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REVIEW ARTICLE

Tomato brown rugose fruit virus: A pathogen that is changing the tomato production worldwide

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Abstract

Tomato (*Solanum lycopersicum* L., family *Solanaceae*) represents one of the most cultivated horticultural crops worldwide, with over 5 million hectares of cultivated area and more than 182 million tons of tomato produced globally. Nevertheless, monoculture conditions, intensive selection, domestication throughout the last decades, international trade of infected propagating material and climate changes intensely favoured the establishment of many pathogens and the rapid spread of new diseases, allowing organisms to establish in new and unfavourable environments. Among different biotic agents, viruses are the most dangerous, because of their rapid diffusion and production losses. Here, we review an emerging viral threat to tomato production, tomato brown rugose fruit virus (ToBRFV), a new highly infectious tobamovirus that is currently causing great concern to tomato global production, especially in those areas where mitigation measures are absent or inadequate and which, in recent years, it has considerably increased its diffusion in new tomato cultivation areas. Through a review of all the existing literature, this article highlights the following aspects: (a) main characteristic of tomato species (origin, taxonomy and genome); (b) main diseases that undermine the tomato production, focusing on viral pathogens; (c) ToBRFV main characteristics (origin and spatiotemporal dispersal, taxonomy, genome organisation, host range and symptoms, transmission, spread and epidemiology, and genetic diversity); (d) detection methods developed and disease management; (e) breeding as a new weapon to control the ToBRFV diffusion. Moreover, future perspectives are highlighted, to understand the epidemiology key factors and the ToBRFV-tomato pathosystem management, in order to develop effective and appropriate control strategies.

KEYWORDS

epidemiology, tobamovirus, ToBRFV, tomato brown rugose fruit virus, tomato production

Andrea Giovanni Caruso and Sofia Bertacca contributed equally to this work.

1 | INTRODUCTION

Tomato (*Solanum lycopersicum* L.), an herbaceous plant originating from western South America (belonging to *Solanaceae* family), represents one of the most cultivated vegetable species worldwide. In 2019, according to the latest data available, over 5 million hectares were allocated for tomato production, with more than a million hectares cultivated just in China, followed by India, Turkey, United States and Egypt, that represent over 60% of world tomato production (Figure 1) (FAO, 2019).

Tomato is produced both for domestic consumption and for international trade, for this reason, it can be found throughout the year. However, market requirements lead to monoculture conditions, that strongly favour the establishment and the recrudescence of many pathogens, which threaten the quantitative and qualitative yield of production (Hanssen, Lapidot, & Thomma, 2010). In addition, the rapid spread of new diseases is also attributed to international trade of infected propagating material associated with the climate changes that has allowed organisms to establish in unfavourable environments (Panno et al., 2021). In particular, among different biotic agents many viruses that infect tomato have been described, and new ones are reported every year (Oladokun, Halabi, Barua, & Nath, 2019). For example, tomato brown rugose fruit virus (ToBRFV) represents an emerging viral threat to tomato production and is currently spreading into new areas, causing great concern to tomato global production, especially in the absence of mitigation measures (Oladokun et al., 2019). Understanding the epidemiology key factors and increasing the knowledge about the ToBRFV-tomato pathosystem management is extremely important in order to find effective and appropriate control strategies. This review presents general aspects of tomato crops, and especially, characterisation, epidemiology and disease management of ToBRFV.

2 | ORIGIN AND TAXONOMY OF TOMATO

The history of tomato began in the 1500s when some European explorers (Spanish and Portuguese) brought back from South America to their respective countries new and uncommon vegetables, such as tomato. Presumably, tomato seeds were first taken to Europe from Mexico in 1519 (Jones Jr, 2007). South America was identified as a certain country of origin of tomato (coastal strip from the equator to about latitude 30° south) but, probably, it was first domesticated in Mexico. Wild tomato plants are native to western South America and are still found along the coast and the high Andes from central Ecuador, northern Chile, Peru, as well as on the Galapagos Islands (Bergougnoux, 2014). In the mid-16th century, it was primarily introduced in European early herbals for the beauty of its fruit and subsequently used for food, mainly in Italy and Spain, as it was initially considered a poisonous fruit, like its relative, the deadly nightshade (*Atropa belladonna* L.). Lastly, the tomato was reintroduced to America in the 18th century, and its importance as a vegetable is still growing.

Tomato belongs to the *Solanum* genus, the largest and most economically important genus in the *Solanaceae* family, that encompasses over 3,000 species, including commonly cultivated herbaceous crops, such as potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), petunia (*Petunia* spp.) and tobacco (*Nicotiana tabacum* L.) (Bergougnoux, 2014). As regards the tomato botanical classification, it was first identified and classified as *Solanum pomiferum* after its introduction to Europe in the 16th century, and subsequently classified by Linnaeus as *S. lycopersicum* in 1753. However, a few years later (1768), Philip Miller changed the name to *Lycopersicon esculentum*, assuming that many differences were present between tomato and potato and eggplant. After this, Hermann Karsten, in 1881, changed the name to *Lycopersicon lycopersicum*. Only in the last years, thanks to the use of new molecular technologies, the designation was changed to *S. lycopersicum* (L.) substantially giving reason to Linnaeus. Today, 500 years after its discovery, tomato is widely spread all over the world, with the main producing countries located in Asia, Europe, North and South America.

3 | TOMATO GENOME

Tomato genome has been fully sequenced in 2012; sequence analyses have provided valuable information on the approximately 35,000 genes that constitute the whole genome and regulate fruit characteristics, such as colour and fleshiness (Tomato Genome Consortium [TGC], 2012).

In particular, genome of the inbred tomato cultivar 'Heinz 1706' was sequenced and collected using a combination of Sanger and High Throughput Sequencing (HTS) technologies (TGC, 2012), revealing a genome size of approximately 900 Mb. The TGC assembled 84% (760 Mb) of the genome into 91 scaffolds aligned to 12 chromosomes and predicted 34,727 genes, 727 of which may be specific to plants with fleshy fruit. In addition, genome sequences of cultivated tomato (*S. lycopersicum* cv. Heinz 1706) and its closest wild relative (*Solanum pimpinellifolium* 'LA1589') were reported and compared to each other. The two tomato genomes showed only 0.6% nucleotide divergence and signs of recent admixture (introgression from *S. pimpinellifolium*). However, 39% of protein-coding regions are shared between *S. pimpinellifolium* and *S. lycopersicum*, which have gained or lost stop codons meaning that although few differences exist between them, these differences highly influence protein translation.

The tomato genome revealed that the floral architecture and fruit texture, size and nutritional quality are the result of gene retention after two sequential paleo-hexaploidy (triplication) events followed by introgressions from wild relatives, recombination, natural selection and domestication that underpin various agriculturally important features (Michael & Alba, 2012). Interestingly, these genome triplications added new gene family members, such as transcription factors and enzymes necessary for ethylene biosynthesis and perception, which mediate important fruit-specific functions (Ranjan, Ichihashi, & Sinha, 2012). Information about tomato genome has helped and will help to improve the economic value of these crops, answering to specific questions related to their development.

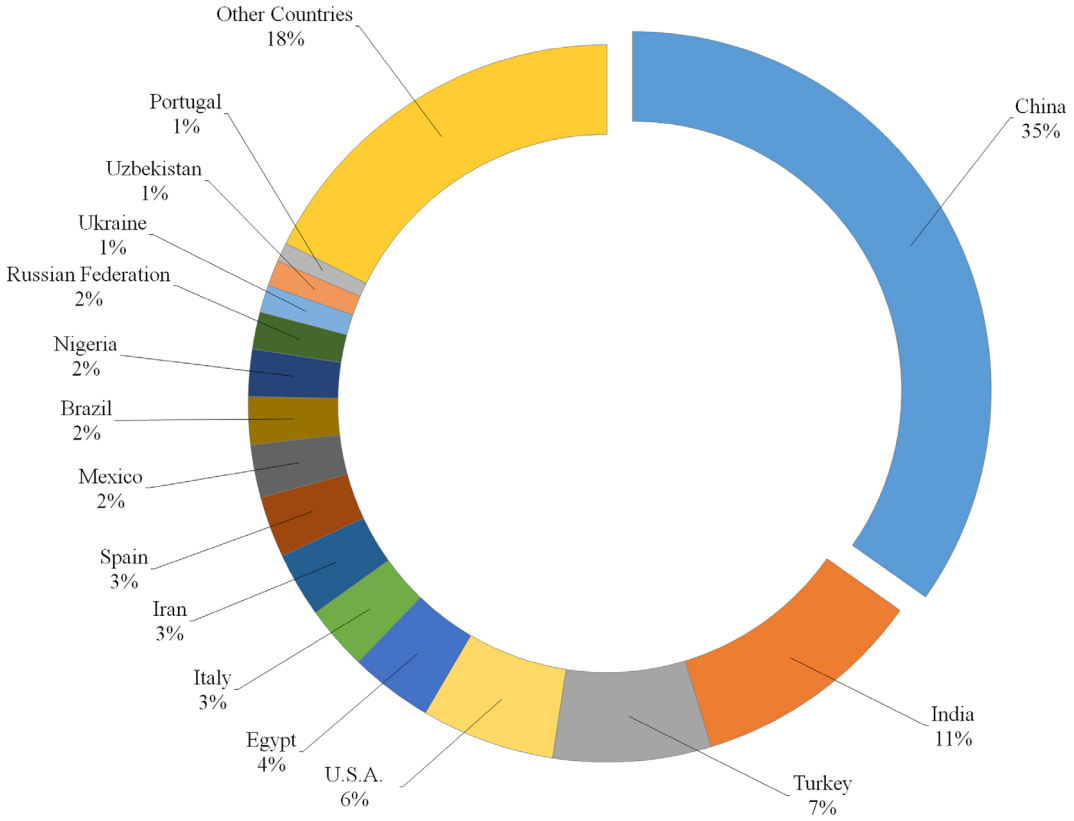

FIGURE 1 Tomato production percentage of the main producing countries worldwide (FAO, 2019)

4 | DISEASES THAT UNDERMINE THE TOMATO PRODUCTION

Tomato cultivars available are characterised by a low genetic diversity, because of the intensive selection and severe genetic bottlenecks caused by evolution and domestication throughout the last decades (Bai & Lindhout, 2007; Blanca et al., 2015); this has led to a significant increase of tomato susceptibility to high disease incidence. In fact, tomato plants are susceptible to more than 200 diseases caused by different pathogens, both in the field and postharvest processing (Singh, Singh, & Kumar, 2017).

Furthermore, the direct effects on plant pathogens and the effect on incidence, severity, temporal and spatial distribution of the diseases are considerably related to the climate changes, such as the rising of CO_2 concentration and of daily and annual temperatures (Burdon & Zhan, 2020; Panno et al., 2021).

The most important tomato diseases are caused by fungi, oomycetes, bacteria, phytoplasmas, viruses and viroids.

In particular, viral diseases play a crucial role, as they are responsible for important production losses affecting both the yield and the quality of the products, estimated in more than USD 30 billion per year (Sastry & Zitter, 2014). The increasing global trades and demand of propagation material, and the spread of agriculture practices such as monoculture and direct human intervention in order to satisfy the rapid expansion of human population, have caused several problems in terms of economic impact. Moreover, viruses represent nearly half of the pathogens that cause emerging and re-emerging plant disease (Anderson et al., 2004), as they can evolve and eventually cause outbreaks, becoming epidemics or even pandemics (Jones, 2021). Tomatoes, as well as many other vegetable crops, are continually exposed to new viral diseases which cause important phytosanitary emergencies.

In the last decades, many viral diseases that threaten tomato productions have occurred worldwide. In detail, regarding the countries of the Mediterranean basin, which is affected by large product and propagation material trades of different vegetable species, several viruses were found, among which the most important are Pepino mosaic virus (PepMV) (Davino et al., 2017; Davino, Davino, Bellardi, & Agosteo, 2008; Jordá et al., 2001; Tiberini, Davino, Davino, & Tomassoli, 2011), tomato spotted wilt virus (TSWV) (Abou-Jawdah, El Mohtar, Sobh, & Nakhla, 2006; Panno et al., 2012), tomato yellow leaf curl virus (TYLCV), tomato yellow leaf curl Sardinia virus and their recombinants (TYLCSV) (Avgelis et al., 2001; Davino et al., 2009, 2012; Panno, Caruso, & Davino, 2018), tomato leaf curl New Delhi virus (ToLCNDV) (Panno et al., 2019a; Ruiz, Simón, Velasco, García, & Janssen, 2015), Parietaria mottle virus (PMoV) (Aramburu, 2001), tomato mosaic virus (ToMV) (Xu et al., 2021) and, more recently, ToBRFV (Alfaro-Fernández, Castillo, Sanahuja, Rodríguez-Salido, & Font, 2021; OEPP/EPPO, 2020a; Panno et al., 2019b).

For this reason, it is important to understand the different virus transmission modes in order to implement the best phytosanitary measures; among these, one of the most important is the seed transmission. Seed transmission involves more than 200 plant viruses, and its frequency is higher in the genera *Potyvirus*, *Potexvirus*, *Nepovirus*, *Ilarvirus*, *Tobamovirus*, *Cucumovirus* and *Bromovirus* (Sastry, 2013). Among these, ToBRFV represents one of the most significant threats to world tomato production.

5 | ORIGIN AND SPATIOTEMPORAL DISPERSAL OF TOMATO BROWN RUGOSE FRUIT VIRUS

In April 2015, ToBRFV was isolated for the first time in Jordan from tomato plants (cv. Candela) grown in greenhouses (Salem, Mansour, Ciuffo, Falk, & Turina, 2016). These plants showed mild foliar symptoms and strong brown rugose symptoms on fruits, with a disease incidence close to 100%, suggesting a viral aetiology based on symptoms and the distribution in the affected fields (Salem et al., 2016). The full-length genome sequence of 6,393 nt (Tom1-Jo) was obtained and compared with those of closely related tobamoviruses.

Phylogenetic analyses and the lower percentage identity obtained from the Jordanian isolate suggested that a new tobamovirus had been isolated, and therefore was considered a member of a new species. Prior to this, in 2014, it was observed a severe outbreak of a new disease in Southern Israel, which subsequently spread within a year in different growing areas towards the South and Southeast/Northern parts of the country. The tomato cultivars carrying the *Tm-2*^{2} resistance gene (which confers resistance to tobacco mosaic virus (TMV) and ToMV) grown in greenhouses showed mosaic pattern on leaves and occasionally narrowing of leaves and yellow-spotted fruits. The complete genome sequence of the new Israeli tobamovirus (TBRFV-IL) showed high sequence identity to the ToBRFV Jordanian isolate previously discovered (Luria et al., 2017).

After the initial findings on tomato plants in Israel and Jordan, several reports have been recorded in different countries. In 2018, ToBRFV was reported in the State of Palestine (Alkowni, Alabdallah, & Fadda, 2019), Mexico (Camacho-Beltrán et al., 2019), Florida (Dey et al., 2021) and California (all plants destroyed, not considered established by the OEPP/EPPO) (Ling, Tian, Gurung, Salati, & Gilliard, 2019), Germany (Menzel, Knierim, Winter, Hamacher, & Heupel, 2019) and Italy (Panno et al., 2019b). In 2019, ToBRFV was reported in Turkey (Fidan, Sarikaya, & Calis, 2019), China (Yan et al., 2019), United Kingdom (Skelton et al., 2019), Netherlands (OEPP/EPPO, 2019a), Greece (OEPP/EPPO, 2019b), Egypt (Amer & Mahmoud, 2020) and Spain (Alfaro-Fernández et al., 2021). Subsequently, in 2020/2021, new outbreaks were reported in France (OEPP/EPPO, 2020a), Czech Republic (OEPP/EPPO, 2020b), Cyprus (OEPP/EPPO, 2020c), Poland (OEPP/EPPO, 2020-d), Austria (OEPP/EPPO, 2021a), Portugal (OEPP/EPPO, 2021b), Belgium (OEPP/EPPO, 2021c), Estonia (OEPP/EPPO, 2021d), Hungary (OEPP/EPPO, 2021e), Malta (OEPP/EPPO, 2021f), Norway (Hamborg & Blystad, 2021), Slovenia (OEPP/EPPO, 2021g), Switzerland (OEPP/EPPO, 2021h), Syria (Hasan, Salem, Ismail, Akel, & Ahmad, 2021) and Saudi Arabia (Sabra, Al Saleh, Alshahwan, & Amer, 2021) (Figure 2). ToBRFV has also been reported in pepper plants grown in Jordan (Salem, Cao, Odeh, Turina, & Tahzima, 2020), Italy (Panno et al., 2020a), Turkey (Fidan et al., 2021; Fidan, 2020), Syria and Lebanon (Abou Kubaa, Choueiri, Heinoun, Cillo, & Saponari, 2021).

To date, because of the fact that the virus was only first reported in 2016 (Salem et al., 2016) and not previously regulated, limited information is available about the ToBRFV distribution and, probably, its presence in other countries has not been officially reported yet. It is possible to hypothesise how the intense movement of infected seeds from one country to another has greatly accelerated the spread of the virus in a short period of time.

6 | TAXONOMY OF TOMATO BROWN RUGOSE FRUIT VIRUS AND GENOME ORGANISATION

ToBRFV is a member of the genus *Tobamovirus*, family *Virgaviridae*. The *Virgaviridae* family includes plant viruses with a single-stranded RNA genome, with a 3′-terminal tRNA-like structure and a replication protein similar to those of the alpha-like super group, and nonenveloped and rod-shaped virions of ~20 nm in diameter and lengths that depend upon the genus (Adams et al., 2017). The genus

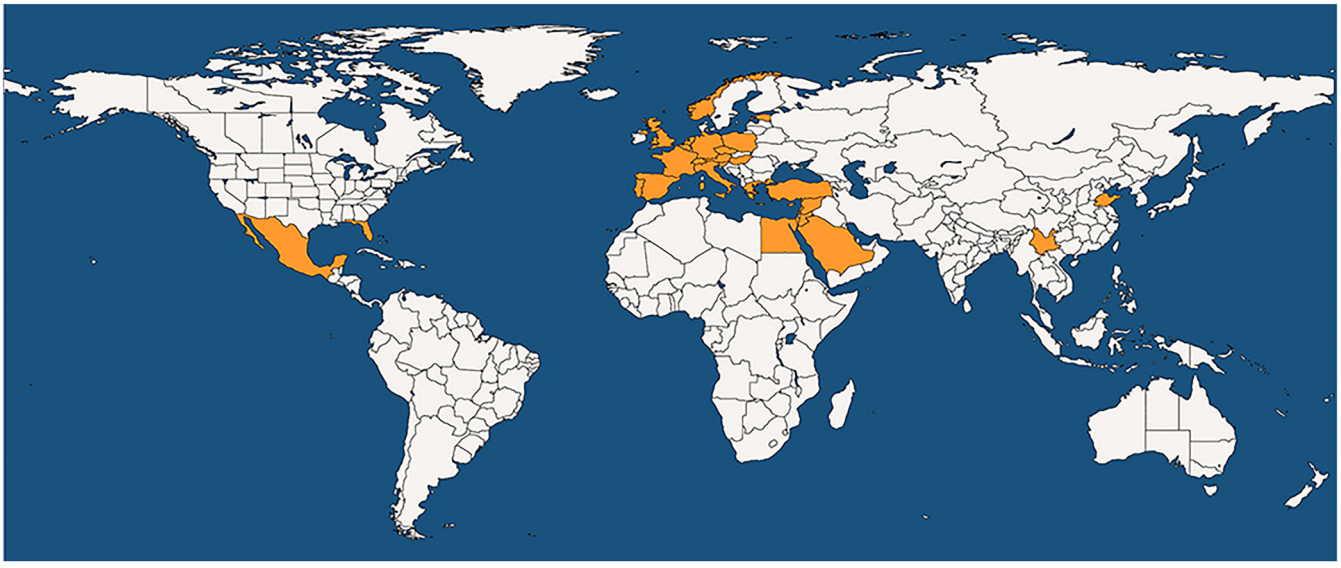

FIGURE 2 Tomato brown rugose fruit virus (ToBRFV) worldwide distribution. In orange all countries where the presence of ToBRFV has been confirmed (revised from EPPO Global Database ToBRFV map)

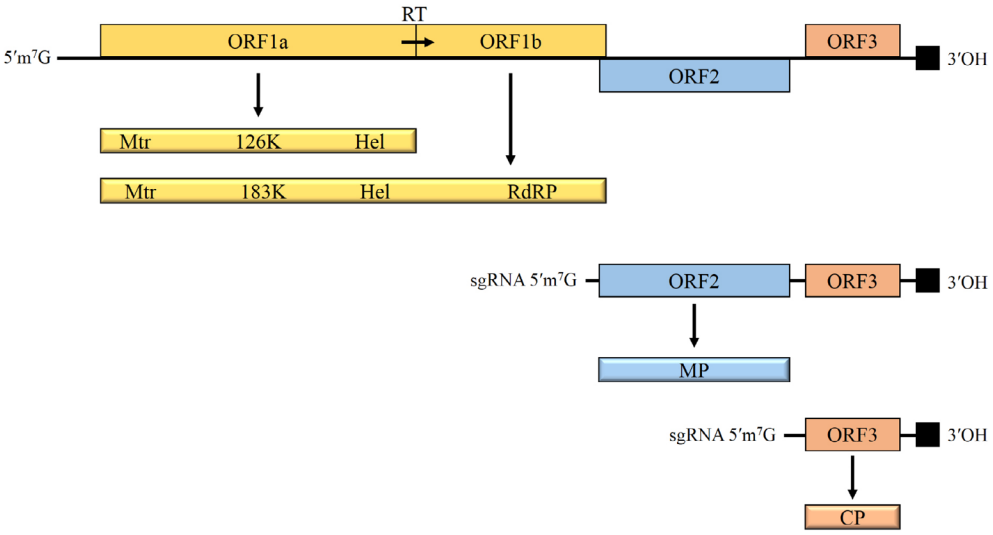

FIGURE 3 Schematic representation of Tomato brown rugose fruit virus genome organisation

demarcation is based on genome organisation, numbers of genome components and the transmission modes. Viruses of this family are classified into the seven genera: *Goravirus*, *Furovirus*, *Hordeivirus*, *Pecluvirus*, *Pomovirus*, *Tobamovirus* and *Tobravirus*. *Tobamovirus* is the largest genus of *Virgaviridae* family for numbers of species; and are the only *Virgaviridae* that have an undivided genome (King, Lefkowitz, Adams, & Carstens, 2011), with a host range usually limited in nature but moderate/wide under experimental conditions (Adams, Antoniw, & Kreuze, 2009). The transmission occurs by plant-to-plant contact and in some cases by seed, although the viral particles are present in the seed coat, sometimes in the endosperm, but never in the embryo.

In detail, ToBRFV has a positive-sense single-stranded RNA (ssRNA+) of ~6,400 nucleotides (nt), with a typical tobamovirus organisation, consisting of four open reading frames (ORFs) that encode two replication-related proteins complexes of 126 kDa (ORF1a) and 183 kDa (ORF1b), with the ORF1b being expressed by the partial suppression of the stop codon, the movement protein (MP) of ~30 kDa (ORF2) and the coat protein (CP) of ~17.5 kDa (ORF3), expressed via the 3′-coterminal subgenomic RNAs (Figure 3) (Salem et al., 2016). ToBRFV viral particles were observed and described using transmission electron microscope (TEM), that permitted to obtain morphological data (Luria et al., 2017).

7 | HOST PLANTS

Since the first identification in 2015 (Salem et al., 2016), different studies were conducted in order to understand the potential ToBRFV host range. ToBRFV currently seems to have tomato and species belonging to *Capsicum* genus as its main host species (Luria et al., 2017; Panno et al., 2020a).

In laboratory conditions (Luria et al., 2017), inoculation experiments showed that ToBRFV has been successfully transmitted to different commercial tomato lines bearing the resistance gene *Tm-2*^{2} (resistance gene against some ToMV isolates). In particular, ToBRFV-infected tomato plants showed the typical symptomatology; this may be because of changes in the structure or function of one or more proteins. In addition, under certain circumstances, ToBRFV is able to infect pepper (*C. annuum*) (Luria et al., 2017; Oladokun et al., 2019). Moreover, different common grasses and weeds are also included in the hosts range. ToBRFV was artificially transmitted on several species of *Nicotiana* genus (*N. tabacum*, *N. benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. occidentalis* subsp. *hesperis*, *N. debneyi*, *N. rustica*), *Solanum nigrum*, *Physalis angulata*, *P. pubescens* and several weeds such as *Chenopodium murale*, *C. amaranticolor*, *C. quinoa*, *C. album*, *Gomphrena globosa*, *Catharanthus roseus*, *Emilia sonchifolia*, *Glebionis coronaria*, *Datura stramonium*, *D. metel* and *Petunia hybrida* (Chanda et al., 2021a; Luria et al., 2017; Salem et al., 2020). In this context, weeds could act as a reservoir for the virus to compromise the cultivated crop. This last aspect should not be underestimated, because these species are very often present in agro-ecosystems and therefore act as potential sources of virus inoculum, representing a greater danger especially if they are asymptomatic (Luria et al., 2017).

Eggplant was reported as a new host of ToBRFV, but only one positive sample was reported in Mexico (EPPO, 2019). Regarding this new host, divergent results were reported; in fact, under experimental conditions, four different research groups failed to transfer the virus in this new host (Chanda et al., 2021a; Fidan, 2020; Luria et al., 2017; Panno et al., 2019c). Moreover, Yan et al. (2021) have not detected characteristic symptoms in systemic leaves of artificially inoculated plants, but were positive for RT-PCR detection. This is the reason why the European and Mediterranean Plant Protection Organization (EPPO) decided to include it among 'doubtful host' (EPPO, 2019). Furthermore, it was demonstrated by Luria and coworkers that potato is not a ToBRFV host plant (Luria et al., 2017).

However, because of the wide range of tomato and pepper commercial cultivars available and the recent ToBRFV discovery, it is still difficult to precisely define many epidemiological aspects.

8 | SYMPTOMS ON TOMATO AND PEPPER

ToBRFV induces different symptoms on tomato plants, which differ in relation to cultivar, growing season, photoperiod, temperature and

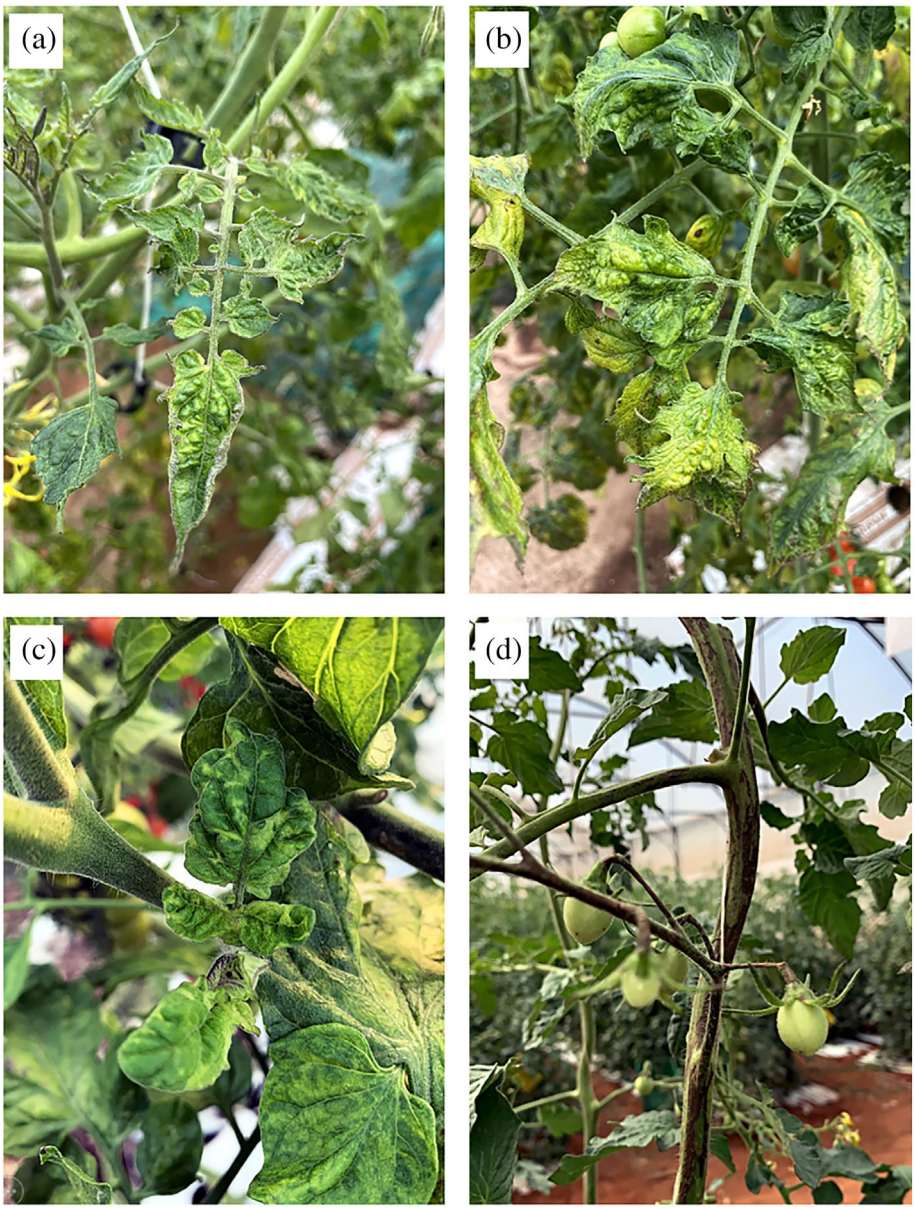

FIGURE 4 Typical disease symptoms generated on tomato crops. (a) Leaves narrowing and dark green bulges on infected tomato plant; (b, c) mosaic and mottling symptoms, leaves interveinal yellowing and deformation induced by tomato brown rugose fruit virus on tomato leaves; (d) longitudinal necrosis on stem

plant age at the time of infection. In tomato, early symptoms are generally observed on young shoots in the upper and lateral parts of the plant. The symptoms observed on the leaves range from mild to severe chlorotic mosaic (Luria et al., 2017), with dark green bulges (Menzel et al., 2019), often associated with interveinal yellowing, deformation, and narrowing of the leaves. Apical brown necrosis of leaflets (Alfaro-Fernández et al., 2021), necrotic lesions on peduncles/calyxes/petioles and longitudinal necrosis on stem/sepals (Oladokun et al., 2019), have been also reported in tomato plants infected by ToBRFV. The leaves may also wither, turning yellow and eventually, in severe cases the whole plant can even collapse and die (Figure 4).

The symptoms on tomato berries consist of yellow spots (often around the calyx) with occasional brown rugose patches (wrinkling) (Salem et al., 2016), deformations and irregular ripening on growing fruit (Panno et al., 2019b), followed by a decrease in berries per branch (Luria et al., 2017); in fact, in both young and ripe berries, symptoms degenerate into marbling, often associated with appearance of wrinkled necrotic areas, which significantly affects the commercial value of the fruit and makes it nonmarketable (Menzel et al., 2019) (Figure 5).

In addition, symptoms observed in field on tomato plants may not be caused by ToBRFV alone; in some cases, they are likely induced by a mixed infection. In fact, ToBRFV has recently been identified in tomato plants together with a strain of PepMV (CH2 strain), revealing severe new symptoms, such as open unripe fruits and yellow patched leaves (Klap et al., 2020). In Mexico, a ToBRFV outbreak was also observed on tomato plants coinfected with TSWV (Ling et al., 2019).

Also, in pepper, the symptoms are quite variable, depending on environmental conditions and genotypes. In ToBRFV-infected pepper plants, it is possible to observe mild mosaic and discoloration of young leaves (especially on the veins), browning of the stem with severe necrosis at the intersection of secondary branches (Panno

FIGURE 5 Characteristic symptomatology on tomato berries, stem and sepals. (a) Marbling and yellow spots form on tomato fruits; (b): tomato berries with brown rugose patches (wrinkled), deformations and irregular ripening; (c): longitudinal necrosis on stem and sepals

et al., 2020a), partial necrosis of the vegetative apex and marbling. Moreover, in some cases, wrinkling and fruit distortion were observed. In pepper genotypes harbouring $L^{1,3,4}$ resistance genes, hypersensitivity response (HR) was recorded with yellowing and fall of the leaves (Luria et al., 2017). Moreover, when these plants are propagated at high temperatures (greater than 30°C), HR response includes necrotic lesions on the roots and stem, inhibiting plant growth and often leading to plant collapse (Luria et al., 2017) (Figure 6).

However, since ToBRFV symptoms are typical of tobamovirus infection, exclusive visual observation of symptoms is not enough for the pathogen identification, especially in the presence of mixed infections by different tobamoviruses and/or genera. For this reason, ToBRFV-symptoms induced in tomato can be confused with infections by viruses belonging to the same genus, such as ToMV, TMV (Alkowni et al., 2019) or, in some case, erroneously associated with phytotoxicity damage. Additionally, early infection and/or certain environmental and agricultural conditions may lead to underestimate the disease incidence in the growing environment.

9 | TRANSMISSION, SPREAD AND EPIDEMIOLOGY OF ToBRFV

Dispersal and spread of ToBRFV is mainly mechanical, but it can also be carried for long distances, from one country to another, via contaminated seeds and berries (Panno et al., 2020b). During farming, short distance transmission occurs through infected propagation material (cuttings, grafts), direct plant-to-plant contact between infected and neighbour uninfected plants (Panno et al., 2020b) and, in the process of ordinary cultivation practices, through wounds made to leaves or to the root-system of seedlings (e.g. following transplanting) (Salem et al., 2016). Infection can also happen through the transfer of infected sap from different surfaces such as human body, clothes,

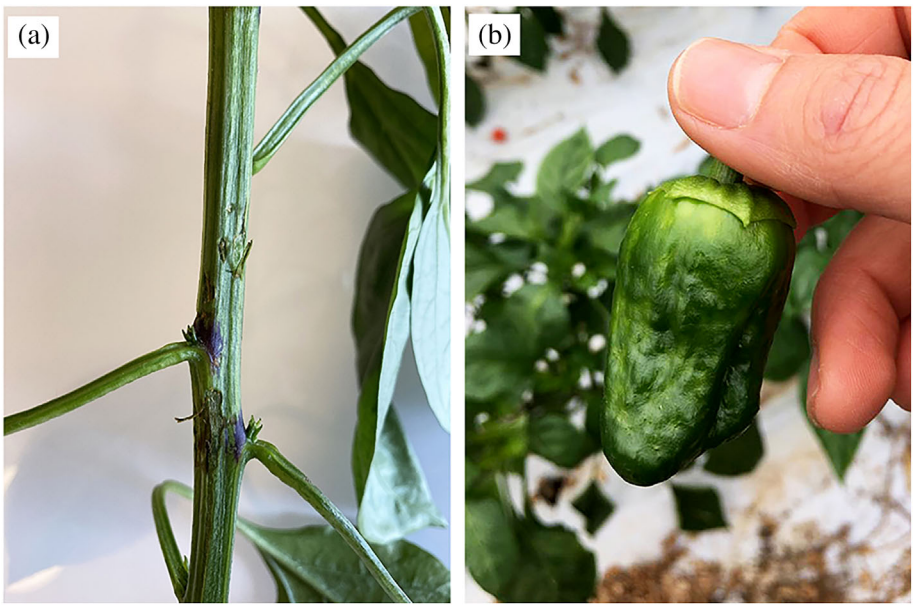

FIGURE 6 Tomato brown rugose fruit virus (ToBRFV) symptoms on sweet pepper. (a) Browning of pepper stem with necrosis at the intersection of secondary branches; (b) marbling, wrinkling and fruits distortion caused by ToBRFV infection

work tools, gloves, shoes, poles as well as through irrigation or drainage water (Li, Liu, & Gu, 2016) and/or nutrient solutions (Wilstermann & Ziebell, 2019). Likewise, after harvesting, ToBRFV *inoculum* can remain infectious also in several surfaces and materials of greenhouse such as wires, glass, concrete and soil (Oladokun et al., 2019).

About seed transmission, according to the study conducted by Davino, Caruso, Bertacca, Barone, and Panno (2020), ToBRFV is classified as a seed-borne virus, since the localization of ToBRFV is in tomato external teguments (the seed coat, contaminated by infected fruit pulp), although in some cases, probably depending on viral accumulation, it also appears to be detected in the endosperm, but never in embryo (Davino et al., 2020). Therefore, ToBRFV seed transmission might occur during the seed germination and emergence of seedlings, from contaminated seed coat through the micro-lesions caused mainly in the cotyledons. However, the transmission rate is relatively low, corresponding to ~2.8% to the cotyledons and ~1.8% to the third true leaf; after transplant, only two infected plants are sufficient to reach a 100% infection rate in a greenhouse, considering the high plant-to-plant transmission rate of ToBRFV (Davino et al., 2020). The seed transmission rate from seeds to their seedlings was also demonstrated by Salem, Sulaiman, Samarah, Turina, and Vallino (2022), with a lower percentage detected (0.08%). The subsequent transplant of infected plantlets contributes to the spread of the virus among them. Thus far, the ability of ToBRFV to be transmitted by Arthropods has not yet been demonstrated. Recent studies have shown that bumblebees, *Bombus terrestris* L. (Hymenoptera: *Apidae*), are able to spread the virus during pollination activity. In this case, ToBRFV transmission modes are mechanical. Bumblebees can transmit the virus by transferring raw lymph using their mandibles, or through their vibrating bodies where the infected pollen adheres (Levitzky et al., 2019), without the ingestion and incubation of the virus. Knowledge on the transmission methods is essential for ToBRFV management, because it facilitates the implementation of preventive measures in affected areas and avoids the introduction of this virus into new countries through infected propagation material. Moreover, in order to effectively control the disease, it is fundamental to understand the ToBRFV epidemiology. First, it is important to note that different ToBRFV isolates have been detected since its first identification in 2015, which however do not show a high differentiation in their ability to infect the horticultural crops, such as tomato and pepper plants. Generally, tobamoviruses can enter in a crop production system, both in greenhouses and open field cultivation, via seeds (Dombrovsky & Smith, 2017). As in the case of ToBRFV, a seed-borne virus, the risks of a new infection are low in frequency, but could have a deep impact in case of high-intensity glasshouse production (Oladokun et al., 2019). In addition, tobamovirus viral particles are significantly stable, in fact, they exhibit a high persistence in irrigation and drainage water and soil (Li et al., 2016), remaining infectious for long periods. Indeed, mechanical transmission (manipulation, working tools, plant-to-plant contact) plays a key role in the virus introduction and subsequent rapid transmission, leading to infection rates close to 100% in the most severe cases. Based on the phylogenetic analysis of the full-length genome sequences today available (see Section 10), it is probably that the virus has been spread in other countries via infected seeds from its place of origin. Due to the lack of knowledge about its presence, the different transmission modes, and the absence of adequate phytosanitary controls and prevention measures, it quickly spread to open field and greenhouse, naturally infecting tomato and pepper crops. Also, there is no scientific evidence for the presence of ToBRFV vectors, but it was demonstrated the role of pollinators, such as bumblebees, that may contribute to mechanically transfer ToBRFV particles during pollination, transferring crude sap with their mandibles from an infected to a healthy plant, or through their vibrating bodies (Levitzky et al., 2019; Panno et al., 2020b). In conclusion, regarding the ToBRFV epidemiology, seed transmission and secondary spread (manipulation, plant-to-plant contact etc.) must be considered as the more critical aspects related to its rapid and uncontrolled spread.

10 | GENETIC DIVERSITY

Different pathways leading to the ToBRFV emergence have been recognised. ToBRFV could be identified as a result of recombination between other tobamoviruses, in fact, the analyses conducted by Salem et al. (2016) may support this hypothesis, as they detected a recombination event in a 314-nucleotide segment of the *Rep* gene, detecting the strain Ohio V of TMV and Tomato mottle mosaic virus (ToMMV) as the major parent and the potential minor parent, respectively. Afterwards, phylogenetic analyses suggest that ToBRFV could have originated from a common ancestor of ToMV and TMV, identifying a host-shifting event of the ToBRFV variant occurred with a relatively low mutation rate within a very short time (Maayan et al., 2018).

Moreover, since ToBRFV has spread so rapidly, within a few years in different countries of the Mediterranean Basin, North America and Asia, there is a very low level of variability among isolates from the different countries where they were first detected, showing a nucleotide percentage identity >99% (Figure 7). In detail, Chanda et al. (2020) demonstrated 99.6–99.9% nucleotide sequence identities among those isolates, by multiple sequence alignment of different full genome ToBRFV isolates from different countries, suggesting a brief evolutionary background of ToBRFV, while a lower identity (<82.2%) to other common tobamoviruses (ToMV, ToMMV, TMV and cucumber green mottle mosaic virus [CGMMV]) was observed. Furthermore, van de Vossenberg et al. (2020) studied the genomic diversity of 50 ToBRFV genomes from different Dutch outbreak locations, demonstrating a high similarity of the obtained ToBRFV sequences, ranging from 99.3 to 100%.

In conclusion, to date, all ToBRFV sequenced isolates reported in the infected areas, are genetically closely related to each other. This indicates probably that all isolates detected so far originated from a unique common ToBRFV ancestor (Oladokun et al., 2019).

11 | DETECTION METHODS

Currently, no commercial tomato and pepper cultivars are known to be resistant to ToBRFV (Davino et al., 2020). Many breeding companies are working to obtain ToBRFV resistant tomato and pepper cultivars, and some claiming to have high resistance (HR) tomato cultivars

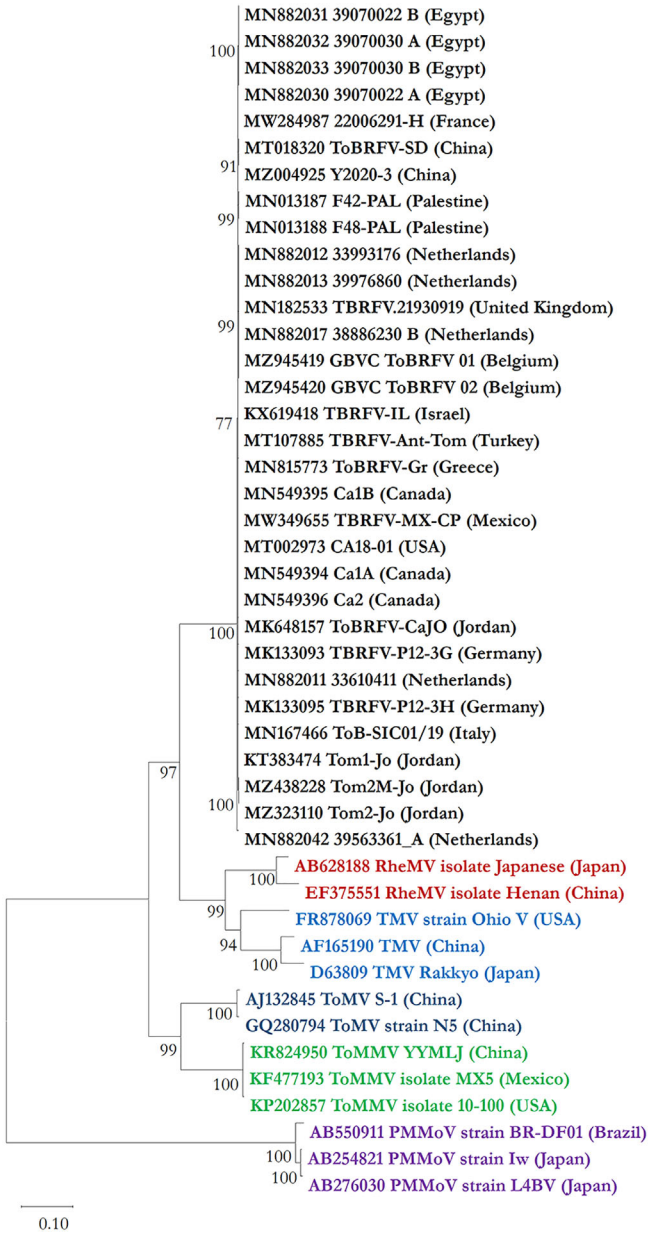

FIGURE 7 Phylogenetic relationships between full genome sequences of tomato brown rugose fruit virus (ToBRFV) available on GenBank database and other common tobamoviruses, inferred by using the maximum-likelihood method (ML) based on the Tamura 3-parameter model (Tamura, 1992) with bootstraps of 1,000 replications. Evolutionary analyses were conducted in MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Colours represent different tobamoviruses: ToBRFV, black; Rehmannia mosaic virus (RheMV), red; tobacco mosaic virus (TMV), pale blue; tomato mottle mosaic virus (ToMMV), green; tomato mosaic virus (ToMV), blue; pepper mild mottle virus (PMMoV), purple

to ToBRFV, but to date, none of them is commercially available. Also, tomato cultivar presenting *Tm-1*, *Tm-2* and *Tm-2*^{2} resistance genes for ToMV and TMV (Pelham, 1966) and pepper varieties with *L*^{1,3,4} resistance genes against tobamoviruses (Tomita et al., 2011) can be severely affected by ToBRFV, leading to new outbreaks and a rapid virus spread that can represent a threat for these important horticultural crops, because of its multiple transmission methods. For this reason, a valuable aid in reducing the introduction and subsequent ToBRFV spread can be given by the application of preventive measures in crop management and by an early and reliable diagnosis (Davino et al., 2020).

Bioassays using indicator plants, such as *Nicotiana* spp., may be used for ToBRFV detection from symptomatic material, but it requires much more time compared with serological and molecular detection methods.

Double-antibody Sandwich-Enzyme-linked immunosorbent assay (DAS-ELISA) was adapted for the detection of ToBRFV infection. Initially, serological assay for TMV detection was used, but they were not species-specific for ToBRFV detection, because of the high conservation of tobamovirus CP, resulting in antibody cross-reactivity between tobamovirus species (Luria et al., 2017). To date, there are available different qualitative serological DAS-ELISA kits for ToBRFV detection in tomato and pepper leaf and seed, using specific ToBRFV polyclonal/monoclonal antibodies, showing low cross-reactivity with other tobamoviruses (TMV and ToMV), provided by Agdia Inc. (Elkhart, IN), Loewe Biochemica GmbH (Sauerlach, Germany), DSMZ (Braunschweig, Germany) and Bioreba (Reinach, Switzerland). They are also available specific lateral flow devices (LFD) that can be used in field, greenhouse and laboratory in order to detect the presence of ToBRFV, obtaining the results in a short time (5–30 min).

Different molecular tests based on conventional RT-PCR assay have been described for specific ToBRFV identification (Alkowni et al., 2019; Ling et al., 2019; Luria et al., 2017; Magaña-Álvarez et al., 2021; Panno et al., 2019b; Rodríguez-Mendoza et al., 2019). Moreover, a quick detection procedure based on real-time RT-PCR TaqMan MGB probe has been developed, combined with different sample preparation procedures, that permit to avoid total RNA extraction and shortening the processing time, thus allowing to drastically reduce the total cost for single analysis (Panno et al., 2019c). It is important to note that, as reported by EPPO Pest Risk Analysis (PRA) (2020), the detection of infectious ToBRFV in tomato and pepper seeds requires minimum 3,000 seeds per lot to be tested, providing 95% of probability of detecting 0.1% of infection. Sarkes, Fu, Feindel, Harding, and Feng (2020) and Rizzo et al. (2021) developed two different methods for ToBRFV detection based on Loop-mediated isothermal AMPlification (LAMP) assay, more sensitive than the currently available RT-PCR assays, that provides reliable results in short time (~35 min) at isothermal conditions (65°C) (Panno et al., 2020c); the results can be easily read by visual colour change observation (Sarkes et al., 2020) or by real time monitoring (Rizzo et al., 2021). Molecular diagnostic techniques for ToBRFV detection are shown in Table 1. Sequencing can also be performed in order to identify ToBRFV after RT-PCR amplification using generic tobamoviruses primers. In addition, complete genome sequences could be obtained by HTS technologies, which analysis can be carried out also for identification of a specific ToBRFV isolate (EPPO, 2020). Recently, two emerging approaches, based on CRISPR/Cas12a technology, were developed for the precise identification of ToBRFV and the discrimination

TABLE 1 Molecular diagnostic techniques developed for tomato brown rugose fruit virus (ToBRFV) detection

Technique	Primer/probe	Sequence 5′–3′	Amplicon size [nt]	Results visualisation	Reference
RT-PCR	F-3666	ATGGTACGAACGGCGGCAG	1,052	Agarose gel electrophoresis	Luria et al. (2017)
	R-4718	CAATCCTTGATGTGTTTAGCAC			
	TBRFV-F-5722	CACAATCGCAACTCCATCGC	458	Agarose gel electrophoresis	Panno et al. (2019b)
	TBRFV-R-6179	CAGAGGACCATTGTAAACCGG			
	ToBRFV-F	AATGTCCATGTTTGTTACGCC	560	Agarose gel electrophoresis	Alkowni et al. (2019)
	ToBRFV-R	CGAATGTGATTTAAAACTGTGAAT			
	ToBRFV-F	GAAGTCCCGATGTCTGTAAGG	842	Agarose gel electrophoresis	Ling et al. (2019)
	ToBRFV-R	GTGCCTACGGATGTGTATGA			
	ToBRFV-FMX	AACCAGAGTCTTCCTATACTCGGAA	475	Agarose gel electrophoresis	Rodríguez-Mendoza et al. (2019)
	ToBRFV-RMX	CTCWCCATCTCTTAATAATCTCCT			
	CP FOR	AGAACAACCGTTCAACGGCAATTTA	359	Agarose gel electrophoresis	Magaña-Álvarez et al. (2021)
	CP REV	CTCAAGATGCAGGTGCAGAGGACCATTGT			
RT-qPCR	ToB5520F	GTAAGGCTTGCAAAATTTCGTTCG	101	Real time monitoring	Panno et al. (2019c)
	ToB5598R	CTTTGGTTTTTGTCTGGTTTCGG			
	ToB-probe	FAM-GTTTAGTAGTAAAAGTGAGAAT-MGB			
	ToBRFV qs1	CAATCAGAGCACATTTGAAAGTGCA	-	Real time monitoring	Menzel and Winter (2019)
	ToBRFV qas2	CAGACACAATCTGTTATTTAAGCATC			
	ToBRFV p1	6FAM-ACAATGGTCCTCTGCACCTG-BHQ1			
	CaTa28 Fw	GGTGGTGTCAGTGTCTGTTT	-	Real time monitoring	ISHI-Veg (2020) (reference not available)
	CaTa28 Rv	GCGTCCTTGGTAGTGATGTT			
	CaTa28 Pr	6FAM-AGAGAATGGAGAGAGCGGACGAGG-BHQ1			
	CSP1325 Fw	CATTTGAAAGTGCATCCGGTTT			
	CSP1325 Rv	GTACCACGTGTGTTTGCAGACA			
	CSP1325 Pr	VIC-ATGGTCCTCTGCACCTGCATCTTGAGA-BHQ1			
	AB-620	CAGATGTGTCGTTGGTCAGAT	144	Real time monitoring	Bernabé-Orts et al. (2021b)
	AB-621	CATCACTACGGTGTAATACTTC			
	AB-622	6-FAM-CGTAGCTTTGTCA-ZEN-AGGCATACCCAAA-IABkFQ			
	ToBRFV-F1	GCCCATGGAACTATCAGAAGAA	92	Real time monitoring	Chanda et al. (2021a)
	ToBRFV-R1	TTCCGGTCTTCGAACGAAAT			
	ToBRFV-P1	FAM-AGTCCCGATGTCTGTAAGGCTTGC-TAMRA			
LAMP	F3	TTGGAGTCTTAGATGTTGCG	-	Visual colour change observation	Sarkes et al. (2020)
	B3	GGACACCGTCAACTAGGA			
	FIP	CCTTCTCCAACTGTCGCAAGTCACATGCTAGGAAGTACCAC			
	BIP	CCGTGAGTTCTGAGTCAATGGTTGAGGCTCACCATCTCTTAA			
	LoopF	CTCCATGCTCATCATACTCCAA			
	LoopB	GCTCAGAACACTGAGGAGATT			
	ToBRFV_F3	TTGGAGTCTTAGATGTTGCG	-	Real time monitoring and visual colour change observation	Rizzo et al. (2021)
	ToBRFV_B3	GGACACCGTCAACTAGGA			
	ToBRFV_FIP	CCTTCTCCAACTGTCGCAAGTTACACATGCTAGGAAGTACCAC			
	ToBRFV_BIP	CCGTGAGTTCTGAGTCAATGGTTATGAGGCTCACCATCTCTTA			
	ToBRFV_LoopF	CTCCATGCTCATCATACTCCAA			
	ToBRFV_LoopB	GCTCAGAACACTGAGGAGATT			
	F3 MP1	TCATAGACTTGTCAAAATCAGAA	-	Real time monitoring	Bernabé-Orts et al. (2021b)
	B3 MP1	GAAGCAAGAGTTGCCTCG			
	FIP MP1	GGACAAAGATTCATCTTCATGAACCCGTCTATGTTCACACCTGTT			

TABLE 1 (Continued)

Technique	Primer/probe	Sequence 5′–3′	Amplicon size [nt]	Results visualisation	Reference
	BIP MP1	AGGTGAATGGAATTTGCCAGATCATTCTCTTATCGACCAAACAG			
	LF MP1	ACCTTGGAGATCATGACACTCT			
	LB MP1	TGTCGTGGTGGTGTCAGTG			
	F3 MP2	GATGGTGGCTATGTACATCT	-	Real time monitoring	
	B3 MP2	ACACAAATTCTAATGACAGCG			
	FIP MP2	TATCGACCAAACAGACACTGACACTTGTGGTGACAGGTGAA			
	BIP MP2	TCACTACCAAGGACGCAGAACAGTAACCCGCAGCAATT			
	LF MP2	CACCACGACAATTATCTGGCA			
	LB MP2	AAGGCAGTTTGGCAAGTACTAGTT			

between ToBRFV and the closely related ToMV (Alon, Hak, Bornstein, Pines, & Spiegelman, 2021), with potential to be applied in field settings (Bernabé-Orts et al., 2021a).

12 | DISEASE MANAGEMENT

The study of epidemiology and different mechanisms involved in the dispersion of different pathogens is one of the most important measures to contain a possible epidemic (Panno et al., 2020b) and to develop efficient disease management strategies (Jeger, Holt, Van Den Bosch, & Madden, 2004).

As regards ToBRFV management, understanding its epidemiology is indispensable to effectively control the disease (Panno et al., 2020b). In fact, ToBRFV epidemic can represent a serious threat because of its different modes of transmission (see Section 10 above) and, therefore, phytosanitary actions are required to avoid its spread to other regions or countries, such as correct crop management and more restrictive measures at international borders using rapid, sensitive and economical diagnosis (Panno et al., 2019c). In order to produce healthy, reasonably ToBRFV-free crops and reduce the disease spreading, it is crucial to identify and apply a combination of simple phytosanitary practices in protected tomato crops (Panno et al., 2020b).

For this reason, it is important to avoid the spread of ToBRFV-infected material to customers or producers. Production, sampling and testing must be done strictly according to international standards for seed and seedling trade. When a possible ToBRFV infection is suspected, it is advisable to contact a specialised laboratory in order to perform a rapid serological or molecular test. It is necessary to know that ToBRFV infection spreads very easily in open field and greenhouses, for example, through worker's footwear and clothing. In addition, extreme hygiene measures should be followed when visiting a grower or seed producers; such measures include restrictions on visits to greenhouses and increased attention to the use of clean or disposable clothing.

It is therefore important to apply specific hygiene and prophylactic measures during routine cultivation operations and/or field visits.

In detail, it is crucial to wear clean clothing at least daily or, when possible, use disposable clothing, wash both hands when entering and leaving the farm using an effective disinfectant, and especially between farm units.

Regarding the use of disinfectants against ToBRFV, Chanda, Shamimuzzaman, Gilliard, and Ling (2021) conducted different bioassay experiments through mechanical inoculation using a ToBRFV inoculum treated with specific concentration of several chemicals at designated exposure time periods, demonstrating the 90–100% efficacy of 0.5% Lactoferrin, 2% Virocid and 10% Clorox, and 3% Virkon against ToBRFV.

It should be avoided bringing jewellery, watches and phones into the greenhouse in order to avoid contamination. Moreover, it is extremely important to regularly disinfect working tools, enter the greenhouse by passing on disinfection mat or sanitising device, and wash or dispose of clothing at the end of a working day, ensuring that disposable clothing is actually discarded. Farmers should always wear disposable gloves before touching any plants; gloves should be removed from the wrist upward so that it ends up inside out. Plant material, gloves, soil, sticks and everything that have been in contact with or come from infected/suspected plants must be disinfected or disposed of in bags or closed containers. Only essential workers should be allowed to enter the field and/or greenhouses, in order to limit visits and the size of the group, avoiding visiting multiple greenhouses during the same day. Moreover, visiting growers during the plant growth phase must be avoided, especially when the old crop is still present at the farms; if visiting a suspected infected greenhouse, clothes should be changed directly before visiting the next grower. No tomatoes or peppers may be brought from home or from one greenhouse to another. Growers must be informed about the hygiene measures to be taken in case you observed risk areas in their greenhouse. Finally, it would be helpful to apply cultural rotation with non-host crops, remove the residues after harvest/weeding, eliminate the infected material like weeds and wild hosts, possibly by controlled burning and use healthy propagation material, such as certified seeds and seedlings, because it is crucial to prevent early infection.

These measures, combined with an early ToBRFV detection both in the field and at international borders, reduce the occurrence and

spread of the disease and allow farmers and regulators to quickly adopt appropriate control measures (Pallás, Sánchez-Navarro, & James, 2018). In addition, subsequent identification of the viral agent through official protocols is required to implement timely measures to remove positive plants. While, in the case of infected seed lots, seed disinfection treatments can be applied. For this purpose, different seed disinfection treatments can be used without compromising the germination percentage; appropriate heat or chemical seed treatments by immersion in a 2.5% sodium hypochlorite solution for 15 min have been found to ensure 100% germination and complete seed disinfection (Davino et al., 2020), while Samarah, Sulaiman, Salem, and Turina (2021) obtained a 100% of disinfection rate ToBRFV-contaminated seeds with 2% hydrochloric acid (HCl) for 30 min or 10% trisodium phosphate (TSP) for 3 hr, alone or in combination.

Another preventive measure (already successfully taken against other viral diseases) is represented by the use of ToBRFV resistant or tolerant cultivar, when they will be available. In addition, as reported by Smith and Dombrovsky (2019), in the near future, for the development of ToBRFV-resistant plants, methods exploiting new molecular biology techniques, such as genome editing, may be applied.

13 | BREEDING FOR RESISTANCE TO ToBRFV: A NEW SCENARIO WITH AN OLD STORY

The first report of disease caused by a tobamovirus in tomato dates back to the early 1900s: G. P. Clinton reported a case of ToMV disease in a protected tomato crop in Connecticut (USA) (Clinton, 1909). At that time the virus was classified as TMV, while it is very likely that the virus in question was TMV, since TMV are rarely found in tomato and because ToMV were considered for long time a strain of TMV.

Only after about 40 years, a source of resistance to ToMV was first described in a wild species of tomato by Porte, Doolittle, and Wellman (1939), who found that the wild species *Solanum habrocaites* were symptomless even though virus was present in their tissues. In this wild species, the *Tm-1* resistance (R) gene, confers a constitutive resistance, effective in the control of the ToMV pathotype 0, by reducing the concentration of the virus from 90 to 100% respectively in the tomato plant and protoplasts. The *Tm-1* gene inhibits viral replication and genetic analysis of the pathotype 1, demonstrating that the *Avirulence* (*Avr*)-gene of ToMV is the gene coding for the viral replicase (*RdRP* gene) and that two concomitant amino acidic substitutions in the wild type determine the capacity to overcome the resistant gene (Meshi et al., 1988). The *Tm-1* R gene was mapped on tomato chromosome 2 (Tanksley et al., 1992).

Shortly after the introduction of hybrids and tomato cultivars with the *Tm-1* resistance gene, ToMV pathotypes 1 became predominant and resistance no longer effective to contain the spread of ToMV in protected tomato crops. In England, pathotype 1 was not found before 1966 but it became widespread in the 3 years following the introduction of the resistant cultivars 'Virocross' and 'Supercross', carrying the *Tm-1* gene (Pelham, Fletcher, & Hawkins, 1970).

Therefore, breeder and virologists started again to collaborate with the aim to search for new sources of resistance in cultivated and wild tomato germplasms against the evolved strain of ToMV (i.e. pathotype 1). The *Tm-2* gene, capable of controlling pathotype 1 of ToMV, has been identified in the wild species *Solanum peruvianum* and was used in generating tomato hybrids (Soost, 1958, 1959, 1963). The *Tm-2* gene is considered more stable than the *Tm-1* gene, although also for this R gene, breaking strain has been reported after few years of release of the new tomato-resistant cultivars.

Again, a new R gene, the *Tm-2*^{2} was discovered in the *S. peruvianum* accession PI 128650 and introgressed in tomato cultivars (Alexander, 1963). Both *Tm-2* and *Tm-2*^{2} R genes are located close to centromere of chromosome 9 and are considered to be allelic, as it is not possible to obtain tomato lines that are homozygous for both genes (Tanksley et al., 1992). Analysis of the nucleotide sequence of ToMV strains able to overcome both *Tm-2* (i.e. pathotype 2) and *Tm-2*^{2} (i.e. pathotype 2^2) R genes, revealed that the ToMV MP is the matching *Avr* protein (Calder & Palukaitis, 1992; Weber & Pfitzner, 1998). Both genes are dominant and encode a member of the CC-NBS-LRR class of resistance proteins (Lanfermeijer, Dijkhuis, Sturre, de Haan, & Hille, 2003). The *Tm-2*^{2} gene has proved to be a very durable resistance gene effective against all ToMV and TMV strains and thus it has been widely targeted in tomato breeding since 1970. This resistance was therefore effective in the control tobamoviruses on tomato plants for almost 50 years, until the first report and consequent spread of ToBRFV in protected tomato crops. Prior to ToBRFV, another tobamovirus had raised concern because of its rapid spread on resistant tomato genotypes. In fact, ToMMV was detected in 2013 as a novel tobamovirus infecting resistant tomatoes in Mexico (Li, Gao, Fei, & Ling, 2013) before the first detection of ToBRFV in 2015 in Jordan (Salem et al., 2016). Although ToMMV was subsequently identified on tomato or pepper plants in Florida (Fillmer, Adkins, Pongam, & D'Elia, 2015) and in State of New York (Padmanabhan et al., 2015), as well as China (Li et al., 2014) and Israel (Turina, Geraats, & Ciuffo, 2016), its prevalence raised less concern than ToBRFV, as some tomato genotypes were found to be totally resistant to ToMMV. In fact, Sui et al. (2017) report that an undefined genotype 'E' of tomato, already resistant to ToMV and TMV, was also extremely resistant to ToMMV, although it is not specified in the work which tobamovirus resistance gene is involved in the control of the virus. Finally, the same scenario occurred once again with the emergence of ToBRFV in protected tomato crops in various places around the world, this time causing more concern because the virus was found to overcome all known tobamovirus resistance genes, in particular the *Tm-2*^{2} gene that, for decades, effectively controlled ToMV and TMV infections. Interestingly, as for the *Tm-2* and *Tm-2*^{2} ToMV resistant-breaking strains, the MP of ToBRFV is the matching *Avr* protein which enable the virus to infect *Tm-2*^{2} transgenic *Nicotiana benthamiana* plants and *Tm-2*^{2} carrying tomato plants (Hak & Spiegelman, 2021). The genetic determinants conferring the ability to overcome the *Tm-2*^{2} gene have been located between amino acids 1 and 216 of the ToBRFV MP (Hak & Spiegelman, 2021). Yan et al. (2021) demonstrated that MP is the virulence determinant for ToBRFV-mediated infection in plants (harbouring

the *Tm-2²* gene), affirming that six residues in the MP central region (residues 60–186) are critical in enabling ToBRFV to evade *Tm-2²*-mediated resistance.

Recently, Zinger et al. (2021) evaluated 160 genotypes for tolerance and resistance to ToBRFV, resulting in the identification of an unexpectedly high number of tolerant genotypes, including cultivated line/hybrids of tomato (27.6% of the genotypes screened) and some *S. pimpinellifolium* accessions (31% of the genotypes screened) and a single genotype resistant to the virus. In particular, two promising genotypes, the tolerant VC532 and the resistant VC554 genotypes, were virologically and genetically characterised. After mechanical inoculation with ToBRFV, symptoms were evaluated according to a disease severity index (DSI) ranging from 0 (no symptoms) to 3 (severe symptoms, as strong mosaic and leaf area reduction of apical leaves), while relative virus titre was evaluated by measuring the optical density (OD) in indirect ELISA analyses, using two discs of 1 cm, obtained from the 4th–5th true leaf for each plant and harvested 30 days after inoculation. The tolerant genotype VC532, after mechanical inoculation of ToBRFV, was characterised by an average DSI of 0.1 ± 0.0, and by an average viral level (AVL) of 724 ± 52 (optical-density – OD × 1,000), while tomato lines LA2706, used as susceptible control, reacted to ToBRFV inoculation giving a DSI of 2.7 ± 0.1 and an AVL of 802 ± 25. The resistant genotype showed no symptoms and extremely low viral levels following ToBRFV inoculation (average DSI = 0.0 ± 0.1, AVL = 0.0 ± 0.0). Based on genetic inheritance and allelic test, a single recessive gene mapped to chromosome 11 was found to control tolerance, whereas at least two genes with additive effect were found to control resistance. In particular, the resistance was composed by 41% of the same locus controlling tolerance and by an additional locus mapped on chromosome 2, near the region of the *Tm-1* gene. These traits were further characterised and genetically analysed to provide a set of DNA markers with the aim to facilitate their introgression into elite tomato cultivars (Zinger et al., 2021).

However, the discovery of this resistance, determined by additive effects of a recessive gene and a dominant gene, represents a novelty in tomato genetics, because effective resistance to viruses described so far is controlled by single dominant genes (Qurat-ul-ain & Eminur, 2020), except for the resistance to Potato virus Y (PVY) and tobacco etch virus (TEV), both controlled by the same single recessive gene *pot-1* (Parrella et al., 2002).

Many *R* genes are dominant, or incompletely dominant, and require specific dominant avirulence (*Avr*) genes in the pathogen for their function (Flor, 1946). This genetic interaction between plant and pathogen led to the current view that such *R* genes encode receptors for *Avr* gene-dependent pathogen molecules (Staskawicz, Ausubel, Baker, Ellis, & Jones, 1995). *R* genes fall into several distinct classes (Hammond-Kosack & Jones, 1997). The *Tm-2²* protein belongs to the coiled coil-nucleotide binding site-leucine rich repeat (CC-NBS-LRR) family and interacts with the MP of ToMV (Lanfermeijer et al., 2003). Upon recognition of these molecules, *R* gene products activate plant defence mechanisms resulting in a hypersensitive reaction (HR), with the aim to isolate the virus from healthy tissues. Since this resistance is not associated with development of visible local lesions, it has been assumed that the programmed cell death (PCD) associated with HR is restricted only to the directly inoculated cells (or few cells around them) or that the *Tm-2²* gene induces an extreme resistance as the *Rx* gene in potato which does not allow viral replication of the potato virus X (PVX) genome, even in directly inoculated cells (Bendahmane, Kanyuka, & Baulcombe, 1999). Therefore, it will be interesting to elucidate the mechanism involved in the resistance to ToBRFV controlled by the R genes so far described, as well the class of resistance genes they belong.

14 | CONCLUSION REMARKS AND FUTURE PERSPECTIVES

In the last few decades, ToMV has been primarily responsible for the forced evolution of tomato. This virus, having spread rapidly throughout the world, has induced breeders to select tomato cultivars with genes of resistance to ToMV, in fact forcing and directing the evolution of this crop, with the aim of mitigating the damage caused by this pathogen. This has led to the gradual abandonment of local cultivars and the destruction of biodiversity, which in many cases can lead to the emergence of new pathogens that skip the mechanisms of resistance or tolerance introduced over the years. Similarly, ToBRFV will direct breeders towards a new forced evolution in order to obtain plants resistant to this pathogen. Thus, we are facing a second major event that will change the evolutionary history of tomato.

Plant viruses being spread by seed, such as ToBRFV, are particularly dangerous because of the possibility of long-distance movement of infected material, from one country to another, in an extremely short time. Therefore, suitable integrated ToBRFV management necessarily requires monitoring of potential secondary hosts, hygiene and prophylactic measures application by workers/farmers when handling plant material and other farm activities, followed by the removal of infected plants, and continuous monitoring of cultural practices (Panno et al., 2020b). Unfortunately, there is a gap in the adoption of phytosanitary measures among different countries. In fact, in Europe, exists a well-organised phytosanitary regulation based on pest risk assessment and analysis, that allowed an effective mitigation of ToBRFV impact and dispersion on production, while in developing countries the application of the control measures is often not adopted or is inadequate (Oladokun et al., 2019). In this scenario, effective phytosanitary actions are required, such as adequate crop management, standardisation of disinfection protocol to be used worldwide for seed movement (Davino et al., 2020) and more restrictive measures at international borders using rapid and sensitive diagnostic methods (Panno et al., 2019c). Therefore, great hope for the future is aimed at the introduction of resistances to ToBRFV in tomato lines and hybrids, especially for those intended for cultivation in protected environments where the problem, because of the biological characteristics of ToBRFV, is particularly evident.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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