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RUGOSE INVASION: RETHINKING TOMATO VIROLOGY IN THE ERA OF TOMATO BROWN RUGOSE FRUIT VIRUS

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ABSTRACT
Tomato brown rugose fruit virus (ToBRFV), a member of the genus Tobamovirus, has emerged as one of the most destructive viral pathogens threatening tomato (Solanum lycopersicum) production worldwide. Capable of overcoming historically durable resistance genes, particularly Tm-2^2 and possessing remarkable environmental stability alongside efficient seed transmission, ToBRFV has rapidly spread across more than forty countries in short period of time. To address this crisis, this prospective review moves beyond a passive summary of current events to propose an actionable roadmap for combating tobamovirus emergence. By anticipating the threat, analyzed the epidemiology and global dissemination and symptomological patterns of the virus. Along with evaluating host–virus interactions to identify novel vulnerabilities specifically, how adaptive mutations in viral movement protein enable the evasion of Tm-mediated resistance. To actively confront resistance defeat, the outline of molecular mechanisms and counter-strategies to outpace viral adaptation. Furthermore, emphasized the deploying next-generation diagnostics, including digital PCR, CRISPR-based detection systems and advanced serological assays. Achieving holistic containment which will require evolving integrated management practices tailored for high-risk agricultural environments. Engineering durable immunity, detailing biotechnological and genetic frameworks for sustained viral control, such as the introgression of wild Solanum resistance loci, engineered NLR variants, RNA-based approaches, and CRISPR-mediated genome editing. Collectively, this framework identifies critical research gaps and provides a strategic outlook for establishing durable resistance and securing global tomato production.

KEYWORDS: Global dissemination, Resistance breakdown, CRISPR based detection.

1. INTRODUCTION
Tomato (Solanum lycopersicum) is one of the most widely cultivated horticultural crops worldwide, with over 5 million hectares under cultivation and more than 182 million tons produced annually (Caruso et al., 2022). Tomato brown rugose fruit virus (ToBRFV) is an emerging tobamovirus that causes mosaic, chlorosis and fruit necrosis in tomato, with 15–55% yield losses (Núñez-Muñoz et al., 2026). Tobamoviruses are characterized by a monopartite, positive-sense single-stranded RNA genome, making them unique within their family because of a nonsegmented genome (Gomaa and Garcia-Ruiz, 2025). These viruses exhibit broad host ranges across diverse plant families and tend to have low genetic diversity, with strong purifying selection acting on their genomes, contributing to their genetic stability (Zamfir et al., 2023). The genus includes economically important species such as Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV) and the emerging Tomato brown rugose fruit virus (ToBRFV), which can overcome genetic resistance in tomato cultivars. Tomato brown rugose fruit virus, a tobamovirus first identified in the Middle East in 2014 (Zois et al., 2025). The virus was detected extensively across floral organs, including petals, stamens, styles, stigmas, ovaries and pollen grains, indicating broad tissue tropism and efficient systemic movement into reproductive structures with widespread localization. The virus was absent from ovules, suggesting a barrier to vertical transmission at the embryo level (Avni et al., 2022). Tissue analysis revealed an uneven distribution of the pathogen, with the highest viral titers consistently found in newly emerged leaves, sepals and fruits compared with older foliage within the fruits themselves, and the mesocarp harbored the greatest viral concentration (Zhao et al., 2025). The virus features a positive-sense single-stranded RNA genome of approximately 6.4 kb encoding replicase proteins, a movement protein, and a coat protein, which facilitates its high stability and efficient transmission (Rezaei et al., 2025). It spreads mechanically through contaminated tools, hands, insects, soil and plant material, evading traditional Tm-2^2 resistance genes (Zois et al., 2025). This further emphasizes the complexity of tracing infection sources due to the presence of mixed-origin seed lots and insufficient phytosanitary traceability (Paraiso et al., 2024). Advanced atomic force microscopy and

nanomechanical mapping revealed that rigid, rod-like viral particles exhibit a high degree of brittleness when subjected to mechanical stress (Puskás et al., 2025).

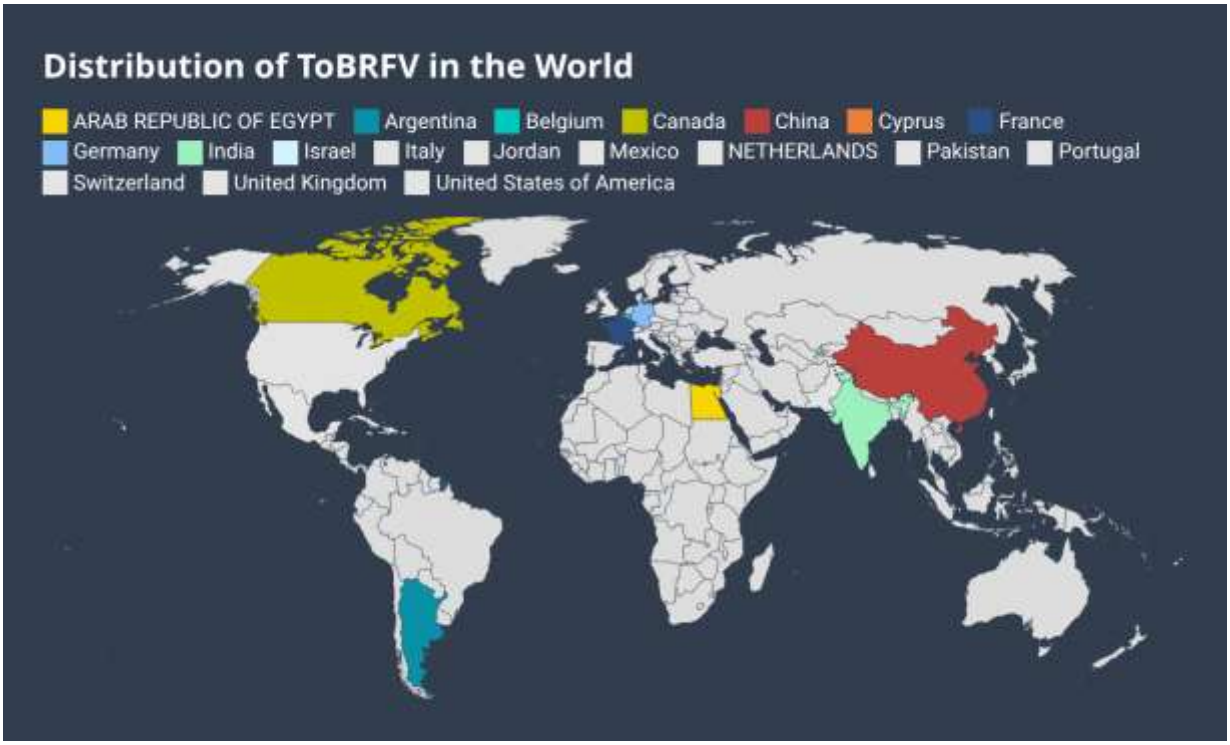

Fig. 1: World map which depicts the diversity and spreads of ToBRFV among the different countries of world. (Map created using https://www.datawrapper.de)

2. Why is ToBRFV still a global threat in the future?

Disease can reach up to 100% incidence in commercial tomato cultivars, contributing to global losses of billions of dollars every year, which makes ToBRFV a major economic and regulatory concern in the coming years (Janssen et al., 2025). The busts of Tm-1, Tm 2 and Tm 2^2 resistance spread through contact with seeds across Europe, Africa, the Americas and Asia EU rules demand tests, but residues confuse things into the future (Giesbers et al., 2024; Paisantham et al., 2025). Multiple incursions, such as Mexico's second wave from Europe, with 0.08–9% seed transmission, continue to occur through trade routes into the future, hitting Mexico, the USA, Canada and China (Fig. 1) (Zamora-Macorra et al., 2025). There are no cases in Southeast Asia, but sewage found worldwide (Canada, US, Slovenia) means that it lingers as a hidden spreader into the future. Owing to its persistence in wastewater at very high concentrations, it is a continuing threat to tomato production systems and is a ubiquitous environmental contaminant (Cobbinah et al., 2025). Mechanical transmission by grafting material, tools, worker contact and other cultural practices can rapidly spread infection through a crop, and persistent inoculum in contaminated structures restricts rotation options for subsequent lantings (Salem et al., 2023). Biosolarization fails after 60 days, with 100% ToBRFV transmission to seedlings through infected debris (Janssen et al., 2025).

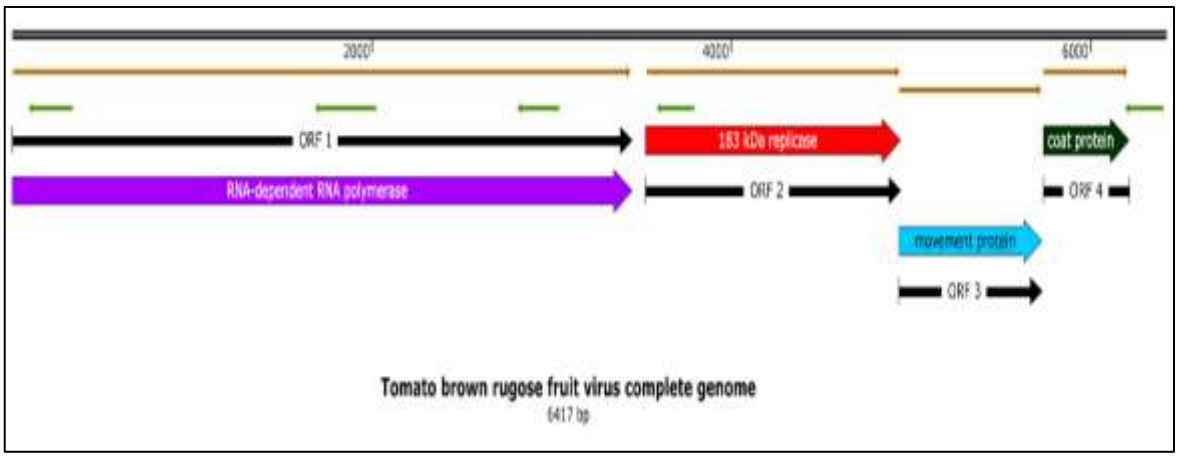

Fig 2: Genome structure Tomato Brown Rugose Fruit Virus in which sequence extracted from GenBank accession number of PV146433.1 in which structure created using snap gene viewer.

3. Genomic Architecture and Global Symptomological Diversity

Tomato brown rugose fruit virus (ToBRFV) features a positive-sense, single-stranded RNA genome that is approximately 6,200 to 6,400 nucleotides in length (Zamora-Macorra et al., 2025). The genomic architecture is flanked by untranslated regions (UTRs), specifically a 5' UTR composed of about 74 nucleotides that is rich in CAA repeats, and a 3' UTR of around 201 nucleotides that forms tRNA-like structures (Yan et al., 2021b; Zamora-Macorra et al., 2025). This viral genome is structured into four distinct open reading frames (ORFs)(Fig 2)(Vargas-Hernández et al., 2022). The first two, ORF1 and ORF2, encode non-structural replicase-associated proteins of 126 kDa and 183 kDa, respectively, which jointly form the RNA-dependent RNA polymerase (RdRP) complex essential for viral replication(Vargas-Hernández et al., 2022; Yan et al., 2021b). The larger 183-kDa protein is produced through a partial readthrough mechanism of a stop codon located between ORF1 and ORF2 (Zamora-Macorra et al., 2025). The remaining two ORFs are expressed from subgenomic RNAs to produce structural proteins (Zamora-Macorra et al., 2025). ORF3 encodes a 30-kDa movement protein (MP) that alters plasmodesmata to enable the cell-to-cell movement of the virus (Vargas-Hernández et al., 2022; Zamora-Macorra et al., 2025). And ORF4 encodes a 17.5-kDa coat protein (CP) that encapsulates the viral RNA, conferring structural stability to the virion and facilitating systemic transmission within the host (Yan et al., 2021b; Zamora-Macorra et al., 2025).

Tomato brown rugose fruit virus causes mosaic patterns and mottling represent some of the most widespread foliar symptoms of the virus, reported across almost all regions with active plant infections. The severity and specific presentation vary globally: China experiences mild to severe mosaic patterns, while Turkey reports severe mosaics accompanied by mottling, and Israel observes pronounced patterns under both natural and experimental conditions (Chao et al., 2021; Fidan et al., 2021; Hak and Spiegelman, 2021; Topcu et al., 2025). Similarly, open-field tomato plants in India (specifically across Karnataka and Maharashtra) have been observed displaying widespread mosaic patterns and mottling (Kavya et al., 2024). The symptoms can also be highly localized or distinctly colored, as seen with the clear mosaic patterns concentrated in the plant heads in the Netherlands and the specific yellow mosaics documented in Belgium (Giesbers et al., 2024). Along with systemic symptoms involving mild to severe mosaic and mottling affect crops in Hungary, while standard leaf mosaics are consistently reported across Germany, Egypt, and Colombia (Amer and Mahmoud, 2020; Ehlers et al., 2022; Jewehan, 2022; Sánchez et al., 2025).

Necrosis, which indicates tissue death and often presents as brown, dried, or wrinkled patches, affects various parts of the infected plants across different regions. In China, brown necrosis appears on pedicles and sepals sometimes rendering them fully necrotic while fruits develop typical brown rugose (wrinkled) lesions (Yan et al., 2021b). Similarly, Turkey reports necrotic, dried spots on the peduncles, calyces, and petioles which heavily triggers premature fruit drop alongside necrotic patches on the fruits themselves (Fidan et al., 2021; Topcu et al., 2025). The characteristic "brown rugose" spots and wrinkled patches on fruit surfaces are also prominently observed in Israel, Canada, Germany and India (Ehlers et al., 2022; Fougere et al., 2025; Hak and Spiegelman, 2021; Kavya et al., 2024). Egypt documents explicit leaf necrosis, the Czech Republic notes necrosis restricted to older leaves in naturally infected plants, and Colombia observes necrosis on shoots and sepals as well as both internal and external necrosis on the fruits (Amer and Mahmoud, 2020; Eichmeier et al., 2023; Sánchez et al., 2025).

Structural distortion of the foliage severely impacts the plant's ability to photosynthesize, presenting through various forms of leaf deformation, narrowing, and blistering across the globe. Dark green, blister-like bulges accompanied by leaf narrowing are frequently reported in both Turkey and Germany, while Colombia observes blistering alongside interveinal yellowing, curling, and overall leaf deformation (Fidan et al., 2021; Sánchez et al., 2025; Topcu et al., 2025). More extreme structural mutations occur in Israel, where severe deformation and elongation can reduce leaves to thin threads or cause epinasty (downward bending), similar to the shoestring leaves, rolled edges and stunting documented in Hungary, or the sharp "needle-tips" observed in Belgium (Giesbers et al., 2024; Jewehan et al., 2022b). Other regions report distinct variations: the Netherlands sees deformation primarily in the plant's head, leading to reduced overall leaf biomass; Egypt notes widespread general leaf deformation; India also reports significant foliar structural issues, with plants exhibiting general yellowing, chlorosis, and distinctly deformed leaves and experimental inoculations in the Czech Republic have resulted in strong leaf deformation, mild blistering, and a stunted, bushy growth habit (Amer and Mahmoud, 2020; Eichmeier et al., 2023; Jewehan, 2022; Kavya et al., 2024).

When the virus affects the fruit, it alters color distribution and ripening processes, ultimately making the produce visually unmarketable. In China, infected fruits develop sunken ring-shapes and yellow spots on their surfaces, similar to the yellow spots frequently concentrated around the calyx observed in Germany (Ehlers et al., 2022; Yan et al., 2021b). Turkey reports rough fruit surfaces that exhibit marbling, chlorotic patches, and yellow or brown spots, while Israel specifically notes prominent yellow and orange marbling (Ehlers et al., 2022; Fidan et al., 2021; Hak and Spiegelman, 2021; Topcu et al., 2025). Furthermore, the virus severely disrupts normal development; Canada reports incomplete or uneven fruit ripening, which closely aligns with the highly irregular ripening patterns, spotting, and discoloration documented in Colombia (Fougere et al., 2025). In Egypt broadly observes general discoloration across affected fruits, while in India, the visual quality of the harvest is similarly compromised by the development of necrotic spots and characteristic brown rugose patches (Amer and Mahmoud, 2020; Kavya et al., 2024; Sánchez et al., 2025).

In some instances, the virus is characterized by asymptomatic or strictly environmental detections. Thailand remains unique, as no agricultural plant infections have been reported; rather, the virus was detected exclusively in environmental wastewater and human sewage (Paisantham et al., 2025). Additionally, naturally infected samples collected in Belgium and the Czech Republic notably showed no fruit symptoms at the time of collection (Eichmeier et l., 2023; Giesbers et al., 2024).

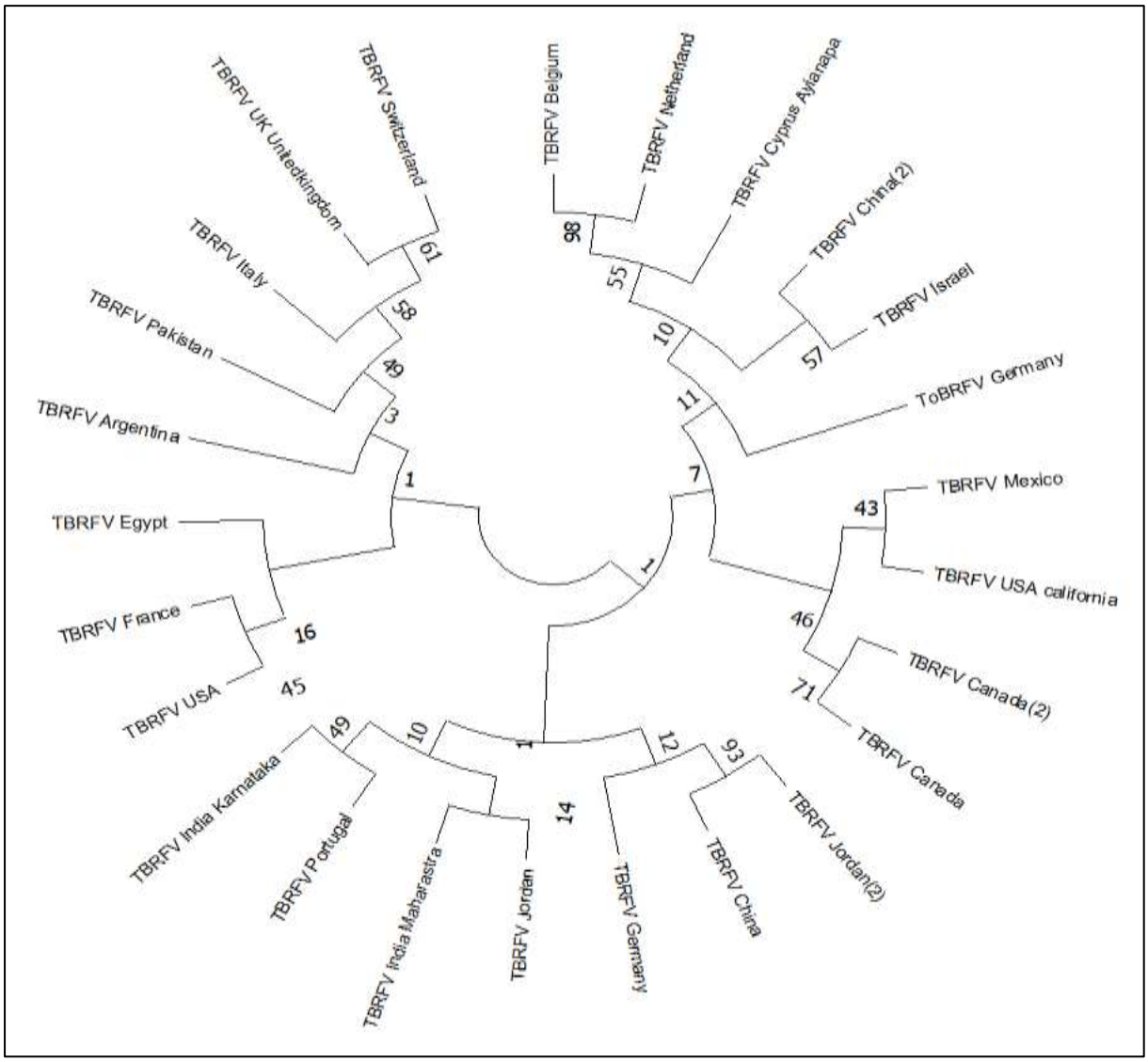

Fig 3: Phylogenetic analysis of ToBRFV genome of different location of world (Image created by MEGA 11 Software)

4. Epidemiology and Global Dissemination Patterns

The introduction of only a few infected plants was sufficient to trigger near-complete infection of the entire crop within a short period, highlighting the highly efficient transmission dynamics of the virus (Panno et al., 2019). ToBRFV has been placed on multiple quarantine lists worldwide, and its global spread and persistence in production systems continue to pose a serious risk (Janssen et al., 2025). Bioassays confirmed that healthy tomato seedlings irrigated with contaminated water successfully acquired the virus through their roots, facilitating systemic infection and eventual manifestation of disease symptoms in the upper foliage (Mehle et al., 2023). Intensive cultural practices such as pruning, tutoring, and harvesting were identified as the primary accelerators of mechanical transmission (González-Concha et al., 2021). Controlled pollination experiments demonstrated that when infected pollen was used to fertilize healthy plants, neither fruits nor seeds carried the virus, confirming that ToBRFV is not transmitted through pollen (Avni et al., 2022). The integration of sequence data with epidemiological metadata supports hypotheses regarding the geographic origin and movement patterns of ToBRFV, offering a powerful tool for tracing outbreaks and understanding transmission dynamics (van de Vossenberg et al., 2021). From an epidemiological perspective, this paper highlights the rapid international expansion of ToBRFV through trade and seed movement, situating the virus within a broader context of primary tomato and pepper virus constraints (cucumoviruses, Begomoviruses, Potyviruses, Tospoviruses and Tobamoviruses), which collectively cause large yield and quality losses worldwide (Besati et al., 2025). By 2021,

seeds, mechanical, bumblebees that infect 50 Chinese tomato cultivars (100% incidence) and are asymptomatic in potato, eggplant, and N. tabacum have spread to 10 countries (Yan et al., 2021b). The virus is transmitted to germinating seedlings through microlesions rather than direct embryonic infection, resulting in transmission rates of 2.8% in cotyledons and 1.8% in the third true leaf. Because the embryo remains uninfected, external seed sterilization is highly viable(Davino et al., 2020) . ToBRFV retains 70% infectivity in perlite and irrigation after 60 days of column leaching, and substrate persistence explains greenhouse epidemics (Mehle et al., 2023). . The pathogen frequently coinfects tomato plants alongside the Tobacco mosaic virus and naturally affects several asymptomatic weed species, including Solanum melongena and Chenopodium species (Jamous et al., 2022). High-throughput sequencing of RNA associated with the bee body enabled the assembly of the viral genome, confirming that ToBRFV persists on pollinators. Dissection studies have revealed that the virus is located predominantly in the abdominal region, suggesting that transmission occurs during buzz pollination, as bees mechanically contact floral tissues (Levitzky et al., 2019).

A marked increase in infection incidence toward the end of the growing season was accompanied by significantly lower Ct values, indicating greater viral accumulation in late-harvested seeds. Several seed batches that showed minimal or no infection early in the season presented substantial infection rates later, with some reaching complete infection across replicates (Zhang et al., 2026). The virus particles retained infectivity on the fabric surfaces and could be efficiently transmitted to plants after contact. Commercial agricultural disinfectants and detergents, including Menno Florades, Fadex H+, and Hortisept Clean Plus, reduced viral infectivity on fabrics by more than 99.9%, whereas commonly used household detergents showed limited efficacy and allowed substantial viral persistence (Ehlers et al., 2022). The phytophagous beetle Henosepilachna vigintioctopunctata affects the transmission dynamics of Tomato brown rugose fruit virus across solanaceous hosts (Wang et al., 2025b). The virus derived from Commelina communis retained full infectivity, successfully inducing typical symptoms in tomato, Nicotiana benthamiana, and the same weed host, confirming its ability as a viral reservoir; hence, identification of a monocot host significantly broadens the ecological range of ToBRFV beyond dicotyledonous species and indicates that common weeds can sustain viral persistence outside cultivated crops (Wang et al., 2025b).

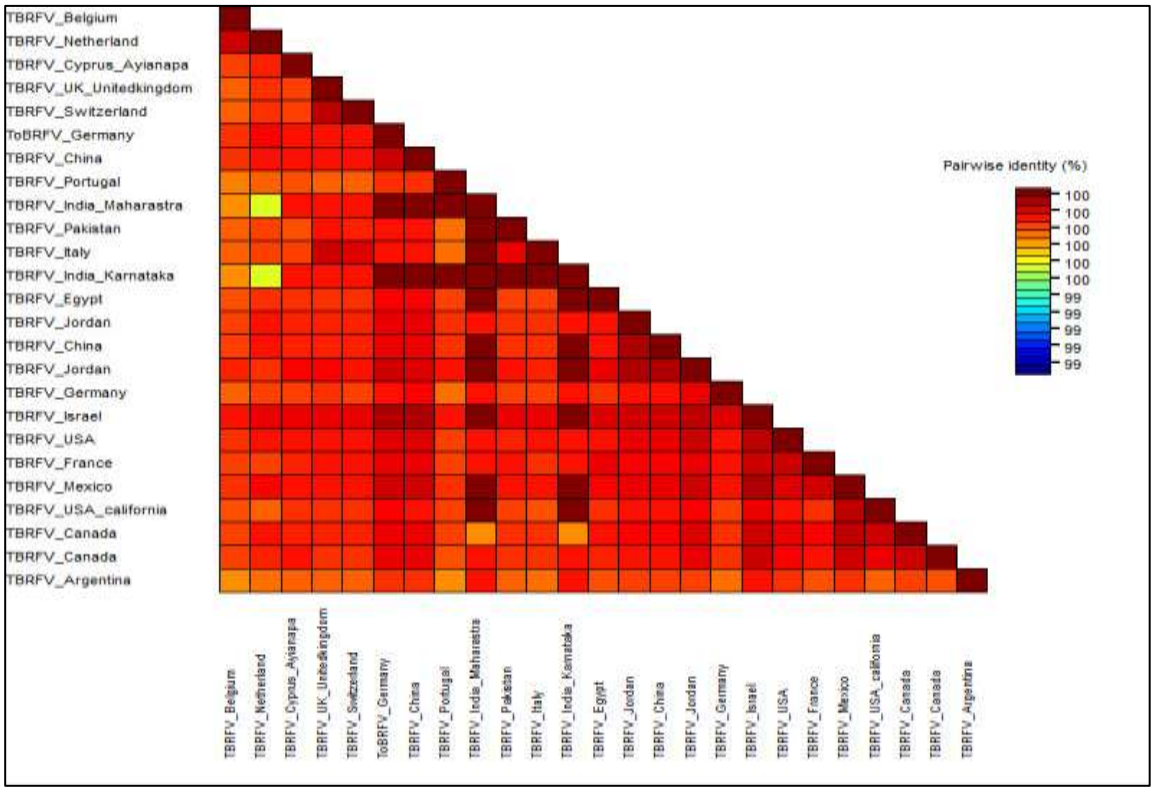

Fig 4: A heat map which visualizes the relative magnitude and patterns of variables across ToBRFV samples. (Map created by using MEGA11 Software)

A systematic survey of weed populations in infected tomato fields identified twelve distinct species that naturally harbor the virus, ten of which represent entirely novel alternate hosts, including Malva parviflora. Most of these natural weed hosts remain completely asymptomatic while the virus accumulates, effectively acting as hidden cryptic reservoirs that bridge consecutive growth cycles (Salem et al., 2022). Extreme acidic conditions (pH 1) reduce infectivity and impair large genome segment amplification, whereas extreme alkaline conditions (pH > 10) severely modify particle morphology, degrade the coat protein, and completely abolish infectivity (Molad et al., 2024).

The circular phylogenetic tree depicts the evolutionary relationships among ToBRFV isolates from different geographic regions (Fig 3). Bootstrap values are shown at the internal nodes, indicating the robustness of each clade. The Indian isolates clustered closely with isolates from Portugal and Jordan, suggesting a shared ancestry or possible epidemiological linkage. Additional well-supported clades include isolates from Europe (e.g., Switzerland, Italy, France, Belgium, Netherlands) and the Middle East (e.g., Israel, Jordan) as well as North and South American isolates (USA, Canada, Mexico, Argentina), highlighting the global diversification of ToBRFV.
The heatmap presents pairwise nucleotide sequence identity (%) among ToBRFV isolates from various global locations, visualized in a symmetric upper triangular matrix format. Isolate labels match those in the companion phylogenetic tree and prior identity matrix, underscoring tight clustering among Indian, European and Middle Eastern strains (Fig 4). The ToBRFV incidence spans North America, South America, Europe, Asia, and Africa, indicating its ability to establish diverse agroclimatic conditions. Notably, widespread occurrences are observed in countries such as the United States of America, Canada, and Mexico in North America, whereas Argentina is present in South America. The European region shows substantial reporting, including Germany, France, Italy, the Netherlands, Belgium, the United Kingdom, Portugal, and Switzerland, suggesting intensive surveillance and reporting systems as well as high levels of international seed trade. In Asia, the virus has been detected in China, India, Jordan, Israel, Cyprus and Pakistan. Its presence in India and Pakistan is particularly significant given the extensive cultivation of solanaceous crops and the potential for rapid regional dissemination. In Africa, detection in Egypt indicates that the virus has also penetrated major tomato-producing regions within arid and semiarid zones. Collectively, the map reflects a pandemic-like distribution pattern, driven primarily by seed transmission, mechanical contact, and global trade networks.

5. Exploitation of Host–Virus Interactions to Identify Novel Vulnerabilities

ToBRFVs are treated primarily as physical particles whose morphology, hydrophobic capsid domains and rod shape govern adsorption and desorption behavior in saturated porous media, which indirectly reflects factors that also favor environmental persistence and long-distance spread (Cobbinah et al., 2025). Codon patterns in coat proteins, movement proteins, and replicases reveal how the virus is tuned into the machinery of tomato plants for better replication and spread (Ghorbani, 2024). The 6.4 kb RNA pumps replicases (126/183 kDa), movement proteins (30 kDa), and coats (17.5 kDa) for spread and packaging. (Vargas-Hernández et al., 2022). Replicase p126 (methyltransferase 280–1365 nt, helicase 2543–3315 nt) along with RNA-dependent RNA polymerase (RdRp) drive RNA synthesis, and the movement protein (MP) enables the spread of plasmodesmata, and the Coat protein leads to seed stability in Solanaceous crops such as tomato and eggplant (Zamora-Macorra et al., 2025). MP binds plasmodesmata to shuttle vRNA despite Tm-2^2, and key aa swaps weaken plant resistance proteins but slow long-distance transport, which balances virulence (Yan et al., 2021b). However, the N-gene hypersensitive response halts the spread of MP (Batuman et al., 2020). A major- effect quantitative trait locus (QTL) is identified on chromosome 11, with a narrow ~22-kb peak interval between 46,825,788 and 46,847,421 bp, tagged by several high-LOD SNPs (e.g., SL4.0ch11_46825788, SL4.0ch11_46847421, and SL4.0ch11_46850215) that collectively explain a substantial proportion of the phenotypic variance in multiple genome–wide association studies (GWASs) and QTL models (Jaiswal et al., 2025). Late-infected plants presented several non-synonymous mutations in the helicase domain of the 126/183-kDa replication proteins, particularly those clustered within regions previously shown to interact with the Tm-1 protein in ToMV, including recurrent substitutions such as Y976H, G983E, D1097N and Y1104F (Kubota et al., 2024).

Solanum elaeagnifolium and Solanum rostratum are susceptible to ToBRFV infection, with S. rostratum supporting greater viral accumulation and displaying clear disease symptoms, whereas S. elaeagnifolium remains largely asymptomatic despite infection (Matzrafi et al., 2023). The sequence variations exhibit clear host-dependent patterns, suggesting that the pathogen undergoes distinct selective pressures to optimize the replication machinery when it colonizes specific cellular environments, such as pepper or watermelon, compared with its primary tomato host (Zamora-Macorra et al., 2025). Microscopic examinations of infected Solanum lycopersicum and Nicotiana tabacum epidermal cells revealed distinct viral inclusions, specifically X-bodies and stacked plaques, which act as intracellular replication factories to facilitate cell–to-cell movement, and field surveys adjacent to infected crops led to the identification of 23 previously undocumented alternative hosts containing common weeds such as Ipomoea purpurea and Clematis drummondii (Vásquez-Gutiérrez et al., 2024). Identification of host microRNA profiles altered during infection indicated that ToBRFV interacts with host gene regulation pathways at the posttranscriptional level. Specifically, miRNAs, including those involved in immune signaling and stress responses, are differentially expressed, suggesting that the virus may manipulate host regulatory networks to facilitate replication and symptom expression (Eichmeier et al., 2023). Comparative profiling between resistant and susceptible genotypes revealed that resistant plants accumulate significantly greater levels of iron and nickel, indicating a link between micronutrient homeostasis and antiviral defense. Additionally, transcriptome analysis revealed differential expression of genes associated with iron regulation and abscisic acid signaling, suggesting coordinated metabolic and hormonal adjustments during infection (Thakare et al., 2024). The movement protein was shown to directly interact with the tomato catalase SiCAT2 through defined amino acid interfaces, suppressing its enzymatic activity and leading to the accumulation of reactive oxygen species, which in turn facilitates viral replication and symptom development. The host response involves an extracellular defense layer in which the subtilisin protease SiSBT5 targets and degrades the viral movement

protein, revealing a previously uncharacterized apoplastic antiviral mechanism (Zhang et al., 2026). The efficacy of biotic bacterial polysaccharides from Pseudomonas fluorescens and Burkholderia gladioli, alongside abiotic elicitors such as salicylic acid and mechanical wounding, in protecting tomatoes against ToBRFV and the simultaneous application of both bacterial elicitors triggered a massive synergistic defense response, increasing the expression of key immunity genes (PR1b, COI1, and NPR1) by hundreds to thousands of-fold (Zohoursoleimani et al., 2025).

6. Molecular Determinants of Resistance Breakdown

6.1 Mechanisms of Viral Evasion

ToBRFV breaks the widely used tomato Tm-2^2 tobamovirus resistance gene, leaving current commercial cultivars largely unprotected (Janssen et al., 2025). The movement protein-encoding gene stands out for its high number of mutation spots and positive selection at key codons such as 123 and 192, allowing it to slip past things such as Tm-2^2 resistance (Ghorbani, 2024). Its stable RNA genome evades Tm-2^2 via movement protein changes, leaving commercial tomatoes wide open (Fig. 6) (Zhao et al., 2024). Host-specific single nucleotide variations (SNVs) in the replicase methyltransferase and helicase domains allow replication in tomato, dodging Tm genes via subtle tweaks that do not result in full mutations (Zamora-Macorra et al., 2025). MP residues such as H67/N125/K129/A134/I147/I168 avoid Tm-2^2 recognition for systemic spread mutations, restoring resistance, pinpointing exact breakdown spots (Yan et al., 2021a). Tm-1 inhibits ToBRFV by binding to 126K/183K helicase mutants whose 2–3 surface substitutions, such as Y976H, G983E, D1097N, and Y1104F breakers, are disrupted, reducing the affinity for efficient replication (Kubota et al., 2024). Stable replicase and MP genes enable Tm jumps in which minimal SNVs hide adaptations (Çelik et al., 2022).

Genomic characterization revealed that ToBRFV possesses a typical tobamovirus genome encoding replication-associated proteins, a movement protein and a coat protein, with the movement protein specifically implicated in overcoming host resistance (Fidan, 2020). The genetic plasticity of ToBRFV is exemplified by its ability to undergo rapid adaptive evolution, such as specific amino acid substitutions within the viral movement protein (e.g., F22Y and N82K) that enable the virus to circumvent dominant resistance genes like Tm-2^2 (Fig 5) (Jewehan et al., 2022a).Comparative whole-genome sequencing of viral isolates from the two cultivar types revealed a single amino acid substitution at position 82 in the viral movement protein, and functional validation through bioassays demonstrated that only the variant carrying this specific mutation was capable of infecting the resistant cultivar, whereas the wild-type isolate remained restricted (Zisi et al., 2024). Protein sequence mapping revealed critical amino acid substitutions, particularly the A134T mutation in the viral movement protein, which is directly associated with increased symptom severity and the ability of the pathogen to overcome conventional Tm-2^2 resistance mechanisms (Sánchez et al., 2025). By employing Oxford Nanopore Technologies to sequence viral genomes from naturally infected tomato crops, critical genetic variations that allow the virus to aggressively evade the widely utilized Tm-2^2 resistance gene can be mapped (Fougere et al., 2025).

6.2 Pyramiding Resistance Genes to Delay Breakdown

Sequencing of Tm-1 alleles revealed nine key amino acids in the N-terminal replicase-binding domain (aa 78–112 under positive selection) that distinguish resistant Tm-1^Sp from susceptible variants, which suggests enhanced affinity for the ToBRFV replicase (Zois et al., 2025). Molecular determinants of resistance breakdown and biotech strategies explain why Tm-2^2's LRR domain recognizes TMV and ToMV MP but fails against ToBRFV MP, so error-prone PCR is used to mutagenize leucine-rich repeats (LRRs) (aa482–861) and screen 8,000 variants for HR upon coexpression with ToBRFV MP in N. benthamiana (Wang et al., 2025a). Five gain-of-function mutants emerged, with Tm-2^2-S723Y and Tm-2^2-N744D inducing strong, specific cell death against ToBRFV MP while retaining TMV MP recognition, demonstrating the evolution of dual-specificity NLRs (Wang et al., 2025a). Because plant viruses continually evolve and demonstrate genetic plasticity by overcoming resistance genes, modern breeding strategies must shift toward developing and combining multiple resistance loci. For example, combining a newly identified tolerance quantitative trait locus (QTL) mapped to chromosome 11 with a locus at the Tm-1 region on chromosome 2 creates an effective resistance that drastically reduces viral load (Zinger et al., 2021). Strategically incorporating such tolerance traits into genetic pyramids is highly advantageous; it harnesses the benefits of tolerance to reduce the evolutionary selection pressure on emerging virulent viral isolates, thereby increasing the overall stability and breadth of the plant's defensive phenotype (Zinger et al., 2021). While new sources of promising genetic resistance to ToBRFV have been identified, physically introgressing these valuable traits into commercial tomato genotypes whether through conventional breeding or biotechnology is a highly laborious process that takes years to complete (Abou Kubaa et al., 2023).

6.3 Leveraging Recessive Resistance and Loss-of-Susceptibility Targets

In contrast to dominant R genes that actively initiate immune responses upon detecting viral effectors, recessive resistance provides a highly durable defense strategy by altering or eliminating the host susceptibility (S) genes such as the eukaryotic translation initiation factors eIF4E and eIF(iso)4E that viruses critically rely on for translation and replication (Sanfaçon, 2015). Expanding beyond traditional eIF4E targets, the targeted mutagenesis of TOBAMOVIRUS MULTIPLICATION1 (SlTOM1) homologs via CRISPR/Cas9 has demonstrated high efficacy against ToBRFV. By utilizing multiplexed gene editing to create quadruple-knockout

tomato mutants, researchers successfully inhibited viral coat protein accumulation and prevented viral replication, highlighting the potential of altering host susceptibility factors to achieve broad-spectrum, durable tobamovirus resistance (Ishikawa et al., 2022). Genetic mapping of ToBRFV-tolerant tomato varieties indicates that this trait is frequently governed by a single recessive gene. By acting as loss-of-susceptibility mutations, these alleles hinder the virus from easily exploiting the plant's cellular machinery for replication. As a result, the plant is protected from severe disease symptoms like fruit wrinkling and deformation, despite the virus continuing to circulate and accumulate systemically within the host (Zinger et al., 2021). Engineering Targeted Loss-of-Susceptibility: Applying CRISPR/Cas9 technology to introduce targeted mutations in eukaryotic translation initiation factors, such as eIF4E1, offers a powerful approach to engineering durable loss-of-susceptibility. By disrupting the host cap-binding proteins essential for the translation of viral RNA, these genome-edited tomato lines severely restrict virus accumulation without incurring the physiological and yield penalties often associated with the dominant hypersensitive response (HR) (Kaur et al., 2020). While ToBRFV readily utilizes single nucleotide variations (SNVs) to alter the conformation of its movement protein and evade Tm-2^2 recognition, it is mechanistically far more difficult for the virus to evolve an entirely new translation pathway or recruit alternative host factors when primary interactors, such as eIF4E or TOM1, have been excised from the tomato genome (Gomez et al., 019).

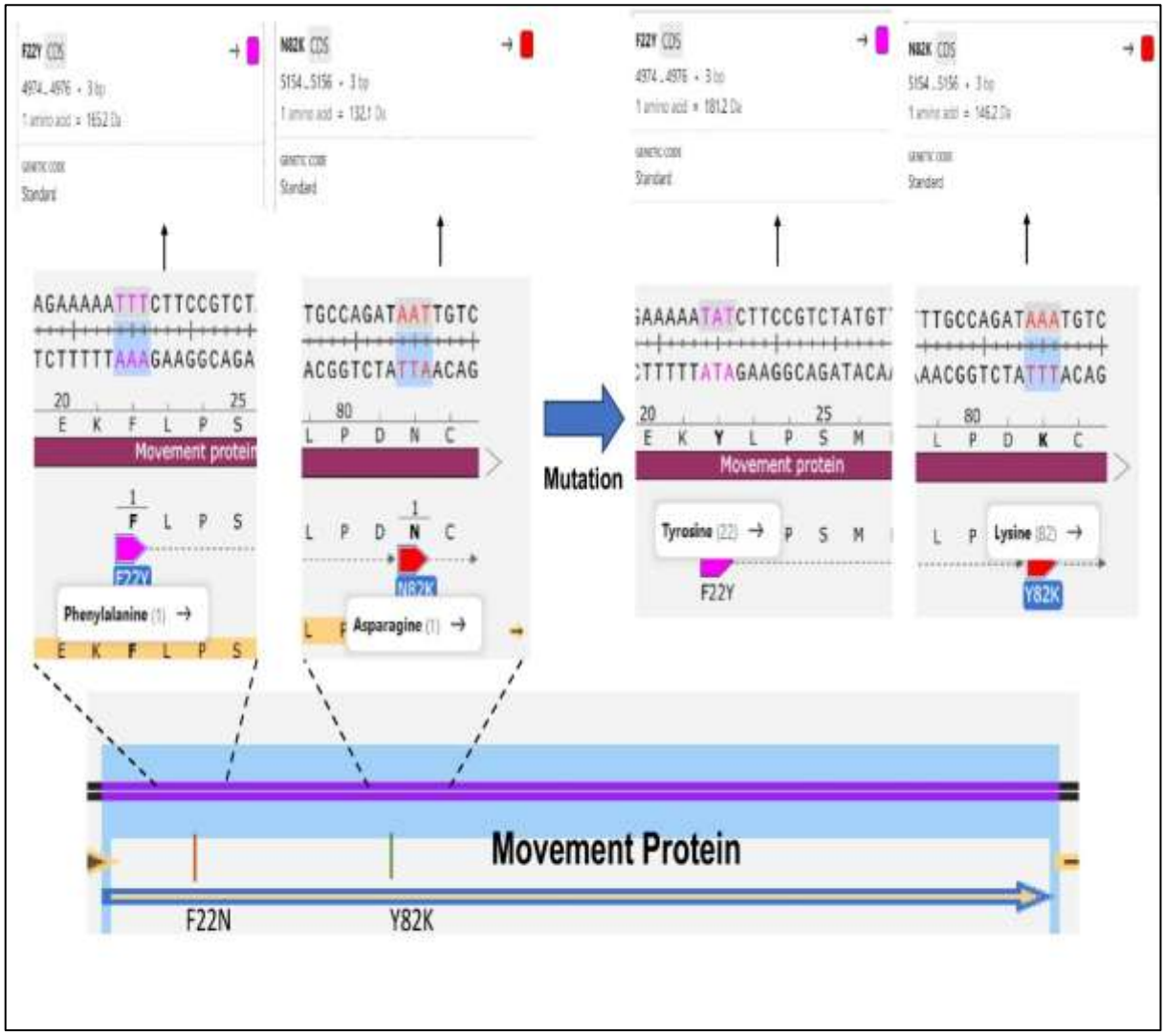

Fig 5: Illustration of specific amino acid substitutions, such as F22Y and N82K, lead to structural changes that allow ToBRFV to circumvent established Tm-2^2 host resistance image created using snap gene viewer and powerpoint application.

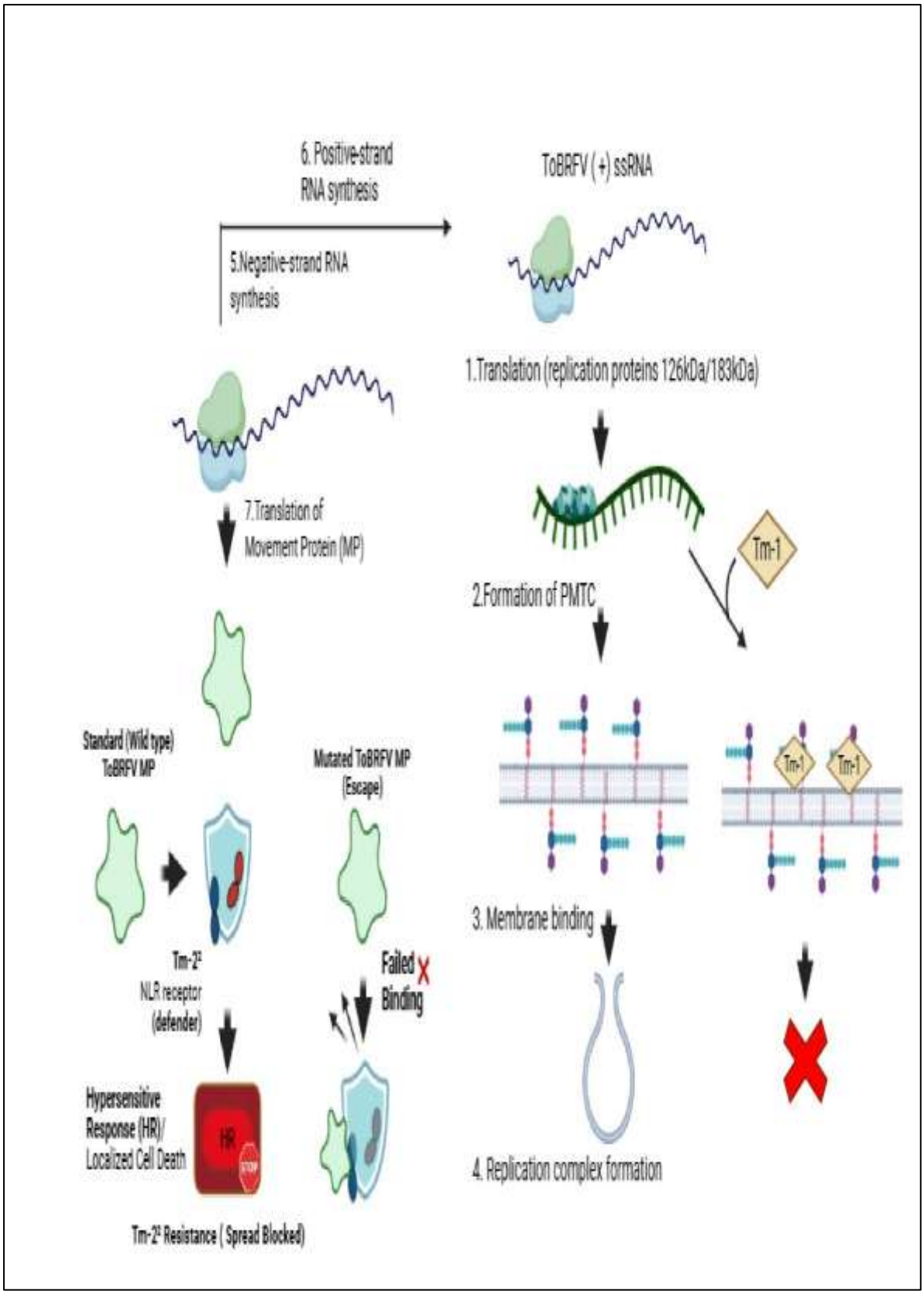

Fig 6: Molecular Mechanisms of ToBRFV Replication and Movement Protein-Mediated Breakdown of Tm-2² Resistance (Image Created in https://BioRender.com)

7. Deploying Next-Generation Diagnostics

Accurate identification of the causative pathogen constitutes a critical initial step in effective disease management, enabling the development of targeted control strategies. While visual assessment of symptoms and identification of associated vectors can assist in diagnosis, multiple viruses may induce similar symptoms, and many vectors are capable of transmitting more than one virus species rather than being pathogen specific. Compared with conventional protocols and commercial kits, a modified SDS-LiCl method yields superior RNA quality and

concentration, enabling reliable downstream RT–PCR detection for ToBRFV (Erdogan et al., 2025). The assay was designed using multiple primers targeting conserved regions of the viral genome, enabling amplification under isothermal conditions without the need for complex thermal cycling equipment that can detect as few as eight RNA molecules and produce visible results within approximately 30–35 minutes through simple colorimetric changes (Sarkes et al., 2020). With respect to diagnostics, traditional ELISA and RT-PCR/RT-qPCR are compared with newer isothermal assays such as LAMP and RT-RAA, which highlight their limitations in terms of their rapid ability to address the fast-moving global ToBRFV problem (Besati et al., 2025). A loop-mediated isothermal amplification (RT-LAMP) approach for detecting Tomato brown rugose fruit virus in commercial tomato seed samples addresses the need for rapid and field-deployable diagnostics (Bados et al., 2024). A loop-mediated isothermal amplification (LAMP) protocol focused on the movement of proteins. These advanced, multitiered detection frameworks provide vital technical infrastructure for early pathogen interception, rigorous seed certification, and the enforcement of international phytosanitary barriers (Bernabé-Orts et al., 2021). A SYBR Green-based RT–qPCR assay with novel primers (NARO2 set) that specifically target ToBRFV while distinguishing it from six other Solanaceae-infecting tobamoviruses through distinct melt curve peaks at 80.9–81.2°C was used (Ota et al., 2025). NARO2 primers targeting conserved ToBRFV regions with ≥5 mismatches to Japanese Solanaceae tobamoviruses (ToMV, TMV, PMMoV, etc.), enabling Ct-based (ToBRFV Ct~11 vs. others ~31) and Tm-based (81°C sharp peak vs. broader 77–78°C) discrimination, were designed (Ota et al., 2025). Digital PCR (ddPCR) results in spots of ~65–127 copies/µL in tomato seeds, which is specific for TMV (Vargas-Hernandez et al., 2022). Nested RT–PCR/ELISA confirmed the expansion of hosts (N. rustica, P. ixocarpa) (Zamora-Macorra et al., 2025). qPCR is the first method in which 77% of inflows are positive, which is better than many viruses (Sherchan et al., 2023). Quadruplex RT–PCR differentiates ToBRFV (604 bp) from TMV, ToMV and TSWV with a sensitivity of 0.02 pg (Yan et al., 2021b). CRISPR-Cas12a- and CRISPR-Cas9--based assays coupled to recombinase polymerase amplification and lateral flow strips achieve visually readable detection of ToBRFV coat protein sequences from infected tomato leaves, in which the CRISPR-Cas12a system has a broader dynamic range and stronger visual signals than Cas9 does; however, both assays discriminate ToBRFV from TMV, demonstrating high analytical specificity (Besati et al., 2025). The recombinant CP thus provides a robust, reproducible antigen platform for scalable serological tools to detect ToBRFV in infected tissues, supporting quarantine and monitoring its global spread (Núñez-Muñoz et al., 2026). New monoclonal antibodies, such as dot-ELISA (1:6400 dilution) and gold strips (1:100,000) specific to ToBRFV, do not cross-hit with TMV/ToMV, which helps with field-level detection (Zhao et al., 2024). However, ToBRFV-CP-IgG-QDs fluoresce at 50 ng/mL, which is 5x more sensitive than ELISA and is specific to Iranian isolates than ToMVs.
Field-deployable immunoassay (Rezaei et al., 2025). Characterized breakthrough curves for ToBRFV in saturated glass-bead columns revealed that ToBRFV's transport behavior correlates more strongly with human adenovirus than does Pepper mild mottle virus, suggesting that ToBRFV is a better environmental surrogate for enteric virus risk assessment (Cobbinah et al., 2025). Seasonal analysis indicated that the ToBRFV lacked significant seasonality and maintained high loads across spring, summer, autumn and winter, whereas the PMMoV exhibited more pronounced seasonal shifts, reinforcing the idea that the ToBRFV is a particularly stable and reliable indicator signal (Li et al., 2026). A multiplex PCR framework capable of simultaneously differentiating among Tomato brown rugose fruit virus, Tomato mosaic virus and Tobacco mosaic virus with absolute species specificity and these optimized primer sets provides plant protection services with a highly accurate, scalable tool for routine laboratory screening to intercept contaminated seed imports and monitor field outbreaks (Karimova et al., 2021). The AmplifyRP XRT assay, which targets the RNA-dependent RNA polymerase gene, achieved significantly higher sensitivity at the femtogram level and showed no cross-reactions, ensuring the precise identification of ToBRFV across diverse isolates (Eads et al., 2023). Whole-genome sequencing further confirmed its persistent presence and high relative abundance compared with other plant, enteric, and bacteriophage viruses, as illustrated in the metagenomic heatmaps, where ToBRFV dominated the viral populations across all the sampling periods (Verma et al., 2023). The inclusion of both endpoint and real-time PCR approaches enabled comparisons of diagnostic robustness under varying laboratory conditions, confirming that certain assays consistently produced accurate detection across diverse sample types and testing environments (Mavrodieva et al., 2025).

8. Integrated Management strategies

8.1 Recent Developments in the Management of virus

Prevention remains a fundamental principle in plant disease management, as the sustainability of agricultural systems is closely linked to the implementation of proper agronomic practices throughout crop production. Effective control of ToBRFV requires the integration of conventional approaches, such as quarantine measures and integrated disease management (IDM), with advanced biotechnological strategies. Atropine and rutin (150 µg/mL) inhibit ToBRFV-CP and RdRp 83% via qRT–PCR, fragmenting virions (TEM) (Salehzadeh et al., 2025). 15 Gy gamma irradiation reduces seed transmission 70%, inducing SAR via ROS signaling without germination loss. Optimal against 20 Gy phytotoxicity (Tokhmechi et al., 2026) Sulfur-rich garlic extracts that deactivate ToBRFV infectivity in bioassays are sources of natural antiviral agents that could be developed into botanical biopesticides for integrated and organic tomato production (Iobbi et al., 2023). Three percent sodium hypochlorite spray followed immediately by a six percent powdered milk solution successfully blocked the transfer of the pathogen to Nicotiana test plants, preventing both local lesion formation and systemic infection (Rodríguez-Díaz

et al., 2022). In vivo, double-stranded RNA was synthesized as a targeted antiviral strategy against ToBRFV, demonstrating its effectiveness in reducing viral infection in tomato plants, in which viral genome fragments were incorporated into a bacteriophage-based system to produce high-quality dsRNA molecules, which were then applied to plants alongside viral inoculation (Weiss et al., 2026).

By integrating physiological measurements with transcriptome and hormone profiling analyses, dufulin-induced resistance against Tomato brown rugose fruit virus, in which treated plants presented reduced viral accumulation alongside enhanced growth performance, increased chlorophyll content and elevated activities of key antioxidant enzymes, including superoxide dismutase, peroxidase, and catalase, indicates strengthened defense capacity (Wang et al., 2026). Antiviral compounds as a potential strategy to control Tomato brown rugose fruit virus by investigating the activity of autoxidized extracts from Combretum micranthum. Catechinic acid and related oxidation products lead to the loss of viral infectivity, accompanied by the absence of detectable viral RNA (Iobbi et al., 2022). The application of an alkaline chlorinated-trisodium phosphate solution (pH > 11.4) to contaminated soil successfully decreased soil-mediated viral transmission to susceptible root-truncated tomato seedlings(Molad et al., 2024). The topical application of 760 mg/L chlorine dioxide significantly curtails viral transmission and replication within susceptible tomato and Nicotiana longiflora plants without inducing phytotoxicity. The oxidizing properties of chlorine dioxide effectively inhibit viral replication and cellular invasion in vivo and provide a viable biochemical strategy to directly suppress host–virus pathogenesis and mitigate the aggressive spread of this pathogen in intensive agriculture (Gutiérrez et al., 2024). Exposing the seeds to a 6% sodium hypochlorite solution successfully eliminated the viral load, preventing transmission to subsequent plant generations (Obaid and Adhab, 2025).

8.2 Biotechnological and Genetic Approaches for Viral Control

From a resistance-breeding perspective, Tm-1 homozygotes can slow ToBRFV multiplication but are unlikely to be durable on their own because resistance-breaking mutants arise readily during prolonged infection (Kubota et al., 2024). Stronger alleles such as Tm-1^I91T or novel genes that more robustly block ToBRFV replication, ideally deployed in combination with other loci (e.g., chromosome-11 QTLs), are needed to build more sustainable resistance against this globally spreading virus (Kubota et al., 2024). The chromosome-11 QTL represents a valuable new source of resistance distinct from classical Tm loci, and the associated SNP markers can be deployed in marker-assisted selection to introgress ToBRFV resistance from S. pimpinellifolium into cultivated tomato backgrounds (Jaiswal et al., 2025). Five Solanum pennellii accessions (LA 0716, LA 0750, G1.1559, G1.1608, and G1.1610) show complete resistance and lack symptoms and viral coat proteins after inoculation (Zois et al., 2025). However, Tm-2²-N744D still triggered HR against MP^K132, and the double mutant Tm-2²-S723Y/N744D conferred broad resistance to both ToBRFV E132/K132 and TMV in stable tomato transgenics, highlighting artificial NLR evolution as a biotech tool for multistrain resistance (Wang et al., 2025a). Extensive screening of wild tomato relatives led to the discovery of the HREZ gene within a Solanum habrochaites accession, which encodes a coiled-coil nucleotide-binding site leucine-rich repeat (CC-NBS-LRR) immune receptor capable of conferring high resistance (Verweij et al., 2026). Advanced serological and molecular testing specifically highlighted four Solanum pimpinellifolium accessions, PI 390713, PI 390714, PI 390716 and PI 390717, as possessing true high-level resistance characterized by drastic suppression of systemic viral accumulation (Jaiswal et al., 2024).

Knocking out four homologs of the TOBAMOVIRUS MULTIPLICATION1 (TOM1) gene in tomato completely suppressed viral coat protein accumulation, indicating effective inhibition of virus multiplication (Ishikawa et al., 2022). Distinct loci on chromosomes 3 and 7 independently modulate shoot resistance and fruit susceptibility, depending on the specific parental background (Rochsar et al., 2025). The S. pennellii Tm-1 allele provides semidominant partial resistance by inhibiting viral replication, but full protection requires an additional recessive locus distinct from known QTLs on chromosomes 9 and 11 (Zois et al., 2025). These findings align with independent reports of ToBRFV resistance loci on chromosome 11 in S. lycopersicum, suggesting that stacking this QTL with other resistance genes, such as Tm-1 variants, could increase durability against an evolving global ToBRFV population (Jaiswal et al., 2025). dsRNA treatment also triggers mild innate immune responses, including callose deposition at plasmodesmata, which can restrict virus movement between cells (Weiss et al., 2026).

9. CONCLUSION

This ToBRFV pattern indicates that phytosanitary measures and regional management practices have partially limited virus spread, preventing widespread establishment across the entire production landscape. Mechanical transmission through contact further accelerates spread within production systems. Therefore, a reliable and highly sensitive quantification method provides a critical mechanism for early pathogen interception, epidemiological tracking and the enforcement of international phytosanitary barriers. Even with advanced breeding, relying entirely on genetics is risky; recent field data shows that just a single amino acid mutation in the ToBRFV movement protein is enough to overcome newly deployed resistant tomato cultivars. Therefore, to successfully delay the breakdown of newly introgressed resistance genes, these genetic strategies must be paired with strict phytosanitary hygiene to keep the baseline viral pressure low in the environment. Also CRISPR not only as a diagnostic tool but also as a future pillar of integrated breeding, engineering and surveillance programs aimed at developing ToBRFV-resilient crops, suggesting that combining CRISPR-based resistance and diagnostics could significantly mitigate the virus global threat status.

CRediT authorship contribution statement
G Rohit: Writing – original draft, Validation, Software, Investigation, Data curation, Conceptualization. **TKS Latha:** Review & editing, Validation, Supervision, Investigation, Data curation, Conceptualization. **Karthikeyan Muthusamy:** Review & editing, Validation, Supervision, Investigation. **Basavaprabhu L. Patil:** Review & editing, Data curation, Validation, Supervision, Conceptualization.**C Indu Rani:** Review & editing, Data curation, Formal analysis, Conceptualization.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements
The authors wish to thank Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India and Division of Basic sciences ICAR-IIHR Bengaluru and All India Network Project on Emerging pest and diseases (AINP) ICAR-IIHR Bengaluru, Karnataka, India for providing guidance and support.

Funding
Not applicable

Ethics declaration
Not applicable

Data availability
Data will be made available in this manuscript.

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