. 2020), interveinal yellowing, deformation, mosaic, and necrosis; fruits mottled with brown roughness. To corroborate the identity of the virus, a rapid assay was performed using Agdia® immunostrip, resulting positive for ToBRFV.

### 2. Inoculation of *ToBRFV*

The inoculation was carried out 30 days after the transplant (ddt) in tomato plants var. Red round® mechanically with ToBRFV according to (Ortíz *et al*., 2021) with some modifications; The raw juice was obtained from the symptomatic diseased tissue, then it was macerated in a cold sterilized mortar, in the presence of a phosphate buffer at pH 7.0, 0.1 M and "celite" as abrasive. Of the plants, only the primary leaves were inoculated using a swab impregnated with the ToBRFV inoculum, rubbing the leaves; and then, they washed with the buffer. As a negative control, healthy plants were rubbed with phosphate buffer and buffer+celite. They were fertilized 3 times a week using macro and microelements of commercial formulations (Pérez & Grajales, 1999) and the observation was made days after inoculation (ddi), to know the incubation period (pdi), as well as the severity of the floors.

### 3. Confirmación de ToBRFV

Identification of ToBRFV was performed by serology, using a DAS-ELISA PathoScreen® Kit. Interpretation of results was performed using a BioTek® ELx405nm spectrophotometer, with a 650nm blank. Generally, positive and negative thresholds can be determined by using 2 times the healthy average. Any samples with a positive value higher than 2 times the healthy average are positive, and samples with a positive value below 2 times the healthy average are negative. An alternative method for threshold calculations is the healthy average plus 3 times the standard deviation of the healthy sample set.

### 4. Severity scale design

The quantification of the leaf area percentage was carried out by designing a severity diagrammatic scale following the methodology established by (Ortega *et al*., 2016) with some modifications. The observation began 10 dai, the sampling was carried out every 15 days after the appearance of the first symptoms until the death of the plant. 5 leaflets were selected by sampling and treatment with different degrees of severity. The leaves were digitized with a RICOH MP C2003 PCL6 printer and with the ImageJ 1.53t program (NIH, USA), the total and affected area of each of the leaflets was quantified. Regarding the percentage of severity (affected area), it was calculated with the formula: $\text{severity} = \left(\frac{\text{Equatorial diameter}}{\text{Polar diameter}}\right)$ (Nutter *et al*., 2006).

### 5. Damage scale

The evaluation of a scale of damage in fruits, began with the harvest of the three treatments, fruits with different symptoms produced by ToBRFV infection were selected. The symptoms to consider were: abortion, brown and necrotic spots (exterior and interior), irregular maturation and roughness.

### 6. Statistic analysis

Four severity classes were determined, with the imageJ 1.33 software, to later process them with the Infostat® statistical software.con el software Infostat®.

### 7. Experimental design

Seeds of tomato var. Red round® in a 200-hole seedbed with peatmoss at a pH of 6.5; 35 days after sowing (dds) the plants were transplanted into 1.5kg plastic pots using

peatmoss+M.O as a substrate with a 1:1 ratio. Three treatments were evaluated, with non-inoculated controls. The isolates to be carried out corresponded to three samples collected in the aforementioned greenhouses: Blindom F1® "TBF1" tissue, Quiroga® Enza zaiden "TQE" tissue, Quiroga® Enza zaiden "FQEZ" fruit. The experiment was established under a completely randomized design with 5 repetitions and the experimental unit consisted of one plant per pot, everything was established at a temperature of 25°C±1.

## III. RESULTS AND DISCUSSION

### 1. Symptom expression

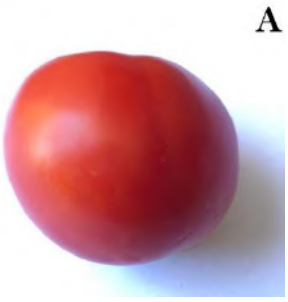
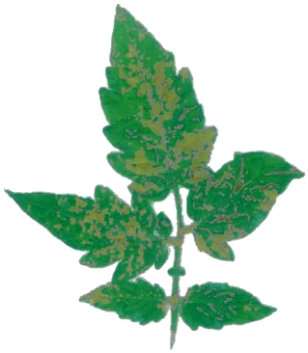
The inoculated isolates differed in leaflet and fruit symptoms (Figure 1), incidence, incubation period, severity (Table 1). None of the isolates matched the severity for ToBRFV, (Luria *et al*., 2017); the isolate "Fruto Quiroga® Enza zaiden (FQEZ)"" showed more severe symptoms, effect by which, plant repetitions did not reach harvest (Figure 1 H), compared to the isolate "Blindom Tissue F1® (TBF1)" showed fewer infections, only apical leaflets with slight curling were observed (Figure 1 D), while in the isolate "Tejido Quiroga® Enza zaiden (TQE)" symptoms similar to the third isolate "FQEZ" were observed but with less severity (Figure 1 F ). In fruits, the severity of symptoms coincided with the leaflets, Salem et al. (twenty-one); when inspecting fruits of the isolated treatment "FQEZ", the epicarp, mesocarp and necrotic locules were observed (Figure 1 A), while the isolated treatment "TBF1" showed mild symptoms of irregular ripening (Figure 1 J). The degree of incidence varied in the three isolates handled, FQEZ showed 100% incidence while TBF1 treatment showed 80%. The incubation period varied from 9 days to 12 dai (Table 1).

*Table 1. Factors observed in tomato isolates.*

| Isolations | Incidence | Incubation period (ddi**) | Severity 80 dai (%) | Symptoms in foliage | Symptoms in fruit. |
|---|---|---|---|---|---|
| **Tejido Blindom F1® (TBF1)*** | 80 | 12 | 40 | Blisters, chlorosis, mosaics, slight leaf curl. | Irregular maturation, necrosis in calyx and petioles. |
| **Tejido Quiroga® Enza zaiden (TQE)*** | 89 | 11 | 55 | Blisters, mosaic, chlorosis, deformation and curling of leaves. | Roughness, brown spots and irregular ripening. |
| **Fruto Quiroga® Enza zaiden (FQEZ)*** | 100 | 9 | 85 | Blisters, severe mosaic, chlorosis, necrosis, deformation and curling of leaves. | Abortion, Brown and necrotic spots (exterior and interior), irregular maturation. |

* isolations in tomato plants; **dai:** days after inoculation.

***Table 2.*** *Diagrammatic scale for ToBRFV according to the affected leaf area in tomato plants.*

| Classes | Severidad % | | |
|---|---|---|---|
| 0 | 0 |  | A  |
| 1 | 0.1 – 3.3 |  | B  |
| 2 | 3.3 – 23.3 |  | C  |
| 3 | 23.3 – 32.2 |  | D  |

| | | | |
|---|---|---|---|
| 4 | 32.2 – 47.1 |  |  |



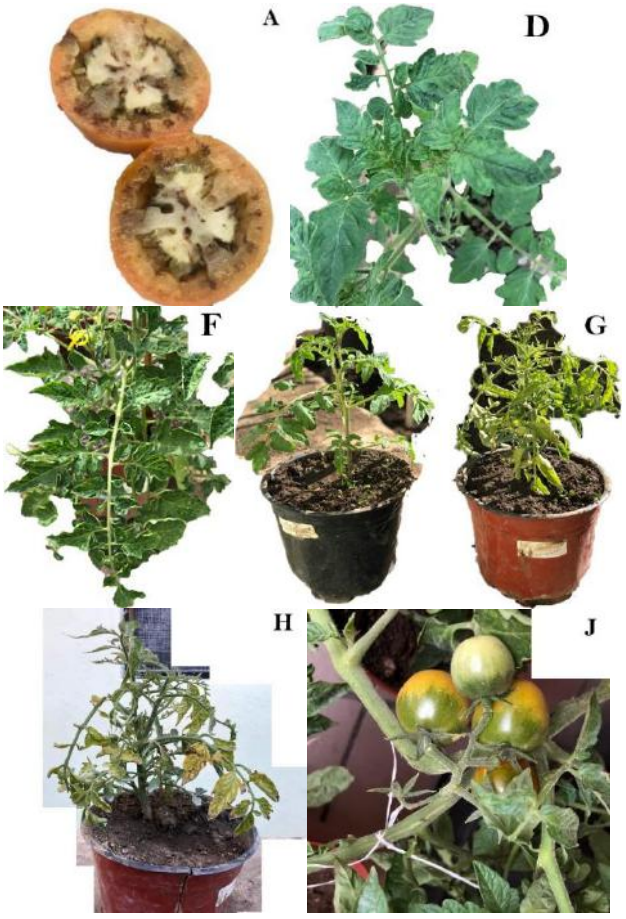



*Fig. 1. Symptoms produced in different isolates from plants infected with ToBRFVDetección por DAS-ELISA y inmunostrip® Agdia*

The presence of ToBRFV was detected in the plant treatments studied, days after inoculation the symptoms to ToBRFV were confirmed using Agia® immunological strips (Figure 2).

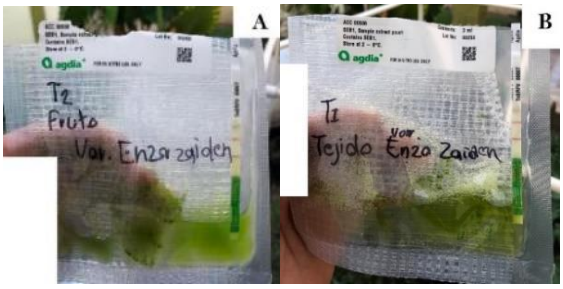



*Fig. 2. Serological diagnosis using Agdia® immunological strips*

**Diagrammatic scale of severity**

Some authors (Kabas et al., 2022) determined different classes for each damage produced by said pathogen in tomato plants, for the comparison of varieties. (Gonzalez *et al*., 2022) designed and validated a leaflet severity scale for ToBRFV from commercial greenhouses. The plants inoculated with ToBRFV varied according to the affectation, from 0 to 47.1% (Table 2). 4 classes were used to define the behavior of the virus in terms of severity; Class 1 and 2: insulation Blindom F1® fabric; Class 3-4: insulation Quiroga® Enza zaiden fabric. The treatment of plants inoculated with the "TBF1" isolate expressed less severity in leaflets and fruits, while the "FQEZ" isolate presented greater severity, causing plant death during the experiment.

## IV. CONCLUSION

It was demonstrated through experimentation that the isolates from commercial greenhouses in Coahuila, Mexico and established under greenhouse conditions belonged to Tomato brown rugose fruit virus. Likewise, the difference in infections produced by the three isolates evaluated was demonstrated.

## ACKNOWLEDGEMENTS

A I thank CONACYT for the scholarship awarded CVU: 1223150, for the study of the Master of Science in Agricultural Parasitology at the Antonio Narro Autonomous Agrarian University.

## REFERENCES

[1] Klap, C.; Luria, N.; Smith, E.; Hadad, L.; Bakelman, E.; Sela, N.; Dombrovsky, A. Tomato Brown Rugose Fruit Virus Contributes to Enhanced Pepino Mosaic Virus Titers in Tomato Plants. Viruses MDPI, 12 (8), 879. 2020. DOI: 10.3390/v12080879.

[2] Salem, N, M.; Cao, M, J.; Odeh, S.; Turina, M.; Tahzima, R. Primer informe sobre el virus del mosaico verde leve del tabaco y el virus de la fruta rugosa marrón del tomate que infectan a Capsicum annuum en Jordania. Planta Dis. 104:601. 2020.

[3] Cambrón, C.; José, M. Primer reporte de Tomato brown rugose fruit virus (ToBRFV) en Michoacán, México. Revista Mexicana de Fitopatología, [Sl], dec. 2018. DOI: http://dx.doi.org/10.18781/R.MEX.FIT.1810-5.

[4] Davino, S.; Caruso, A. G.; Bertacca, S.; Barone, S.; Panno, S. Tomato Brown Rugose Fruit Virus: Seed Transmission Rate and Efficacy of Different Seed Disinfection Treatments. Plants, 9 (11), 1615. 2020. DOI: 10.3390/plants9111615

[5] Pérez-Grajales, M.; Castro-Brindis, R. Guía para la Producción Intensiva de Jitomate en Invernadero. Texcoco: Universidad Autónoma Chapingo, v.3, 58p. 1999.

[6] Nutter, Jr. F.W.; Esker, P.D.; Coelho-Netto, R.A. Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data. European Journal of Plant Pathology, Ámsterdam, v.115, p.95-103, 2006. DOI: https://doi.org/10.1007/s10658-005-1230-z.

[7] Kabas, A.; Fidan, H.; Kucukaydin, H.; Atan, H, N. Detección de especies de tomates silvestres e híbridos interespecíficos para resistencia/tolerancia al virus de la fruta rugosa marrón del tomate (ToBRFV). Revista Chilena de Investigaciones Agropecuarias, 82 (1), 189-196. 2022.

[8] González, C, L, F.; Ramírez, Gil, J. G.; Mora, Romero, G, A.; García-Estrada, R, S.; Carrillo-Fasio, J, A.; Tovar-Pedraza, J, M. 2022. Desarrollo de una escala para la evaluación de la gravedad de la enfermedad y el impacto del virus de la fruta rugosa parda del tomate en el rendimiento del tomate. Revista Europea de Patología Vegetal ,1-14. 2022.

[9] Ortega-Acosta, S.A.; Velasco-Cruz, C.; Hernández-Morales, J.; Ochoa-Mar¬tinez, D.L.; Hernández-Ruiz, J. Escalas logarítmicas diagramáticas para evaluar la severidad del manchado de hojas y cálices de Jamaica. Revista Mexicana de Fitopatología, Texcoco, v.34, n.3, p.270-285, 2016. DOI: https://doi.org/10.18781/r.mex.fit.1606-6.

[10] Nutter, Jr. F.W.; Esker, P.D.; Coelho-Netto, R.A. Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data. European Journal of Plant Pathology, Ámsterdam, v.115, p.95-103, 2006. DOI: https://doi.org/10.1007/s10658-005-1230-z.