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Review Article

Tobamovirus fructirugosum an emerging disease*:* review and current situation in Mexico

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Background/Objective. *Tobamovirus fructirugosum* species (ToBRFV) is considered a worldwide quarantine pest that limits the production of *Solanum lycopersicum* and *Capsicum annum*, currently present in three countries of the American continent. The objective of this work was to deepen in the genetic variability of ToBRFV with respect to the different isolates, the physico-molecular and symptomatic characterization, the traditional and more current methods implemented for diagnosis, the range of virus reservoir hosts, and the epidemiology.

Results. ToBRFV was generated from a mutation resulting from genetic recombination with TMV, considered the main progenitor and ToMMV secondary progenitor. Phylogenetic analyses report the existence of five clades with respect to the genetic diversity of ToBRFV. The first primers for detection were designed in 2015 that encode replication, movement and capsid proteins. Serological methods can be used for preventive diagnosis, while molecular and NGS can confirm virus infection even at low concentrations in the plant. Sixteen weed families and host crops are reported from 47 countries. To achieve an effective strategy, it is necessary to reduce inoculum sources, develop compounds that inhibit mechanical transmission and develop tolerant genotypes.

Conclusion. ToBRFV is distributed nationally and represents a phytosanitary risk for Mexico; the exhaustive analysis of the study of diagnostic techniques, host range, dissemination, epidemiology and control strategies, contributes to the knowledge of ToBRFV.

Key-words: Diagnosis, epidemiology, ToBRFV, virus progression, genetic variability.

Introduction

Tobamovirus research has gained importance across agronomic and scientific fields due to the viruses' genetic diversity, transmission mechanisms, adaptability, host range evolution, new taxonomic classifications, and virus-plant interactions (Aiewsakun and Katzourakis, 2016). The genus *Tobamovirus* is believed to have emerged 140-120 million years ago, coinciding with the rise of angiosperms (Gibbs, 1999). Initially, these viruses existed in wild plants without plant-pathogen interactions, maintaining ecological homeostasis. However, the onset of agriculture transformed them into pathogens affecting solanaceous plants and other families (Lartey *et al*., 1996).

The *Virgaviridae* family encompasses 5 phytopathogenic virus genera, including the globally significant *Tobamovirus*, which comprises 37 species (Adams *et al*., 2017; Dombrovsky *et al*., 2017a). Economically important tobamoviruses include: tobacco mosaic virus (TMV, *Tobamovirus tabaci*), tomato mosaic virus (ToMV, *Tobamovirus tomatotessellati*), tobacco mild green mosaic virus (TMGMV, *Tobamovirus mititessellati*), pepper mild mottle virus (PMMoV, *Tobamovirus capsici*), tomato mottle mosaic virus (ToMMV, *Tobamovirus maculatessellati*), and tomato brown rugose fruit virus (ToBRFV, *Tobamovirus fructirugosum*) (EPPO, 2023). TMV, the first identified tobamovirus (Ivanovsky, 1892), caused an epidemic in Dutch tobacco-producing regions before spreading worldwide (Shen *et al*., 2013). Later discoveries include yellow tailflower mild mottle virus (YTMMV, *Tobamovirus anthocercis*) in Australia, affecting ornamental solanaceous species (Wylie *et al*., 2014), and ToMV (Skotnicki *et al*., 1976) and ToMMV infecting tomato and pepper plants in Mexico (Li *et al*., 2013). While all tobamovirus species are aggressive emerging pathogens causing significant crop losses, the tomato brown rugose fruit virus stands out for its economic impact. Notably, it is considered the first plant virus to cause a global pandemic since SARS-CoV-2 (Salem *et al*., 2023).

Origin and Genetic Diversity of ToBRFV

Viral species of the genus *Tobamovirus* are significant pathogens in solanaceous crops, notable for the symptoms they induce and the need for specific serological methods for their identification. Nevertheless, some symptoms produced are similar among the causative species, such as TMV, ToMV, ToMMV, and ToBRFV (Alon *et al*., 2021). Some studies report that TMV, ToMV, and ToBRFV can produce mixed infections in tomato plants, complicating diagnosis (Jamous *et al*., 2022; Yan *et al*., 2021a). ToBRFV was first identified in 2014 in the Ohad province of southern Israel. Subsequently, an outbreak in tomato nurseries in Jordan led to the isolation named "Tom1-jo", and the new virus was designated tomato brown rugose fruit virus (Salem *et al*., 2016; Luria *et al*., 2017). Through phylogenetic analyses including

TMV, ToMV, ToMMV, and BPMV, Salem *et al*. (2016) validated ToBRFV as a new species within the genus *Tobamovirus*. Luria *et al*. (2017), using Koch's postulates, transmission electron microscopy (TEM), partial host range determination, and antisera analysis to rule out cross-reactivity with other viruses, concluded that the ToBRFV-IL isolate showed high identity with the Jordanian isolate.

Current biogenomic studies on ToBRFV, following new outbreaks, have demonstrated genetic variability, with over 99% identity among different isolates (Chanda *et al*., 2020). Eichmeier *et al*. (2023) found 99.3 to 100% similarity among 50 ToBRFV genomes, while Zhang *et al*. (2022) clearly differentiated ToBRFV isolates from TMV and ToMV in a study of 78 genomic sequences. Yan *et al*. (2021b) reported over 99.6% similarity among Tom1-Jo "KT383474.1", ToBRFV IL, and ToBRFV MX isolates, suggesting a common ancestor for all ToBRFV isolates (Oladokun *et al*., 2019). Additionally, recombination of ToBRFV with other viral species has been identified, indicating ToMMV as a secondary progenitor and the TMV Ohio V strain as the main progenitor (Salem *et al*., 2016).

A phylogenetic analysis based on the ORF4/CP gene (Figure 1) grouped 34 isolates from Turkey in the first clade, while 49 isolates were placed in the

Figure 1. Phylogenetic analysis of ToBRFV sequences reported in NCBI. For the reconstruction of the phylogenetic tree, Molecular Evolutionary Genetics Analysis version 11 software was used with the Neighbor joining model and 10,000 replicates (Bootstrap). With a genetic distance of 0.02.

second (Çelik *et al*., 2022). Two French isolates "MW284988.1, MW284987.1" were classified in the third clade, while 12 isolates from various regions including USA (MT002973.1), Mexico (TBRFV-MX-CP "MK319944.1, MW349655.1"), Netherlands "MW314116.1", Italy "OK62464678.1", Belgium (OM515231.1), and United Kingdom (TBRFV.21930919) formed the fifth group (Van de Vossenberg *et al*., 2020). Abrahamian *et al*. (2022) revealed a phylogenetic analysis of isolates from USA, Mexico, and Peru, reporting that most of these group in clade three, evidencing limited genetic diversity among isolates worldwide. Eichmeier *et al*. (2023) analyzed the complete virus genome, identifying the Czech ToBRFV isolate "OP413740.1" as belonging to an independent group within the fifth clade, distinguishing it from other European isolates. This overview underscores the complexity of ToBRFV genomics and the influence of multiple factors on its evolution and relationship with hosts.

Physical and Molecular Characteristics of ToBRFV

ToBRFV has rigid rod-shaped particles 300 nm long and 18 nm in diameter. The genome is a positive-sense single-stranded RNA (ssRNA+) of ~6,400 nt. It contains four distinct open reading frames (ORF1, 2, 3, and 4), encoding two replication-related protein complexes of 126 kDa (ORF1a) and 183 kDa (ORF1b), the movement protein (MP) of ~30 kDa (ORF2), and the coat protein (CP) of ~17.5 kDa (ORF3), expressed through coterminal subgenomic RNAs (Eichmeier *et al*., 2023). The 126 kDa protein can act as an RNA silencer, while the 30 kDa MP facilitates cell-to-cell virus translocation (Zhang *et al*., 2022). It has been noted that the ToBRFV control strategy focuses on the CP, which plays a crucial role in viral particle assembly and long-distance movement within the host plant (Ishikawa *et al*., 2022).

Description of Symptoms

ToBRFV, a distinctive tobamovirus, induces a wide array of symptoms in its hosts. The severity and type of symptoms vary based on the host plant species, season, temperature, growth conditions (greenhouse or open field), and plant age at infection (Caruso *et al*., 2022). These symptoms, both direct and indirect, significantly diminish fruit quality and commercial value, leading to substantial crop losses (Menzel *et al*., 2019). Symptom expression varies with crop type and environmental factors (Figure 2A and C). Tomato plants exhibit systemic symptoms, while species like *Nicotiana tabacum*, *N. glutinosa*, *N. clevelandii*, *N. benthamiana*, and *Chenopodium amaranticolor* primarily display localized symptoms (Chanda *et al*., 2021; Vásquez-Gutiérrez *et al*., 2024). Although tobamoviruses generally

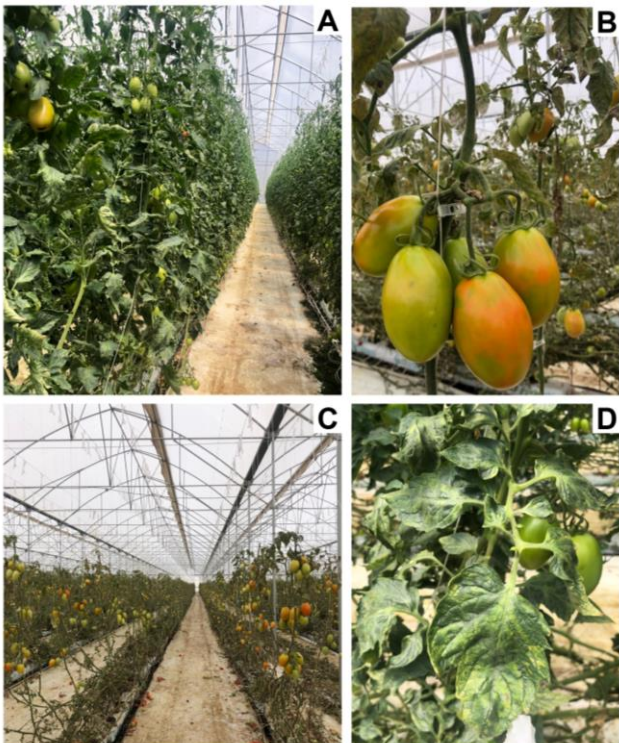

Figure 2. Symptoms manifested in tomato plants by ToBRFV grown in greenhouse. A) Tomato plants at 180 days after sowing showing high incidence of ToBRFV; B) Irregularities in fruit ripening; C) Plants in a state of collapse due to severe ToBRFV infection; D) Presence of mosaic patterns, mottling, and blistering on leaves.

cause similar symptoms such as mosaics and yellowing, ToBRFV stands out for its particularly severe syndrome. Foliar symptoms include mosaics, blistering, curling, deformations (Figure 2D), reduced leaf size, and stunted growth. Fruits exhibit deformation, brown spots, mottling, irregular ripening (Figure 2B), yellow spots, and both external and internal necrosis (González-Concha *et al*., 2023; Jewehan *et al*., 2022a; Vásquez-Gutiérrez *et al*., 2023a; Zhang *et al*., 2022). This symptom diversity highlights the critical need for effective identification and management of ToBRFV to mitigate its impact on agricultural productivity.

Diagnostic methods

Diagnostic methods begin with the identification of plant symptoms. The observation of dark mosaics, deformation and narrowing of leaves in young apical shoots, and brown rugosity on fruits confirms the presence of ToBRFV (Alfaro-Fernández *et al*., 2021). Currently, there are various alternatives for ToBRFV

identification and detection, which can be classified into different types: differential plant diagnosis, transmission electron microscopy (TEM), serological methods, reactive strips, and molecular techniques (González-Garza, 2017).

Diagnosis using Differential Plants. Tobamoviruses exhibit distinct symptomatic patterns that distinguish them from other genera (Roistacher, 1991). Initially, ToBRFV was thought to naturally infect only pepper and tomato (Luria *et al*., 2017). However, recent studies have expanded our understanding of its host range. Cultrona *et al*. (2024) demonstrated that ToBRFV can naturally infect *Convolvulus arvensis* and *Polycarpon tetraphyllum*, which serve as reservoirs. Furthermore, Vásquez-Gutiérrez *et al*. (2024) identified and confirmed through ELISA that ToBRFV can naturally infect 21 different host species.

ToBRFV's host range encompasses over 40 species across four families: *Amaranthaceae*, *Apocynaceae*, *Asteraceae*, and *Solanaceae* (Table 1). Its host range similarity to ToMV reflects their close phylogenetic relationship (Chanda *et al*., 2020). Differential plants enhance ToBRFV diagnosis by leveraging the hypersensitive response (HR), though mixed infections with other tobamoviruses can complicate interpretation (González *et al*., 2017). When mechanically inoculated, experimental plants exhibit an HR that isolates ToBRFV particles, limiting systemic spread (Fidan *et al*., 2021).

Experimental host range studies for ToBRFV primarily focus on species from *Chenopodiaceae*, *Amaranthaceae*, and *Solanaceae* families. Notably, it does not

Table 1. Hypersensitivity response in the range of hosts susceptible to ToBRFV

Host/Observed symptomatology	References
Amaranthaceae	
Gomphrena globosa[3, 18], *Amaranthus viridis*[14]	Salem *et al.,* 2022
Asteraceae	
Emilia sonchifolia[9, 10, 21], *Glebionis coronaria*[3, 20], *Lactuca serriola*[4], *Verbesina encioloides*[14], *Bidens pilosa*[14], *Helianthus annus*[14], *Sonchus oleraceus*[14], *Titonia tubaeformis*[14]	Sabra *et al*., 2022; Salem *et al*., 2022; Matzrafi *et al*., 2023; Luria *et al*., 2017; Chanda *et al*., 2021a; Vásquez-Gutiérrez *et al*., 2024
Labiatae	
Marrubium vulgare[13, 21]	Vásquez-Gutiérrez *et al*., 2024
Solanaceae	
Salanum lycopersicum[3, 11, 23], *S. eleagnifolium*[1], *S. rostratum* [4, 20, 21, 22], *S. melongena*[1], *S. arcanum*[8, 19, 20, 22, 24], *S. Cheesmaniae*[7, 16, 19, 22], *S. habrochaites*[3, 7, 16], *S. nigrum*[3, 4, 12, 18, 20], *S. pennellii*[3, 8, 22], *S. peruvianum*[3, 8, 16, 22], *S. pimpinellifolium*[3, 8, 16, 22], *S. tuberosum*[1, 7, 21], *S. sitiens*[3], *Nicotiana glutinosa*[5, 10], *N. tabacum* Samsun [3, 5, 10, 20], *N. occidentalis* subsp. *Hesperis*[5, 15], *N. benthamiana*[2, 6, 5, 15, 21, 22], *N. clevelandii* [3, 10, 21] *N. sylvestris*[3, 10], *N. rustica*[2, 12, 14, 28], *N. longiflora*[12], *N. glauca*[14], *Petunia hibrida*[3], *Physalis angulata*[3], *P. pubescens*[5, 7], *Datura stramonium*[10, 14], *Capsicum annum*[3, 5, 8, 13, 21]	Matzrafi *et al*., 2023; Salem *et al*., 2022; Yan *et al*., 2021a; Sabra *et al*., 2022; Jewehan *et al*., 2022b; Chanda *et al*., 2021a; Zhang *et al*., 2022; Vásquez-Gutiérrez *et al*., 2024; Luria *et al*., 2017; Ortiz-Martínez *et al*., 2021

Table 1. Continue.

Host/Observed symptomatology	References
Chenopodiaceae	
Chenopodium berlandieri[12, 18], *Ch. amaranticolor*[14], *Ch. album*[8, 12, 17] *Ch. quinoa*[10, 12, 17, 18], *Ch. glaucum*[12], *Ch. Murale*[14]	Chanda *et al*., 2021a; Luria *et al*., 2017; Sabra *et al*., 2022; Sabra *et al*., 2022; Salem *et al*., 2016; Sabra *et al*., 2022; Vásquez-Gutiérrez *et al*., 2024
Convolvuláceae	
Ipomoea purpurea[4,7]	Vásquez-Gutiérrez *et al*., 2024
Apocynaceae	
Catharanthus roseus[1]	Chanda *et al*., 2021a
Malvaceae	
Malva parviflora[21], *Malva neglecta*[14], *Malvastrum coromandelianum*[14]	Salem *et al*., 2022; Vásquez-Gutiérrez *et al.,* 2024
Nyctaginaceae	
Mirabilis jalapa[4, 21, 7, 24]	
Oxalidaceae	
Oxalis latifolia[14]	
Resedaceae	
Reseda luteola[14]	
Araliaceae	
Hedera hélix[14]	
Plantaginaceae	Vásquez-Gutiérrez *et al.,* 2024
Plantago lanceolata[14]	
Polygonaceae	
Polygonum convolvulus[14]	
Ranunculaceae	
Clematis drummondii[4, 7. 21, 24]	
Euphorbiaceae	
Ricinus comunis[14]	

[1]asymptomatic; [2]plant death; [3]mosaic; [4]mosaic, [5]necrosis, [6]blistering, [7]Leaf distortion, [8]deformation, [9]local black spots, [11]plant stunting [12]Necrotic local lesions, [13]necrotic spots, [14]chlorotic local lesions, [15]Plant death, [16]blister formation, [17]chlorosis, [18]necrotic ring spot, [19]leaf narrowing, [20]mottling, [21]yellowing, [22]leaf curling, [23]stunting, [24]leaf deformation.

infect species from *Brassicaceae*, *Cucurbitaceae*, and *Verbenaceae* (Yan *et al*., 2021a; Chanda *et al*., 2021a). While most indicator plants display both systemic and local symptoms, some species like *Solanum tuberosum* and *S. melongena* were initially reported as asymptomatic (Yan et al., 2021b). However, Vásquez-Gutiérrez *et al*. (2024) recently observed that *S. tuberosum* plants in contact with ToBRFV-infected tomatoes developed visual symptoms including yellowing, leaf deformation, and size reduction, subsequently confirmed by ELISA.

Alternative hosts. ToBRFV has proven to be a pathogen with a wide range of potential hosts, including crops and various weeds (Chanda 2021a; Zhang *et al*., 2022; Matzrafi *et al*. 2023) (Table 1). Weeds play a significant role in the spread of ToBRFV inoculum (Matzarafi *et al*., 2023), highlighting the importance of integrated management that includes weed control to limit virus propagation.

Microscopic diagnosis. Microscopic methods are crucial for diagnosis but can be imprecise due to variability in viral particle size among species (Luria *et al*., 2017). Optical microscopy, particularly when combined with fluorescence techniques, supports tobamovirus diagnosis through viral inclusions in plant tissues (Pepperkok and Ellenberg, 2006). Virus classification using inclusions is done by genera, as most tobamovirus species form hexagonal and needle-shaped inclusions like tobacco mosaic virus (Khamphirapaeng *et al*., 2017), while potato virus Y (species *Potyvirus yituberosi*) produces amorphous spherical bodies (Guo *et al*., 2022). ToBRFV inclusions were previously undescribed; recently, Vásquez-Gutiérrez *et al*. (2024) reported X-bodies, rounded and stacked plates as infection responses to ToBRFV in tomato and tobacco tissues. Transmission electron microscopy (TEM) has contributed to ToBRFV's morphological identification since its first sighting (Luria *et al*., 2017; Zhang *et al*., 2022) and is considered a classic method for visualizing plant tissues (Home *et al*., 2018). TEM diagnosis aids in characterizing viral particles based on morphological features (Kitajima *et al*., 2004). However, TEM can be imprecise as viral particles vary in size, complicating identification (Luria *et al*., 2017). Electron microscopy also detects cellular alterations during ToBRFV infections related to viral inclusions. Despite being recently identified, TEM proved valuable for ToBRFV's viral characterization and particle observation.

Luria *et al*. (2017), Cambrón-Crisantos *et al*. (2019), Eichmeier *et al*. (2023), Fidan *et al*. (2021), Levitzky *et al*. (2019), and Mahillon *et al*. (2022) sampled infected tomato leaflets and fruits cv. Mose and Ikram with unusual symptoms, then purified the unknown virus following Cohen *et al*. (2000). They found viral particles averaging 265.6±56.2nm long and 19±1.41nm in diameter, noting that particles differ based on sample origin and can be short or elongated rods.

Serological detection of ToBRFV. Serological detection of ToBRFV employs techniques like Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA), *in situ* immunofluorescence, and Western blot. Previously, these methods lacked specificity in distinguishing ToBRFV from related species like TMV and ToMV, possibly due to cross-reactivity between antibodies and coat proteins (CP) of different tobamoviruses. Luria *et al*. (2017) serologically characterized ToBRFV, generating antibodies for virus CP detection using DAS-ELISA (1:12,000 dilution). Their analysis showed slight cross-reactivity with TMV

and PMMoV. Field diagnosis typically uses symptomatic leaflets, increasing DAS-ELISA accuracy.

Yan *et al*. (2021b) developed monoclonal antibodies specific to ToBRFV CP, improving diagnostic sensitivity and specificity. Eads *et al*. (2023) validated an Agdia® DAS-ELISA with high analytical sensitivity (64 to 320 pg mL^{-1}) and specificity for ToBRFV, noting cross-reactivity with TMV, ToMV, and ToMMV at higher concentrations. Luria *et al*. (2017) implemented Western blot diagnosis using ToBRFV-specific polyclonal antibodies, analyzing CP band intensity via t-student test.

In situ immunofluorescence, enhanced by specific fluorophores for laser scanning microscopy, enables direct ToBRFV detection in infected samples, differentiating between tobamoviruses (Klap *et al*., 2020). This technique often uses Alexa Fluor 594®, a bright, stable red-spectrum fluorophore (Salem *et al*., 2022). Another variant involves staining seeds, plant tissues, and roots for fluorescence in situ hybridization and microscopic detection (Ragasová *et al*., 2022).

These serological methods contribute to accurate diagnosis and characterization of ToBRFV's physicochemical effects on infected hosts, offering valuable tools for virus management and control.

Diagnosis with test strips. The quest for rapid virus diagnosis in field conditions has gained importance (Fillmer *et al*., 2015; Li *et al*., 2019). Following ToBRFV's emergence (Salem *et al*., 2016; Luria *et al*., 2017), rapid immunochromatographic assays using test strips for plant virus detection have become significant due to their sensitivity and field applicability (Byzova *et al*., 2009). Agdia® test strips for ToBRFV specifically detect isolates from Israel, the Netherlands, Italy, Germany, and Mexico, validating field monitoring results (Levitzky *et al*., 2019; Eldan *et al*., 2022). Despite a ToBRFV detection limit of 200 ng mL^{-1}, cross-reactions with related viruses occur. These strips may cross-react with TMV, ToMV, and ToMMV at low concentrations, necessitating validation through molecular assays (Wilstermann and Ziebell, 2019). Eads *et al*. (2023) recently determined a 1:259,000 dilution limit for ToBRFV detection in infected tissue with 64-320 pg mL^{-1} of purified virus. Their validation of Agdia® strips revealed slight cross-reactivity with TMV and ToMV at a 200 ng mL^{-1} detection limit for purified virus. However, the strips showed no cross-reaction with other ToBRFV-related species. Notably, all 384 ToBRFV-positive samples tested positive using these strips (Figure 3 A and B).

Molecular techniques for ToBRFV detection. Early detection through molecular diagnosis of ToBRFV presents an opportunity to reduce the risk of virus entry and spread (Luigi *et al*., 2022). Consequently, various protocols based on nucleic acid amplification have been developed, including: reverse transcription polymerase

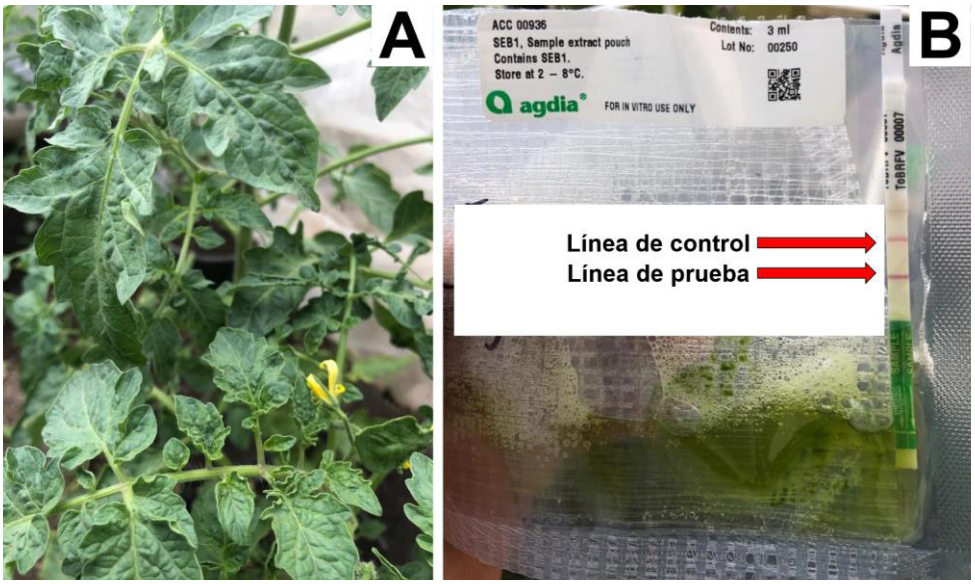

Figure 3. Rapid detection procedure for ToBRFV using Agdia® immunological strips. A) Selection of symptomatic tissue (young leaves); B) Macerated sample and positive reaction to ToBRFV, showing the control line and the test line (both in red color).

chain reaction (RT-PCR), quantitative reverse transcription PCR (RT-qPCR), and digital PCR (ddPCR) for detection in leaflets, fruits, and seeds (Panno *et al*., 2019b; Rodríguez *et al*., 2019; Chanda *et al*., 2021b; Yan *et al*., 2021b; Menzel & Winter, 2021). The development of these molecular techniques has gained relevance in expanding real-time virus diagnostic strategies. Due to result variation and the efficacy of existing molecular methods, new techniques have been continuously developed to allow specific detection of genes in ToBRFV viral proteins. These primers, described in Table 2, are used for preventive diagnosis of ToBRFV, reducing the risk of entry into countries where the virus is absent.

Vargas-Hernández *et al*. (2022) implemented a digital droplet polymerase chain reaction (ddPCR) assay that enables the detection of the virus using recombinant plasmids encoding a specific gene of the virus coat. This is the first report of ToBRFV detection in tomato seeds using ddPCR. However, the technique has not been widely implemented for field application. Another recent method is the application of AmplifyRP XRT, a technique based on the molecular principle, which utilizes recombinase polymerase amplification. This technique is designed to be a hybrid tool for field and/or laboratory detection. Therefore, it is considered a highly sensitive and precise technique, with an analytical sensitivity of 16 fg μL^{-1} and an analytical specificity that encompasses all ToBRFV isolates, without producing cross-reactions (Eads *et al*., 2023). Detection based on loop-mediated isothermal amplification (LAMP) offers an efficient and robust alternative for ToBRFV

Table 2. Primers Reported for the International Detection of ToBRFV.

Detection frabric	Initiators	Nucleotide sequences 5´ to 3´	White region	Amplicon size	Authors
Leaflets and fruits	ToBRFV-F ToBRFV-R	AACCAGAGTCTTCCTATACTCGGAA CTCWCCATCTCTTAATAATCTCCT	RdRp[1]	475 bp	Rodríguez *et al*., 2019
	TBRFV-F-5722 TBRFV-R-6179	CACAATCGCAACTCCATCGC CAGAGGACCATTGTAAACCGG	CP[1]	458 bp	Panno *et al*., 2019a
Leaflets	AB5520F AB5598R	GTAAGGCTTGCAAAATTTCGTTCG CTTTGGTTTTTGTCTGGTTTTCGG	CP[1]	101 bp	Panno *et al*., 2019b
Leaflets and fruits	ToBRFV-F-Alk ToBRFV-R-Alk	AATGTCCATGTTTGTTACGCC CGAATGTGATTTAAAACTGTGAAT	RdRp[1]	560 bp	Alkowni *et al.,* 2019
Leaflets and fruits	ToBRFV-F ToBRFV-R	GAAGTCCCGATGTCTGTAAGG GTGCCTACGGATGTGTATGA	CP[1]	842 bp	Ling *et al*., 2019
Leaflets, fruits and seeds	ToBRFVqs1 ToBRFVp1 ToBRFVqas2	CAATCAGAGCACATTTGAAAGTGCA FAM-ACAATGGTCCTCTGCACCTG-BHQ1 CAGACACAATCTGTTATTTAAGCATC	CP[2]	96 bp	Menzel and Winter, 2021
Leaflets	CP FOR CP REV	AGAACAACCGTTCAACGGCAATTTA CTCAAGATGCAGGTGCAGAGGACCATTGT	CP[4]	359 bp	Magaña-Álvarez *et al*., 2021
Leaflets, fruits and seeds	CaTa28-FW CaTa28-Pr CaTa28-Rv	GGTGGTGTCAGTGTCTGTTT FAM-AGAGAATGGAGAGAGCGGACGAGG -BHQ1 GCGTCCTTGGTAGTGATGTT	MP[3]	139 bp	International Seed Federation, 2020
	CSP13251Fw CSP1325 Pr CSP1Rv	CATTTGAAAGTGCATCCGGTTT HEX-ATGGTCCTCTGCACCTGCATCTTGAGA -BHQ1 GTACCACGTGTGTTTGCAGACA	CP[3]	100 bp	International Seed Federation, 2020
Leaflets and seeds	AB-620 AB-621	CAGATGTGTCGTTGGTCAGAT CATCACTACGGTGTAATACTTC	MP1 y MP2, ORF[5]	144 bp	Bernabé-Orts *et al*., 2022*
Leaflets	ToBRFV-R1 ToBRFV-P1	GCCCATGGAACTATCAGAAGAA TTCCGGTCTTCGAACGAAAT	MP[3]	92 bp	Chanda *et al*., 2021a*
Leaflets, fruits and seeds	F ToBRFV_F3 ABRFV_B3	TTGGAGTCTTAGATGTTGCG GGACACCGTCAACTAGGA	MP[6]	279 bp	Sarkes *et al*., 2020; Rizzo *et al*., 2021
	F-3666 R-4718	ATGGTACGAACGGCGGCAG CAATCCTTGATGTGTTTAGCAC	RdRp[1]	1052 bp	Luria *et al*., 2017
Leaflets	ToBRFV-1534-F ToBRFV-3733-R	AGATTTCCCTGGCTTTTGGA ATCATCGCCACCAAATTTTC	RdRp[1]	1052 bp	Yan *et al*., 2019

Table 2. Continue.

Detection frabric	Initiators	Nucleotide sequences 5´ to 3´	White region	Amplicon size	Authors
Leaflets	ToBRFV MP1-59-F1 ToBRFV MP1-59-R	GAAGTTTGTTTATAGATGGCTCTTGTTA-AGGGTAAA GTATCCACTATCGATGAGTTTTACACCTT-TAAGTAAATTGAC GTCAATTTACTTAAAGGTGTAAAACT-CATCGATAGTGGATAC	MP[1]	15 bp	Yan *et al*., 2021a[A]
	ToBRFV MP60-126-F ToBRFV MP60-126-R	AAAGGAGTTAAGCTTATTGATGGTGGC-TATGTACAT TGCGTCCTGGGTGGTGATGTTGTAATTTG-GAACGACT	MP[1]	15 bp	
	ToBRFV MP127-186-F ToBRFV MP127-186-R	GACGGAGGTCCCATGACTACCAAGGACG-CAGAAA TTCTTCTGTAAGTTCCATGGGCCCTCCATC	MP[1]	15 bp	
	ToBRFV MP187-266-FToBRFV MP187-266-R1	GACGGAGGTCCCATGGAACTAT-CAGAAGAAGTTGTTGATG TTGTGTAAGATCTATTTAATACGAATCT-GAATCGGC	MP[1]	15 bp	
	ToBRFV-CP-detection-FToBRFV-CP-detection-R	ATGTCTTACACAATCGCAACTC TCAAGATGCAGGTGCAGAG	CP[1]	1019 bp	
	q-ToBRFV CP-Fq-ToBRFV CP-R	AAATCAGGCGAACCCG GCAGAGGACCATTGTAAACC	CP[1]	173 bp	
	q-ToBRFV RdRp-Fq-ToBRFV RdRp-R	CAATACCTTGGTCAACGAT TTGGGCATACAGCAGTG	RdRp[1]	329 bp	

Technique: [1]RT-PCR endpoint; [2]RT-qPCR; [3]RT-qPCR multiplex, [4]SEqPCR; [5]CRISPR/Cas; [6]LAMP; [A]Primers used in the study reported by the corresponding author; *real-time monitoring; RdRp: RNA-dependent RNA polymerase; MP: movement protein.

diagnosis. Sarkes *et al*. (2020) describe the principle of this technique, which is based on the specificity of a set of primers; F3: TTGCAAGTCTTAGATGCG, B3: GGACACCGTCAACTAGG with a size of 279 bp. FIP(F1c+F2): CCTTCTCCAACTGTCGCAAGTCACATGCTAGGAAGTACCAC, BIP (B1c+B2) CCGTGAGTTCTGAGTCAATGGTTGAGGCTCACCACCATCTC TTAA and loopF; CTCCATGCTCATCATACCCAA. LAMP assays are performed

in a WarmStart LAMP colorimetric master mix (NEB Canada). The reaction program consists of a single step, where tubes are incubated at 65°C for 30 min, after which the reactions are visualized and the results are recorded photographically. Positive results confirm the presence of the virus when there is a color change in the reaction mixture from pink to yellow. These results indicate that LAMP can detect six molecules in a 25 µL reaction, being more specific than RT-PCR. On the other hand, LAMP-PCR has been used in combination with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology for the specific detection of ToBRFV in the field (Rizzo *et al*., 2022).

Next-generation sequencing (NGS) has gained relevance with the emergence of new omic technologies. NGS remains dominant in plant virus detection due to its high throughput and low cost (Dumschott *et al*., 2020). The importance lies in the ability to generate enormous data and its rapid processing (Mehetre *et al*., 2016). NGS shares processes that are common for plant virus detection, such as the extraction of nucleic acids (DNA/RNA) from infected samples, the subsequent fragmentation of the nucleic acid for library preparation, and finally, the development of several synthetic primers in the fragmented DNA for the consecutive development of different sequencing chemistries and platforms for ToBRFV analysis (Luria *et al*., 2017; Riesenfeld *et al.,* 2004). NGS coupled with MiSeq Illumina, known as second-generation technology, allows the detection of ToBRFV at low concentrations in the plant, even in asymptomatic infections, through metagenomic analysis (Mehetre *et al*., 2021). The technique could be used for the mass monitoring of emerging viruses, limiting their spread and impact on economically important crops.

Spread of the virus

The tomato brown rugose fruit virus was first identified in Israel in 2014 in tomato plantations under shade mesh, according to Luria *et al*. (2017). However, it was not until 2015 that the first report of its presence in tomato greenhouses in Jordan was made, where atypical symptoms were observed compared with other viruses; on leaves, mosaics, blistering, and on fruits, yellow spots to brown rugosity (Salem *et al*., 2016). Molecular analyses confirmed the presence of a new tobamovirus in tomato plants, which was identified as tomato brown rugose fruit virus (Salem *et al*., 2016; Luria *et al.,* 2017)*. The appearance and severity of symptoms in fruits affected by ToBRFV constituted a significant obstacle to their commercialization, and the absence of adequate diagnostic methods along with epidemiological plans delayed the implementation of quarantine control measures (Zhang *et al*., 2023). After its discovery, the virus spread rapidly worldwide, mainly through contaminated seeds, as Israel and Jordan are prominent seed exporters.

This marked the beginning of the global expansion of the virus (Van de Vossenberg *et al*., 2020). Currently, ToBRFV is present on five continents: America, Asia, Africa, Europe, and Oceania, covering 47 countries where it has been reported in **Solanum lycopersicum* *and *Capsicum annum* crops (Figure 4). Although its presence has not been officially confirmed in Oceania, it has been indicated that seeds from Australia were contaminated with ToBRFV, suggesting its existence in the region (EPPO, 2023; Zhang *et al.,* 2022).

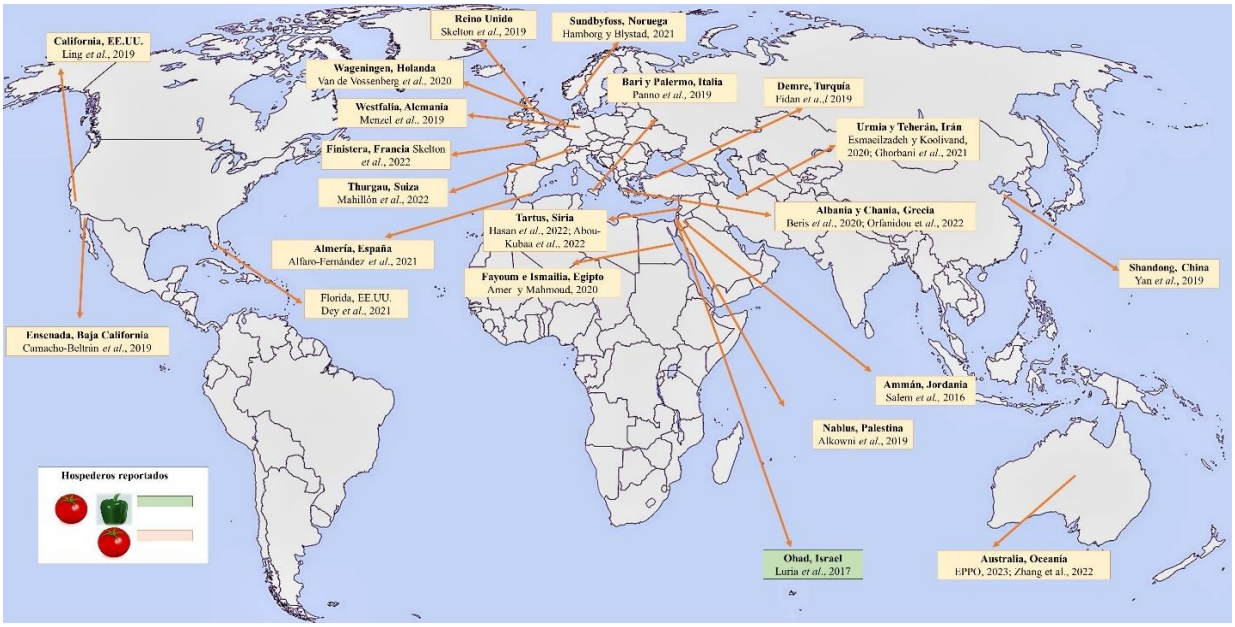

Figure 4. ToBRFV records in producing regions of the World, indicating its presence in 47 countries distributed across the continents of America, Asia, Africa, Europe, and Oceania. Affected countries are marked with an arrow and highlighted according to the first host found.

With the importation of seeds to Mexico, in 2017, symptoms of a disease that spread mechanically in tomato greenhouses were evidenced. In July 2018, the first case of ToBRFV was registered in Ensenada, Baja California. The analyzed samples showed 100% identity with the 1052 bp region of the RdRP gene of the ToBRFV isolates identified in Israel and Jordan, according to the sequences deposited in GenBank (KX619418 and KT383474.1) (Camacho-Beltrán *et al*., 2019). In September 2018, the virus was also detected in tomato and chili pepper greenhouses in the Yurécuaro region of Michoacán, confirmed by RT-PCR with a 100% identity to the Israeli strains (Cambrón-Crisantos *et al*., 2019). Likewise, Ling *et al.* (2019) in September of the same year, reported the presence of ToBRFV in a greenhouse with tomato plants from Baja California. This evidence shows that the virus spread rapidly through the tomato and pepper production areas in Mexico,

currently being widely distributed throughout the national territory, according to official reports (Figure 5).

Figure 5. Records of ToBRFV in the Mexican Republic where tomato brown rugose fruit virus was detected. The numbers 1, 2, 3, etc., represent the chronological sequence of virus detections, starting with the first confirmed case in Mexico.

Epidemiology

The dynamics of the spread and evolution of the disease caused by ToBRFV in tomato crops are articulated through different epidemiological phases, reflecting an inherent complexity in the behavior of the virus. The polycyclic ToBRFV disease has a constant production of inoculum mediated by the processes of virus replication within the host cells (Smith and Dambrovsky, 2019), as well as the rapid dispersal and subsequent infection of new individuals (Madden *et al*., 2007). The epidemiological development stages of ToBRFV can be described (Figure 6) during infection and disease progression in tomato plants. It begins with an initial phase (Figure 6A), where the infection rate is relatively low, which is attributed to the pathogen's acclimatization phase to its new environment and host. The initial phase is usually triggered by the germination of contaminated seeds; the

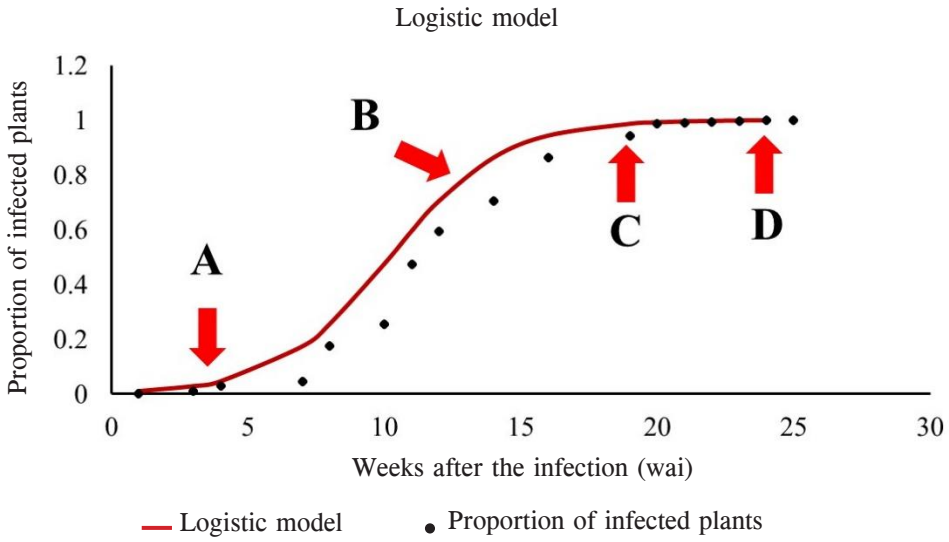

Figure 6. Evolution of a ToBRFV epidemic in tomato crops (Hypothetical design). A) Start of infection; B) Growth phase or exponential stage; C) Peak infection point; D) Deceleration phase or stationary phase.

hypocotyl activates the replication of the virus, which was in a latent state in the cotyledons (Dombrovsky *et al*., 2017b). As the disease progresses, an exponential growth phase is observed (Figure 6B), during which the transmission speed and viral replication intensify drastically, in direct correlation with the observed incidence. Given the high mechanical transmission capacity of ToBRFV and the start of cultural practices in the crop, the virus spreads rapidly (Levitzky *et al*., 2019). During this period, the relative rate of the disease increases in such a way that it produces a maximum transmission point (Figure 6C), driven by secondary infections in the set of evaluated plants. Crop management practices, together with insufficient disinfection measures, create a scenario where the incidence can reach 100% (Jeger *et al*., 2018; Klein *et al*., 2023). At the end of this phase, the epidemic enters a deceleration or stationary phase (Figure 6D), indicating the moment when the disease has infected the maximum possible number of individuals within the plant population. Unlike other members of the *Tobamovirus* genus, ToBRFV is capable of causing particularly severe infections (Temple *et al*., 2023), which can be fatal to the plant, especially in the presence of mixed infections with other tobamoviruses. (Vásquez-Gutiérrez *et al*., 2023b; Abou *et al*., 2023).

The symptoms caused by ToBRFV in tomato are influenced by biotic and/or abiotic factors, such as precipitation, humidity, and temperature, which play a crucial role in the incidence and severity of the virus (Nolasco *et al*., 2023). González-Concha *et al*. (2023) highlight that the symptomatology in tomato

plants grown in open fields differs significantly from those grown in greenhouses, with high temperatures exacerbating both the incidence and severity of the virus (Salem *et al.,* 2016). Menzel *et al*. (2019) reported the appearance of dark green protuberances on tomato leaflets under high temperature conditions, while Oladokun *et al*. (2019) identified necrotic lesions on leaflet peduncles and stem necrosis. Panno *et al*. (2019a) observed calyx and leaflet petiole necrosis in tomato. Although tobamoviruses generally do not act as necrotrophic parasites with their hosts, ToBRFV constitutes a notable exception to this trend (Caruso *et al*., 2022). Under favorable conditions, this virus has the ability to cause the collapse of its hosts, demonstrating a distinct and more severe pathogenic impact compared to other members of its genus (Dombrosky and Smith, 2017).

Management strategies

In the absence of specific methods for the control and management of ToBRFV, various cultural strategies have been implemented that partially reduce the spread of the virus in the work area. These strategies include the use of virus-free seeds, avoiding the reuse of substrates (García-Estrada *et al*., 2024), or, failing that, applying thermal inactivation and disinfection treatments for ToBRFV (Michael *et al*., 2022; Samarah *et al*., 2021; Ling *et al*., 2022), removing symptomatic plants after transplanting, keeping the work area free of weeds that can act as virus reservoirs, and considering crop rotation specifically with legumes. In addition, it is crucial to keep workers located in specific areas (Ehlers *et al*., 2022b), properly clean workers' clothing and shoes, and disinfect tools before and after entering the production area (Ehlers *et al*., 2022a). These are some mitigation strategies that could contribute to good management of ToBRFV. Although there are currently no chemical products specifically effective against ToBRFV in host plants, disinfectant products have been investigated to reduce its incidence and severity in the field (Iobbi *et al*., 2022; Nourinejhad-Zarghani *et al*., 2023; Pablo *et al*., 2022). For example, the use of hydrogen peroxide (HP) in polyvinyl alcohol (PVA)/polyvinylpyrrolidone (PVP) hydrogel for controlled release in field applications has been reported. The release rate of HP hydrogel through direct and indirect contact with soil (gas phase) has shown low phytotoxicity and high efficacy against ToBRFV in tomato and tobacco plants (Eldan *et al*., 2022; Liao *et al*., 2013). The use of resistance inducers applied directly to the crop to counteract the damage caused by ToBRFV is still limited due to the recent emergence of the virus. However, recent studies have evaluated (Ortiz-Martínez and Ochoa-Martínez, 2023) the effect of 14 elicitors and biostimulants on agronomic parameters of morphology, yield, and fruit quality in tomato plants infected with ToBRFV. They determined that Virablock® 3G50, Optifert®, Silicant®, and Haifa ProtekTM improved the evaluated agronomic

parameters, while Haifa ProtekTM induced a larger root system. ToBRFV is considered the first tobamovirus capable of breaking the resistance to the *Tm-2*, *Tm-1*, and *Tm-22* genes that confer resistance to TMV, ToMV, and ToMMV in solanaceous plants (Hak and Spiegelman, 2021). Therefore, the selection of wild materials for the application of genetic improvement with resistance to ToBRFV has provided favorable results in research, as indicated by Kabas *et al*. (2022), who evaluated tomato materials including 10 wild species and 11 interspecific F1 hybrids derived from *Solanum habrochaites* and *S. pennellii*. These were tested with ToBRFV isolates using the biological test method. In the end, they found that *S. pimpinellifolium* (LA1651), *S. penellii* (LA0716), and *S. chilense* (LA4117A, LA2747) could be tolerant to ToBRFV with a severity index (SI) lower than 19.6, 28.3, 35.0, and 35.2%, respectively. Additionally, there are reports indicating that ToBRFV is capable of systemically infecting pepper plants carrying *L1* or *L2* genes resistant to ToMV (Eldan *et al*., 2022), although in pepper with *L2* and *L4* genes, ToBRFV produces local lesions as resistance to the virus (Fidan, 2021). Pelletier and Moffett (2022) indicated that *Nicotiana tabacum* cultivar BY-2 conferred resistance to ToBRFV through the recognition of the viral P50 and CP fragments, respectively; this could have been achieved by the resistance mediated by the *N* and *N*' genes by not inducing a hypersensitivity response (HR) in *N. tabacum*. This highlights the limited availability of genetic materials, both wild and commercial, that offer resistance to ToBRFV, a crucial aspect for mitigating losses in tomato and pepper crops (Avni *et al*., 2021; García-Estrada *et al*., 2022). It is important to mention that, although there are genotypes in the market considered resistant, they do not always counteract the infection by ToBRFV. In other words, genotypes established at normal temperatures (20 to 28 °C) may tolerate the pathogen, but at extreme temperatures (30 to 48 °C), they may manifest as susceptible. Therefore, it is necessary to consider the validation of resistance to ToBRFV before its establishment in production zones (González-Concha *et al*., 2023; Nolasco *et al*., 2023).

Legal control and regulations

The phytosanitary problem of the presence of ToBRFV prompted the United States in 2018, through the California Department of Food and Agriculture (CDFA) and the Department of Agriculture (USDA), to assess the risks that the presence of ToBRFV would entail. With this, they established restrictions on imports of vegetative material and tomato and pepper seeds that could allow the dispersal of the inoculum. Despite these measures, ToBRFV was detected for the first time in tomato greenhouses in Southern California, USA (Ling *et al*., 2019). In Mexico, after the detection of the virus, the National Epidemiological Surveillance System

(SINAVEF) implemented measures that classified the pest status as "Actionable and eradicable transient pest (AETP)," given that there were no reports by the International Standards for Phytosanitary Measures (ISPMs) (FAO, 2023) to prevent its spread. These actions included: diagnosis, elimination of plantations with the presence of ToBRFV, and destruction of contaminated propagative material (seeds). However, the results were not satisfactory, so in that same year, the status changed to "Quarantine pest, under official control (QPOC)." Regarding the appearance of the virus in our country, the following hypothesis has been proposed: The inoculum came from a block of seeds imported from Jordan to Israel (Luria *et al*., 2017), which was distributed throughout the Mexican republic, being detected for the first time in Baja California and later in Michoacán. From 2019 to date, the virus has been considered a "Regulated non-quarantine pest (RNQP)," so regulations for seeds and plant material have continued to be implemented to reduce its impact on Mexican production. In 2019, the first circular was issued aimed at seeds, seedlings, and fresh fruits of tomatoes and peppers for export purposes and the absence of ToBRFV (SENASICA, 2023). Finally, the European Union issued an Implementing Decision notification (EU) 2019/1615, which establishes emergency measures to prevent the introduction and spread of ToBRFV in the EU. For this reason, in 2021, Circular No. 040 was published for the export of tomato and pepper seeds destined for the EU. These guidelines continue to be respected today for the import and export of seeds to the USA, EU, and Mexico.

Conclusions

The lack of knowledge about ToBRFV following its detection resulted in rapid spread worldwide, as it is a pathogen that can be transmitted through seeds and plant debris, facilitating greater dissemination. Currently, five clades are reported based on the existing genetic diversity. ToBRFV infects 16 families of weeds and host crops distributed across 47 countries, with *Solanum lycopersicum* being the most economically significant. Genetic resistance is a promising alternative for developing resistant genotypes; however, studies in this area are currently limited. The current status of ToBRFV in Mexico and its presence in tomato-producing regions reveals that the fundamental problem lies not only in contaminated seeds but also in the phytosanitary measures implemented for detecting emerging diseases. This review emphasizes the need for a comprehensive analysis of ToBRFV, from its genetic diversity to specific management strategies. The absence of corrective measures for viral diseases could lead to the recurrence of similar scenarios, underscoring the importance of conducting further research focused on emerging viral diseases, such as ToBRFV.

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