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Molecular detection and identification of the tomato brown rugose fruit virus infecting greenhouse-grown tomatoes and peppers in Yazd Province, Iran

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Abstract

Objective

Tomato brown rugose fruit virus (ToBRFV, *Tobamovirus fructirugosum*, family *Virgaviridae*) is an important pathogen affecting tomatoes and peppers. The objectives of this study are to characterize the ToBRFV infecting these crops, determine its incidence, and study the symptomatology of affected plants, as well as possible ways that the virus may get into the greenhouses in Yazd Province of Iran.

Materials and methods

A total of 136 plant samples exhibiting viral and virus-like symptoms on leaves and fruits, as well as 13 asymptomatic samples from seedlings at one month of age, were collected from greenhouse-grown tomatoes and peppers in different regions in Yazd Province during 2023 and 2024. Total RNA was extracted from leaves and fruits using a commercial kit (Pars Tous, Iran) and subsequently subjected to reverse transcription polymerase chain reaction (RT-PCR).

Accordingly, 14 ToBRFV isolates, chosen based on plant cultivar, geographical area, and isolation source were used for molecular studies, and their coat protein (CP) gene sequences were deposited in GenBank. Subsequently, the sequences were phylogenetically analyzed, along with 67 ToBRFV isolates from GenBank.

Results

Of the 136 symptomatic samples, an 800 bp amplicon was amplified from 98 samples in RT-PCR reactions using specific primers targeting the CP gene of ToBRFV. The findings revealed a significant infection rate (65.77%) of tomatoes and peppers grown in greenhouses with ToBRFV in Yazd Province. The CP-based phylogenetic tree analysis of 67 ToBRFV isolates from GenBank and 14 isolates from this study grouped them into three phylogenetic clusters (I, II, and III) with low bootstrap validity. Out of the 14 ToBRFV isolates, four were placed in cluster II, while the rest were not included in any of the specified clusters. Moreover, phylogenetic analysis conducted with the CP gene demonstrated that these isolates exhibit a high nucleotide identity of approximately 98% when compared to other previously documented isolates, which indicates a limited genetic diversity level (0.005).

Conclusions

The virus usually enters greenhouses through asymptomatic infected seedlings, with symptoms becoming apparent as the plants grow. Inside the greenhouses, the virus is transmitted through mechanical means, gardening operations, and the interaction of workers with the plants. The lack of ToBRFV detection in specific symptomatic samples indicates that the symptoms observed could be due to other viruses, co-infections, or non-viral elements. Furthermore, it is possible that other viruses are present alongside the identified virus in positive samples requires further study.

Keywords: infected seed, infected seedling, mechanical transmission, tomato brown rugose fruit virus

Paper Type: Research Paper.

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Introduction

Tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) represent the most significant vegetable crops globally, cultivated in both field and greenhouse environments (Baenas et al., 2019; Quinet et al., 2019). Plant viruses are a major risk to agricultural production, leading to considerable reductions in both yield and quality.The plant virus genera that hold significant economic importance for these crops include *Potyvirus*, *Cucumovirus*, *Tospovirus*, *Begomovirus*, and *Tobamovirus*.Viral infections not only decrease the yield and quality of fruit but also compromise their marketability, resulting in substantial economic damage (Hanssen & Lapidot, 2012; Jones & Naidu, 2019). The genus *Tobamovirus* is notably important as it contains several damaging pathogens, such as the tobacco mosaic virus, tomato mosaic virus, tomato mild mottle virus, pepper mild mottle virus (PMMoV), and cucumber green mottle mosaic virus (ICTV, 2021). Alongside the well-known tobamoviruses, the emergence of new viral diseases poses significant risks to the cultivation of tomatoes and peppers. Over the last ten years, the tomato brown rugose fruit virus (ToBRFV), a recently identified member of the Tobamovirus family, has emerged as a significant pathogen affecting tomatoes (Salem et al., 2016). Since that time, the virus has been identified in over 50 nations across the globe (EPPO, 2025). Currently, it is regarded as a significant danger to worldwide tomato cultivation, which covers more than five million hectares, due to its fast spread and severe effects on crop yield and quality (Caruso et al., 2022; FAOSTAT, 2020; Jones, 2021; Oladokun et al., 2019). Similar to other members of the Tobamovirus group, ToBRFV has a positive-sense, single-stranded RNA genome measuring approximately 6.4 kb, which encodes four open reading frames (ORFs). ORF1 and ORF2 synthesize replication proteins of about 126 kDa and 183 kDa, respectively; the larger protein is generated from the readthrough of the termination codon of ORF1, and together, they assemble the RNA-dependent RNA polymerase (replicase) complex.ORF3 is responsible for encoding the approximately 30-kDa movement protein, which facilitates cell-to-cell transport. Meanwhile, ORF4 encodes a coat protein (CP) of approximately 17.5 kDa, necessary for the assembly of virions, systemic movement, and the success of infection. (ICTV, 2021; Salem et al., 2023; Zhang et al., 2022). The virus is efficiently transmitted both mechanically and via seeds, enabling regional outbreaks, as well as long-distance spread (Davino et al., 2020; Salem et al., 2023). Its ability to overcome widely used resistance genes, *Tm* in tomato and *L* in pepper, has significantly reduced the effectiveness of existing genetic resistance strategies (Zhang et al., 2022). Infected plants often exhibit severely reduced fruit yield and quality, with direct consequences for economic returns and marketability. The expression of symptoms in tomatoes that are infected is influenced by factors such as cultivar, season, photoperiod, temperature, and age. Mosaic patterns, chlorotic mottling of diverse intensity, leaf blistering, and narrowing are frequently observed as foliar symptoms (Luria et al., 2017; Salem et al., 2023; Zhang et al., 2022). Conversely, fruits usually show discoloration, necrotic spotting, rugosity, and deformation, with the severity of these symptoms influenced by genotype, the timing of infection, and environmental conditions (Menzel et al., 2019; Salem et al., 2023; Zhang et al., 2022). Infections in pepper are usually marked by a mild mosaic appearance on the leaves, chlorosis affecting young foliage, necrosis of the stems, and deformities in the fruit (Salem et al., 2023). The losses in yield due to ToBRFV are considerable, with reports indicating reductions between 15% and 55%, regardless of whether the plants possess the *Tm-2²* resistance gene (Avni et al., 2021). These attributes emphasize ToBRFV as a considerable risk to the global production of solanaceous crops. Reports of ToBRFV outbreaks have been noted in numerous regions where tomatoes are grown, often causing severe losses in yield. Considering its high rate of transmission and worldwide repercussions, ToBRFV is currently viewed as one of the most critical viral threats to tomato cultivation (Avni et al., 2021; EPPO, 2020; Jones, 2021; Oladokun et al., 2019).

Given the worldwide epidemiological significance of the tomato brown rugose fruit virus (ToBRFV), particularly its presence in Iran, this research aimed to identify and characterize ToBRFV affecting greenhouse-grown tomatoes and peppers in Yazd Province. In addition, the study was designed analyze the genetic diversity of the virus through the coat protein (CP) gene and determine its prevalence in the region.

Materials and methods

Plant materials: To identify and study ToBRFV in Yazd Province, surveys were conducted in tomato and pepper growing greenhouses from January 2023 to June 2024 in different regions of Yazd Province. A total of 136 plant samples with viral and viral-like symptoms on leaves and fruits, and 13 asymptomatic samples related to one-month transplants, were collected from 12 tomato and pepper growing regions.

RNA isolation and RT-PCR: Total RNA was obtained from 20–40 mg of leaf tissue through the use of the Pars Tous Total RNA Extraction Kit (Pars Tous, Iran), following the manufacturer's guidelines with minor adjustments specific to plant material.In summary, the tissue was homogenized in RL buffer, subsequently subjected to chloroform extraction and centrifugation at 13,000 rpm for 12 minutes at 4 °C. The aqueous phase was mixed with ethanol, transferred to a spin column, rinsed with PW buffer, and eluted using 50 µL of DEPC-treated water.RNA was stored at −70 °C until use. Moloney murine leukemia virus reverse transcriptase (M-MLV-RT) was used to synthesize first-strand cDNA, as summarized by Sharifi et al. (2008). To amplify the full-length CP genome of ToBRFV isolates, the resulting cDNA and the primer pair, ToBRFV-F (5′-GAAGTCCCGATGTCTGTAAGG-3′)/ToBRFV-R (5′-TGCCTACGGATGTGTATGA-3′) (Ghorbani et al. 2021) were used for RT-PCR assays. A single-step RT-PCR reaction was employed to amplify the CP gene on a Techne TC 312 thermal cycler (Techne, Cambridge, UK). The protocol began with a denaturation step at 98°C for 3 minutes, followed by 35 cycles comprising denaturation at 94°C for 1 minute, annealing at 54°C for 1 minute, and extension at 72°C for 1 minute, finishing with a final extension of 10 minutes at 72°C.

Sequencing and phylogenetic analysis: A total of fourteen representative isolates were chosen for molecular characterization and sequencing from different cultivars of tomato and pepper originating from different geographical regions. Using the High Pure PCR Product Purification Kit (Roche Diagnostics), approximately 800 bp PCR products from each isolate were purified, followed by nucleotide sequencing reactions performed by Bioneer (South Korea) on an ABI 3730XL DNA Analyzer. The CP gene sequences of ToBRFV isolates were analyzed in comparison to the sequences available in GenBank through the use of the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/BLAST). The MEGA software version 11.0 was employed to generate the maximum likelihood phylogenetic tree based on the Kimura 2-parameter model, with 1000 bootstrap replicates included (Tamura et al., 2021). In order to construct the phylogenetic tree, 81 complete coat protein (CP) gene sequences of ToBRFV were examined, which comprised the 14 isolates sequenced in this research, 14 Iranian isolates that were reported earlier, and 54 sequences sourced from global databases. The dataset included isolates from Asia (26), the Americas (9), Europe (17), and Africa (2, both originating from Egypt). Moreover, one Tomato mosaic virus sequence (JX121575.1, Ker.Tom.41) was designated as an out-group. The pairwise identities were calculated using the SDT v1.3 software (Muhire et al., 2025). Genetic diversity (π) among ToBRFV sequences was also calculated using MEGA. The DnaSP v5 software was used to estimate the ratio of non-synonymous to synonymous substitutions ($dN/dS = \omega$) in ToBRFV sequences, as well as to study natural selection (Librado & Rozas, 2009).

Results

Virus detection and the CP gene sequencing: To identify the presence of ToBRFV in Yazd Province, a total of 149 plant samples were collected from tomato and pepper greenhouses, followed by RT-PCR assays to confirm viral presence. The findings revealed that ToBRFV was detected in six tomato cultivars (4129, 3725, PARDIS, CAPTAIN, PINK, TERMEH) and eight pepper cultivars (132, 9908, 9325, BIGO, KASPEL, RIMINO, RUBIK, SHAMSHIRI) (Table 1). Infected tomato plants showed a variety of symptoms, including mosaic patterns, yellowing, chlorosis, rugosity, blistering, leaf deformation, tiny leaves, and vein banding. Fruit symptoms on infected tomatoes included chlorosis, mottling, necrotic and brown spots, deformation, and reduced size. Pepper plants exhibited various symptoms including deformation, chlorosis, mosaic, tiny leaf, rugosity, blistering, and vein banding. Additionally, the fruit displayed symptoms such as deformation, reduced size, and mottling (Figure 1). The nucleotide sequences derived from the ToBRFV isolates were deposited in the NCBI GenBank database (Table 2).

Phylogenetic studies: According to the phylogenetic tree, the ToBRFV sequences were categorized into three significant clusters (I, II, and III) (Figure 2).

Table 1. Sampling, host plants, and the number of ToBRFV positive samples in greenhouse-grown peppers and tomatoes in Yazd Province, Iran

Region	Host	Line/variety	Number of samples[a]		Number of positive sample	
Yazd-Fahraj	Tomato	4129	1*		1	
	Tomato	3725	2		0	
	Tomato	4129	10		6	
	Pepper	132	2		1	
	Pepper	9908	2		1	
	Pepper	BIGO	1		0	
	Pepper	SHAMSHIRI	5		3	
Yazd-Khavidak	Tomato	3725	8		3	
	Tomato	4129	6		2	
	Tomato	PARDIS	1		1	
	Tomato	CAPTAIN	2		2	
	Pepper	9325	3		2	
	Tomato	4129	1*		1	
	Tomato	3725	2		0	
Yazd-Mollabashi	Tomato	3725	8		6	
	Tomato	4129	2		2	
	Tomato	CAPTAIN	4		3	
	Tomato	PARDIS	3		2	
	Pepper	9325	8		6	
	Pepper	9908	3		2	
Yazd-Dehnoo	Tomato	3725	1		0	
	Pepper	9325	3		2	
Yazd-Ahamadabad	Tomato	3725	2		2	
	Tomato	CAPTAIN	1		0	
	Pepper	9325	1		0	
Yazd-Arnan	Tomato	PINK	1*		1	
	Tomato	PINK	3		3	
Yazd-Chaheyazdani	Tomato	CAPTAIN	2		0	
Yazd-Ashkezar	Tomato	4129	3		2	
	Tomato	TERMEH	3		3	
	Tomato	CAPTAIN	3		2	
	Pepper	9325	2*		1	
Mehrize	Tomato	4129	5		4	
	Tomato	TERMEH	2		1	
	Tomato fruit	PINK	2		2	
	Tomato	PINK	2*		2	
	Pepper	KASPEL	2		1	
Mehrize Nursery	Tomato	4129	2		1	
	Tomato	3725	1		1	
	Pepper	RIMINO	1		1	
	Pepper	RUBIK	1		1	
	Pepper	9325	1		1	
Marvast	Tomato	3725	3		2	
	Tomato	4129	9		7	
Taft	Tomato	3725	7		4	
	Tomato	4129	6		5	
	Pepper	9325	5		3	
	Pepper	KASPEL	3		2	
Total			105 (Tomato)	44 (Pepper)	71 (Tomato)	27 (Pepper)
			149		98	

[a] Leaves and fruits (*) of symptomatic plants were sampled.

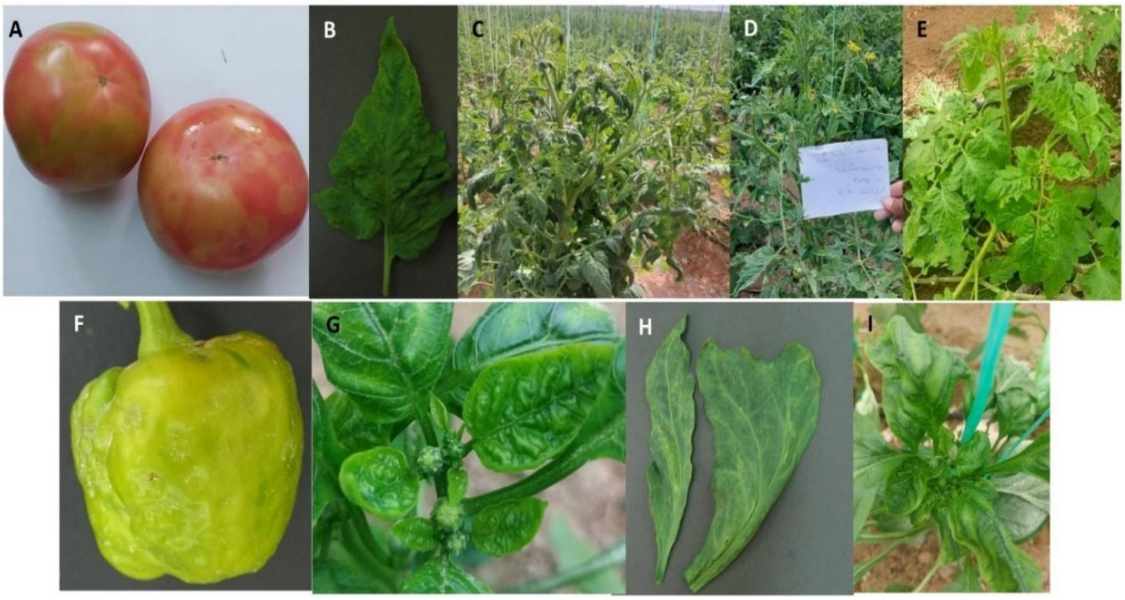

Figure. 1. Association of the tomato brown rugose fruit virus with greenhouse-grown tomato and pepper expressing various symptoms in Yazd Province. A: Brown spot lesions on tomato fruit, B: Mosaic pattern and rugosity (wrinkling) on tomato leaves, C: Severe leaf rugosity, plant deformation, and leaf malformation in tomato, D: Leaf distortion and rugosity symptoms in tomato, E: Rugosity, mosaic leaf pattern, and stunting in tomato, F: Fruit deformation, chlorotic areas, and reduced growth in pepper, G: Rugosity (wrinkling) of pepper leaves, H: Leaf deformation in pepper, and I: Rugosity, vein banding, and leaf malformation in pepper

Table 2. Features of the ToBRFV isolates, including GenBank accession numbers that were molecularly characterized in the current study

Isolate name	Origin sampling	Line/Variety	GenBank accession No.	Source
Yaz.Kha.To	Kharidak	3725-Tomato	PP916473	Leaf
Yaz.Mo.To1	Mollabachie	4129- Tomato	PP916474	"
Yaz.Ma.To1	Marvast	3725- Tomato	PP916475	"
Yaz.Fa.To	Fahraj	4129- Tomato	PP916476	"
Yaz.Mo.To2	Mollabachie	4129- Tomato	PP916477	"
Yaz.Me.To	Mehrize	PINK- Tomato	PP916478	Fruit
Yaz.Ma.To2	Marvast	4129- Tomato	PP916479	Leaf
Yaz.Me.To2	Mehrize	4129- Tomato	PQ310342	Seedling
Yaz.Me.To3	Mehrize	4129- Tomato	PQ310341	"
Yaz.Ash.Pe	Ashke zar	9325-Pepper	PQ310346	Fruit
Yaz. Me.Pe1	Mehrize	9325- Pepper	PQ310343	Seedling
Yaz. Me.Pe2	Mehrize	RUBIK- Pepper	PQ310344	"
Yaz.Ta.Pe	Taft	KASPEL- Pepper	PQ310347	Leaf
Yaz.Fa. Pe	Fahraj	9908- Pepper	PQ310345	"

It is noteworthy that the 10 Iranian isolates from the current study, along with 14 previously identified isolates from other regions of the country, did not align with these well-defined clades; instead, they constituted a highly polytomic branch with low bootstrap support, suggesting that the phylogenetic relationships are unresolved. These included five pepper-derived isolates, including Yaz.Me.Pe1 and Yaz.Me.Pe2, from nursery plants of cultivars 9325 and RUBIK in Mehriz, as well as isolates from cultivars 9908 and KASPEL. The isolates, Yaz.Me.To (tomato, cv. PINK) and Yaz.Ash.Pe (pepper, cv. 9325), were also clustered in this unresolved branch. In Group III, the isolates Yaz.Mo.To1, Yaz.Mo.To2, Yaz.Ma.To1, and Yaz.Ma.To3, originating from tomato cultivars 4129 and 3725 collected in Mollabashi (Yazd) and Marvast, formed a cluster. Also Group III comprised 14 Iranian isolates that had already been reported; these included isolates from Khuzestan–Dezful (pepper), Hormozgan (tomato), and a number of pepper and tomato isolates (Pep-27, PEP6, ZAR-K-20, TOM4, TOM5, TOM6, Iran-1, and ToBRFV-IR). Importantly, ToBRFV-CaJO, the only non-Iranian isolate in this group, was also assigned to this Group. It was obtained from pepper in Jordan (Salem et al., 2020), as well as the previously reported IR-Pep isolate from pepper in Yazd Province (Bananej et al., 2024). Moreover, 33 isolates from various geographical areas and host plants, such as tomato, watermelon, black nightshade (*Solanum nigrum*), wild potato (*S. chacoense*), pepper, and wild tomato (*S. pimpinellifolium*), were found in Group I (Group I is shown collapsed and in green (Figure. 2)(Supplementary table 1)), while five isolates were found in Group II. High genetic similarity was found between all isolates based on pairwise sequence comparisons. The lowest nucleotide identity (98.1%) was observed between the Iranian isolate Yaz.Me.To2 and three foreign isolates (39563361 A (Netherlands), F48-PAL, and F42-PAL (Palestine)). Conversely, 100% identity was observed between multiple Iranian and global isolates. Across all 81 sequences, the nucleotide identity ranged from 97.5% to 100%, while the amino acid identity ranged from 95% to 100% (Figure 3).

Genetic diversity: The predicted nucleotide diversity values for the three primary phylogenetic groups were 0.0000 (Group I), 0.0025 (Group II), and 0.0070 (Group III) (refer to Table 3). Among the various geographic populations, isolates originating from the American continent displayed the least diversity ($\pi = 0.0014$), while those from Yazd Province demonstrated the highest diversity value ($\pi = 0.0103$). In comparison, the average nucleotide diversity among non-Iranian isolates was measured at 0.0022, whereas Iranian isolates exhibited a significantly elevated value of 0.0093. The Iranian isolates sourced from Yazd recorded an ω value of 0.4068, in contrast to the global population which had a value of 0.16612. Among the populations studied, the Asia–Africa group demonstrated the most significant ω ratio at 0.6304, in contrast to the

American population, which had the least ratio of 0.0868. Group III, which included four isolates sequenced in our investigation, has the highest ω ratio (0.3567) of all the phylogenetic groupings.

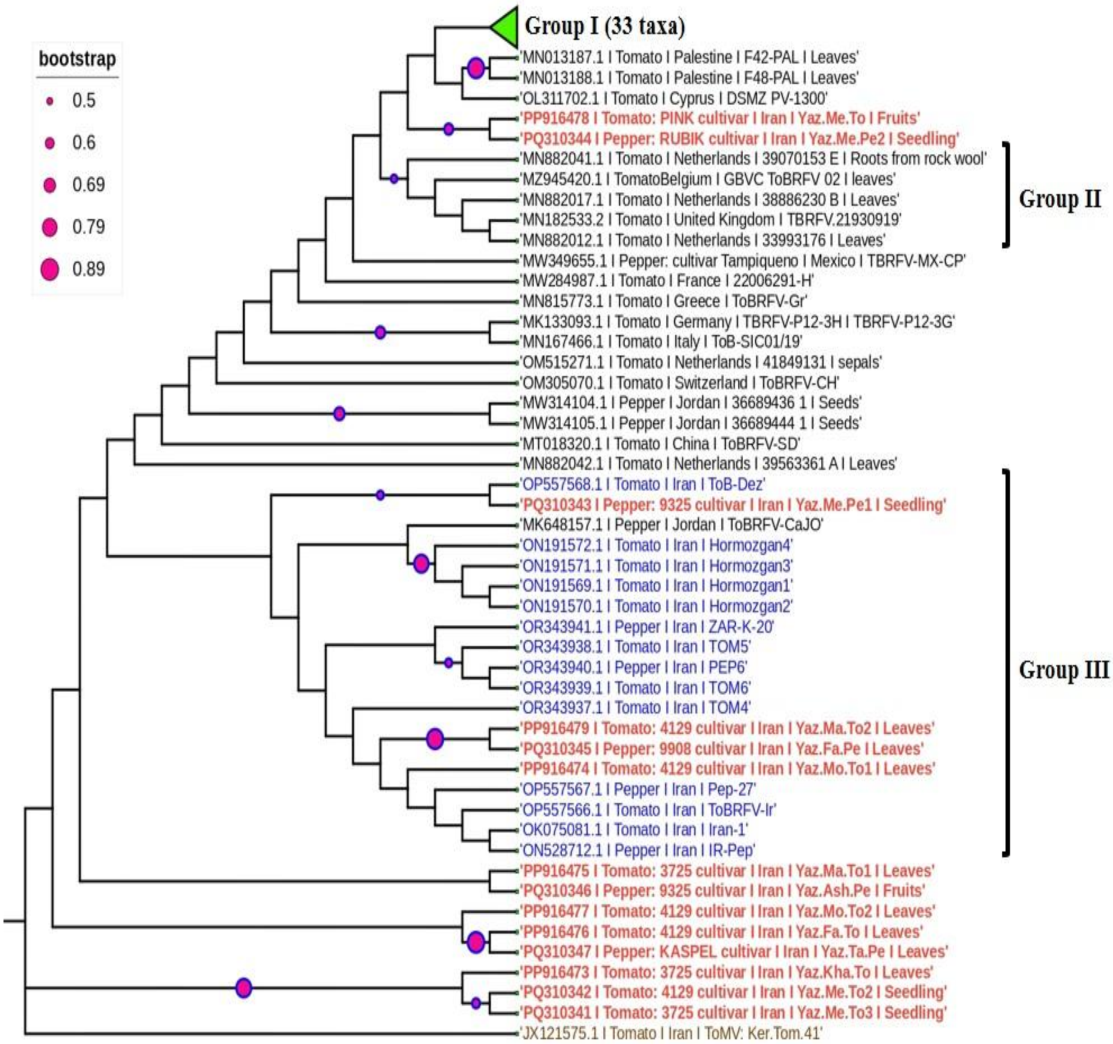

Figure. 2. Figure 2. Maximum likelihood phylogenetic tree of the coat protein gene from the tomato brown rugose fruit virus (ToBRFV) isolates identified in this study, along with related isolates from GenBank. The tomato mosaic virus sequence (accession no. JX121575) was used as an outgroup. The ToBRFV isolates characterized in this study are depicted in red. Sequence labels indicate GenBank accession number, host plant–cultivar (if available), country of origin, isolate name, and source of isolation (if available). The tree was generated using the MEGA program with 1000 bootstrap replications; values below 50 are not displayed

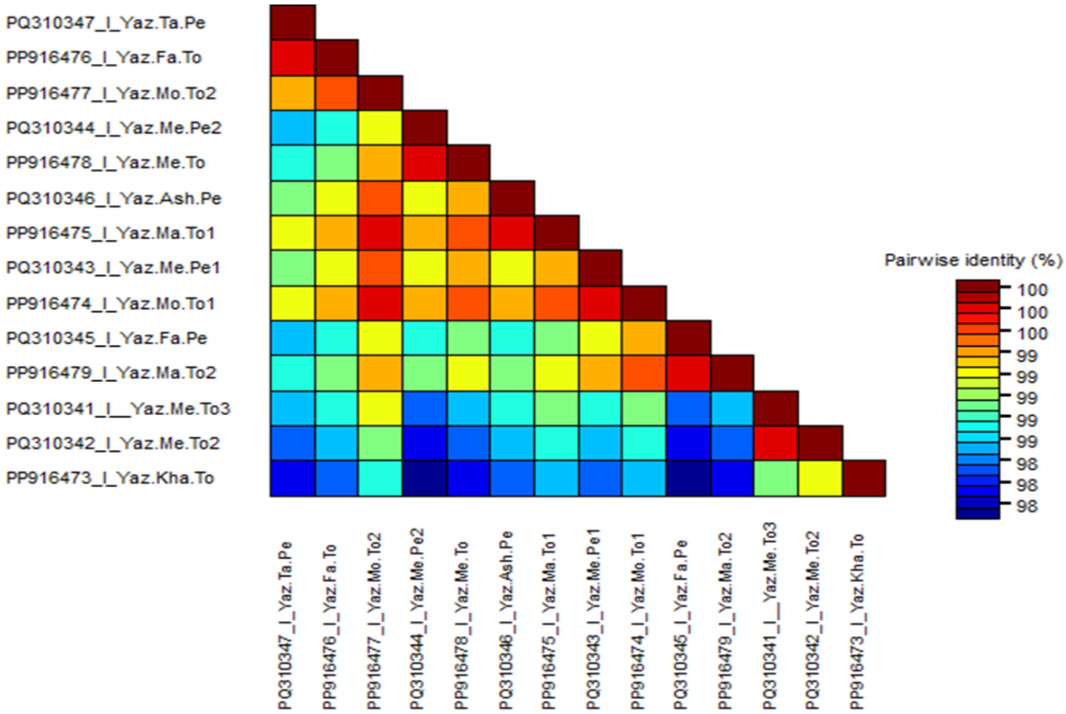

Figure 3. Sequence-based pairwise nucleotide identity of the CP genes of the tomato brown rugose fruit virus (ToBRFV) isolates identified in this study, calculated using the SDT v1.3 program. Pairwise identity values ranged from 97.5% to 100%

Table 3. Genetic diversity (π) and mean number of amino acid substitutions in the coat protein gene of ToBRFV isolates in various regions, including phylogenetic groups

Geographic populations/ phylogenetic groups	Number of sequences	dN[a]	dS[b]	dN/dS	Genetic diversity (π)
Total	81	0.00297	0.01364	0.21774	0.0055
World[c]	53	0.00103	0.0062	0.16612	0.0022
Iran[d]	28	0.0065	0.01833	0.3546	0.0093
Yazd	15	0.00773	0.019	0.4068	0.0103
Europe	17	0.00126	0.01226	0.1027	0.0038
America	9	0.00051	0.00587	0.0868	0.0014
Asia-Africa	28	0.00116	0.00184	0.6304	0.001
Group I	31	0.00000	0.00000	0.0000	0.0000
Group II	5	0.00000	0.01062	0.0000	0.0025
Group III	19	0.00488	0.01368	0.3567	0.007
Other isolates	26	0.00535	0.01856	0.2882	0.0085

[a]: Nonsynonymous substitutions estimated with the Jukes and Cantor (1969) correction
[b]: Synonymous substitutions estimated with the Jukes and Cantor (1969) correction
[c]: Multiple sequence alignment was conducted on 53 selected nucleotide sequences from various countries worldwide, excluding Iranian isolates
[d] Multiple sequence alignment was conducted on 14 Iranian nucleotide sequences previously deposited in GenBank, together with the 14 Iranian isolates sequenced in this study

Discussion

ToBRFV was initially identified in Iran in 2021, nearly simultaneously by Ghorbani et al. (2021) and Esmaeilzadeh and Koolivand (2022) in greenhouse-grown tomatoes in the Isfahan and Tehran Provinces. Subsequently, it was detected in tomatoes from an additional eight provinces, with an isolate also reported in Yazd Province (Bananej et al. 2024). These reports, along with our data, show that ToBRFV is quite common. More than 60% of the tomato and pepper samples we tested from Yazd were found to be positive. This study provides the inaugural thorough report on the emergence of ToBRFV in tomato and pepper greenhouses within Yazd Province. This research identified various symptoms in the plants, such as mild yellowing of the leaves and more serious symptoms including mosaic patterns, leaf distortion, and fruit rugosity. These results align with comparable studies carried out both in and outside of Iran, validating a common issue (Esmaeilzadeh et al., 2023; Celik et al., 2022; Salem et al., 2023; Zhang et al., 2022). In certain cases, symptoms were notably intense, possibly due to co-infections with additional viruses, a recognized element that exacerbates the development of symptoms. Previous studies have documented co-infections with two or three viruses in tomato greenhouses in Yazd Province (Massumi et al., 2009; Mirrahimi et al., 2015), indicating the potential influence of virus–virus interactions on disease severity. However, in our study, mixed infection testing was performed only for PMMoV in association with ToBRFV using RT-PCR, and all results were negative for the simultaneous presence of both viruses (data not shown). Furthermore, several samples exhibiting viral or virus-like symptoms tested negative for ToBRFV by RT-PCR. Considering that more than 312 viruses, viroids, and satellite viruses have been documented to infect tomatoes (Rivarez et al., 2021) and 68 have been identified to infect peppers (Ojinaga et al., 2022), it is probable that additional reported agents may also induce symptoms. The molecular characterization of 81 ToBRFV isolates, which includes those examined in this study, demonstrated a high nucleotide similarity of approximately 98% and a low genetic diversity of 0.005, aligning with earlier research findings (Abrahamian et al., 2022; Bananej et al., 2024; Caruso et al., 2022; Celik et al., 2022; Esmaeilzadeh et al., 2023; Salem et al., 2023; Yilmaz & Batuman, 2023; Zhang et al., 2022). Although Iranian isolates displayed slightly higher diversity than the global average, all phylogenetic clades identified here exhibited low bootstrap support. No significant correlation was identified between phylogenetic grouping and host species (e.g., tomato and pepper). The significant nucleotide similarity observed between Iranian isolates and those from far-off regions indicates that continental classifications and weakly supported phylogenetic groupings provide minimal insight into the population structure of ToBRFV. This is consistent with earlier studies that geographic origin and grouping based on either the CP gene or the whole genome have no impact on the virus's evolutionary way (Esmaeilzadeh et al., 2023;

Celik et al., 2022). The significant conservation of CP sequences globally supports the hypothesis of a recent and rapid worldwide dissemination, possibly facilitated by the translocation of infected seeds and plant material (Abrahamian et al., 2022; Caruso et al., 2022; Esmaeilzadeh et al., 2023). ToBRFV was first described in greenhouse tomatoes in Jordan (Salem et al., 2016) and is suspected to have originated in South America (van de Vossenberg et al., 2021), where solanaceous crops have been cultivated for thousands of years (Olmstead, 2013). The divergence of a Peruvian isolate in complete genome phylogenies lends further weight to this hypothesis (Abrahamian et al., 2022; van de Vossenberg et al., 2021). In our study, ToBRFV was detected in seedlings and fruiting plants grown from imported seeds originating from the Netherlands, Spain, Thailand, Turkey, India, and the United States, implicating seed-borne introduction into Iran. The international seed trade hinders the ability to pinpoint a unique origin for the virus, and despite thorough investigations, its source continues to be unclear (Salem et al., 2023; Zhang et al., 2022). Significant introductions consist of seed imports into the Netherlands from Peru, China, and Jordan (Branthôme, 2021; HortiDaily, 2020), along with contaminated fruits from Mexico entering the United States (Abrahamian et al., 2022). Seeds exported from Australia, India, Ethiopia, and Japan have also been found to be positive for ToBRFV, despite the absence of official notifications from these countries (EPPO, 2020, 2022; Zhang et al., 2022). These findings validate that the global transfer of seeds significantly contributes to the extensive dissemination of seed-borne viruses (Rizzo et al., 2021; Salem et al., 2023). ToBRFV spreads rapidly in greenhouse systems through mechanical contact, propagation materials, plant debris, contaminated substrates, recirculating irrigation water, and worker activities (Dombrovsky & Smith, 2017; Oladokun et al., 2019). After the introduction mediated by seeds, it is probable that asymptomatic infected seedlings function as the primary sources of inoculum, with outbreaks intensified by horticultural practices and the mobility of workers. In agreement with Bananej et al. (2024), our greenhouse-based sampling and the high incidence rates observed underscore the exceptional infectivity of ToBRFV. Seed transmission plays a pivotal role in initiating infection foci, while local spread in protected cultivation is largely driven by frequent mechanical contact (Salem et al., 2023).

Conclusions: This investigation verifies a considerable occurrence of ToBRFV in Yazd Province, with over 60% of the analyzed tomato and pepper samples showing positive results for the virus.The symptom spectrum observed aligns with global reports, indicating that severe cases may be associated with mixed viral infections; however, no co-infection with PMMoV was identified. Molecular analyses conducted on 81 isolates, which include 14 that were sequenced in this study, demonstrated a remarkably high nucleotide identity (~98%) and minimal genetic diversity (π=0.005), suggesting a recent and swift global dissemination. Phylogenetic clustering demonstrated low bootstrap support and a lack of association with host species or geographic origin, indicating a limited phylogenetic resolution for elucidating the population structure of ToBRFV. The identification of ToBRFV in plants cultivated from imported seeds emphasizes seed-borne transmission as a crucial and primary means of introduction into Iran. Alongside global evidence, this highlights the significance of international seed transfer in the extensive spread of the virus. The rapid local transmission of the virus within greenhouse systems is enabled by mechanical contact, contaminated materials, and common horticultural practices. Effective management, therefore, requires both strict seed health testing and stringent hygiene protocols in protected cultivation systems.

Author Contributions

Reza Farajeyani: Field and laboratory works. Hossain Massumi: Supervision, methodology, writing, reviewing & editing. Mohammad Maddahian: Writing draft paper, data analyzing and editing. Seyyed Alireza Esmaeilzadeh-Hosseini and Akbar Hosseinipour: Counseling, reviewing & editing.

Data Availability Statement

Sequencing data generated in this study have been deposited in the NCBI under accession numbers: PP916473-PP916479 and PQ310341-PQ310347.

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Ethical Considerations

Ethical approval was not required for this study because it focused solely on plant materials. All experimental procedures were performed in accordance with applicable biosafety and phytosanitary regulations for quarantine plant viruses, and the experiments were carried out in certified containment facilities to ensure that no quarantine viruses were released into the environment.

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Conflict of interest

The authors declare that there are no conflicts of interest present.

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چکیده

هدف: ویروس پژمردگی قهوه‌ای گوجه‌فرنگی (ToBRFV, *Tobamovirus fructirugosum*) از پاتوژن‌های مهمی است که گیاهان گوجه‌فرنگی و فلفل را تحت تأثیر قرار داده و آلوده می‌نماید. اهداف این مطالعه شامل تعیین خصوصیات مولکولی ToBRFV، میزان پراکنش، بررسی علائم گیاهان آلوده و همچنین بررسی مسیرهای احتمالی ورود این ویروس به گلخانه‌های استان یزد در ایران می‌باشد.

مواد و روش‌ها: طی سال‌های ۱۴۰۳- ۱۴۰۲ در مجموع ۱۳۶ نمونه گیاهی دارای علائم ویروسی و شبه‌ویروسی روی برگ‌ها و میوه‌ها، به‌همراه ۱۳ نمونه بدون علائم از نشاءهای یک‌ماهه گیاهان گوجه‌فرنگی و فلفل گلخانه‌ای در مناطق مختلف استان یزد

جمع‌آوری شدند.آران‌ای کل از برگ‌ها و میوه‌های نمونه‌برداری شده با استفاده از کیت تجاری (Pars Tous, Iran) استخراج و سپس با استفاده از آزمون نسخه‌برداری معکوس-واکنش زنجیره‌ای پلیمراز (RT-PCR) آلودگی آن‌ها نسبت‌به ToBRFV مورد بررسی قرار گرفت. بر این اساس، ۱۴ جدایه ویروسی بر پایه رقم گیاهی، منطقه جغرافیایی و منبع جداسازی جهت مطالعات مولکولی انتخاب و ترادف ژن پروتئین پوششی (CP) آن‌ها در بانک ژن ثبت گردیدند. سپس این توالی‌ها به‌همراه ۶۷ جدایه ToBRFV، انتخاب شده از بانک ژن، جهت مطالعات تبارزائی مورد تجزیه و تحلیل قرار گرفتند.

نتایج: واکنش RT-PCR با استفاده از پرایمرهای اختصاصی، از میان ۱۳۶ نمونه دارای علائم، در ۹۸ نمونه منجر به تشکیل باند ۸۰۰ جفت‌بازی مربوط به ژن ToBRFV–CP گردید. نتایج این تحقیق بیانگر میزان بالای آلودگی (۶۵/۷۷ درصد) گیاهان گوجه‌فرنگی و فلفل‌های کشت‌شده در گلخانه‌های استان یزد به این ویروس می‌باشد. بر مبنای درخت تبارزائی رسم شده مبتنی بر ژن CP مربوط به ۶۷ جدایه ToBRFV انتخاب شده از بانک ژن به‌همراه ۱۴ جدایه حاصل از این مطالعه، جدایه‌ها در سه شاخه (I، II و III) با اعتبار سنجی پایین گروه‌بندی گردیدند. از میان ۱۴ جدایه مذکور، چهار جدایه در گروه II قرار گرفتند. این در حالی‌است که سایر جدایه‌ها در هیچ‌یک از گروه‌های مشخص‌شده گروهبندی نگردیدند. علاوه بر این، نتایج حاصل نشان دهنده تشابه ترادف نوکلئوتیدی بالای (حدود ۹۸٪) جدایه‌های معرفی شده در این تحقیق با سایر جدایه‌های گزارش‌شده می‌باشد، که بیانگر تنوع ژنتیکی پائین (۰/۰۰۵) در میان جدایه‌های ToBRFV است.

نتیجه‌گیری: ToBRFV معمولاً از طریق نشاءهای آلوده بدون علامت وارد گلخلنه‌ها می‌شود و با رشد گیاه علائم ظاهر می‌گردند. پس از ورود به گلخلنه، ویروس از طریق انتقال مکانیکی، فعالیت‌های باغبانی مانند هرس توسط کارگران گسترش می‌یابد. اینکه ToBRFV در برخی نمونه‌های دارای علائم شناسایی نشد، نشان می‌دهد که ویروس‌ها یا عوامل بیماریزای دیگری نیز ممکن است در ایجاد علائم نقش داشته باشند. علاوه بر این، ممکن است ویروس‌های دیگری همراه با ویروس شناسایی‌شده در نمونه‌های مثبت وجود داشته باشند که این موضوع نیز نیازمند بررسی بیشتر است.

کلیدواژه‌ها: انتقال مکانیکی، بذر آلوده، نشاء آلوده، ویروس چروکیدگی قهوه‌ای میوه گوجه‌فرنگی

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