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Review

Breeding Tomato for Durable Resistance to ToBRFV: Genetic Resources, Resistance Architecture, and Deployment Strategies

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Abstract

Tomato brown rugose fruit virus (ToBRFV) has overcome durable *Tm-2*^{2}-mediated extreme resistance and emerged as a globally prevalent pandemic pathogen, severely limiting tomato production. This breeding-oriented review critically summarises recent advances in ToBRFV resistance research. ToBRFV evades host defence via mutations in the movement protein and the replicase helicase, thereby defeating *Tm-2*^{2}-mediated extreme resistance and, at least under experimental challenge, overcoming *Tm-1*, respectively. In the best-characterised sources, reduced-escape resistance rests on an oligogenic epistatic architecture, with the chromosome 2 *Tm-1* locus acting as a key hub paired with a donor-dependent secondary recessive locus, typically on chromosome 11, whilst also acting as a distinct locus in some wild donors. Resistant wild and cultivated germplasm are categorised and ranked, with strict differentiation between genuine resistance and viral tolerance. Critical introgression bottlenecks, including linkage drag and associated fruit quality penalties, are emphasised. Comparative evaluation shows that rational gene stacking is essential for durable reduced-escape resistance (field validation pending). Inconsistent phenotyping standards across studies compromise result comparability, thereby necessitating a unified evaluation framework. Priority research directions include cloning the chromosome 11 resistance gene, implementing comparative omics profiling, and establishing standardised resistance assessment criteria to advance the development of durable ToBRFV-resistant tomato varieties.

Keywords: disease resistance breeding; durable resistance; gene pyramiding; marker-assisted selection; movement protein; *Tm-1*; *Tm-2*^{2}; *Tobamovirus*; tomato brown rugose fruit virus

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1. Introduction

Tomato is among the most economically valuable vegetable crops worldwide [1], its tobamovirus resistance having long been sustained by the deployed *Tm-2*^{2} gene. The newly emerged tomato brown rugose fruit virus (ToBRFV) broke this half-century-stable extreme resistance and, first detected in 2014–2016 [2,3], ToBRFV has since spread to more than 50 countries in the most recent surveys [4], up from 37 countries across four continents documented by 2023 [5]; by 2024, it had affected 31 EPPO countries, compared with 9 in 2020 [6], causing severe yield and commercial losses and prompting stringent international quarantine measures [7–9] (Figure 1).

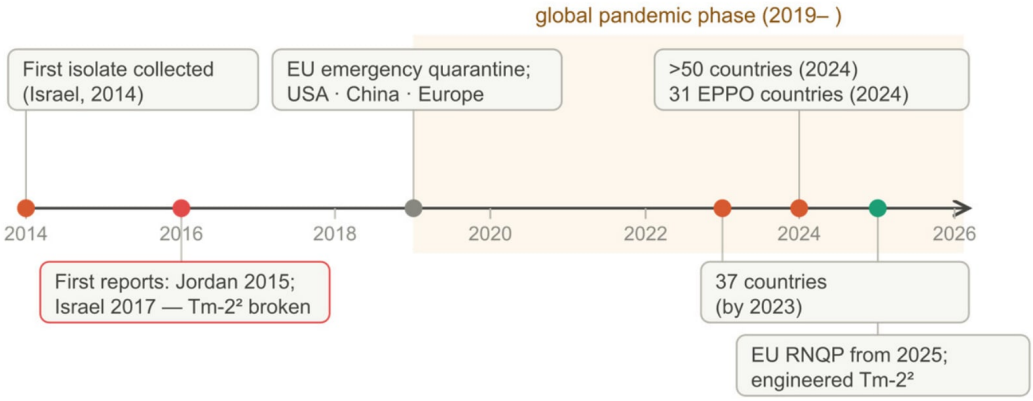


Figure 1. Global emergence and spread of ToBRFV, 2014–2026. First collected in Israel (2014) and reported in Jordan (2015) and Israel (2017) [2,3], with the Israeli isolate being the one shown to overcome *Tm-2*^{2}-mediated extreme resistance, it has since rapidly spread to the USA, China, and Europe, prompting EU emergency quarantine (2019)—more than 50 countries are affected worldwide [4], with 37 documented by 2023 [5] and 31 EPPO countries affected by 2024 compared to 9 in 2020 [6]. By 2025, the EU had reclassified it as a regulated non-quarantine pest, and the first peer-reviewed engineered *Tm-2*^{2} resistance was demonstrated [9].

ToBRFV's stable virions and multiple transmission routes circumvent conventional phytosanitary controls: it spreads via seed contamination, mechanical contact, insects (pollinators and chewing pests), contaminated water, and weed reservoirs, including seed-borne *Solanum nigrum* [10–16]. No antiviral agents are available for field use, and cross-protection with attenuated strains, sometimes proposed as an interim measure, is discussed in Section 7.6. Conventional sanitation and quarantine are therefore insufficient—the EPPO pest risk analysis has concluded that eradication is not always possible and that new resistant varieties could minimise its impacts [6]—making durable genetic resistance the primary and most reliable strategy for long-term management in commercial tomato production.

Existing tobamovirus reviews have focused on viral diagnostics, molecular immunity, and engineered resistance and have largely neglected germplasm evaluation and resistance deployment in tomato breeding [17–22]. This breeding-focused review fills that gap, integrating resistance-breaking mechanisms with practical tomato breeding, detailing the resistance/tolerance distinction, the two-locus epistatic architecture and its deployment trade-offs, the available germplasm, standardised phenotyping, the key bottlenecks, and a phased breeding roadmap.

To frame the evidence underpinning this review, we briefly describe the literature sources and boundaries of the present review. The literature was identified from the main bibliographic databases (PubMed, Web of Science, Scopus, and Google Scholar), from patent databases (Espacenet, Google Patents), and by backward and forward citation screening, combining terms for the virus, resistance genes, and breeding strategies discussed in Sections 2–10. Peer-reviewed primary studies of ToBRFV-resistant or tolerant phenotypes, resistance genetics, or deployment experience in tomato, pepper, and *Nicotiana* were included; conference abstracts and non-peer-reviewed reports were excluded. The searches were not run as a single dated systematic query, and no fixed cut-off date is claimed; the literature was revisited as the manuscript was revised. This is a critical narrative synthesis, not a systematic review: no record-level screening, risk-of-bias assessment or meta-analysis was performed, and the ordinal scores discussed in Sections 4.5 and 7.7 are expert gradings rather than quantitative syntheses.

2. The Pathogen Through a Breeder's Lens

2.1. Biology and Transmission

ToBRFV is a canonical tobamovirus (genus *Tobamovirus*, family *Virgaviridae*), possessing a ~6.4 kb positive-sense RNA genome encoding two replication proteins (126/183 kDa), a 30 kDa movement protein (MP), and a 17.5 kDa coat protein (CP) [17,23]. Two viral proteins carry the review's argument: MP is recognised by *Tm-2*^{2}, and its variation defeats recognition by that gene; the replicase is inhibited by *Tm-1*, and its variation overcomes *Tm-1* (Section 3; [24]). The replicase p50 fragment is also recognised by the tobacco *N* gene (the helicase domain is the classical *N* determinant in TMV; [25]) for which no resistance-breaking ToBRFV variant has been reported to our knowledge; this resistance is nevertheless thermolabile and collapses at 30 °C [26] (Section 7.4), so the absence of a reported break is a statement about the record, not about durability.

Its transmission biology explains the failure of conventional sanitation strategies. Seed transmission is predominantly external: the virus localises to the seed coat and, occasionally, the endosperm but not the embryo itself, and although it reaches the pollen, transmission to the progeny through pollination has not been demonstrated [11,12,27,28]. Progeny can still be infected from contaminated seeds at a low cultivar-dependent rate [29]. Seedling infection is therefore largely preventable by disinfection, but a seed lot carrying the virus on the seed coat remains an introduction pathway even when embryo transmission is negligible. Virions persist for ≥6 months on some glasshouse surfaces [13] and ≥184 days in soil (undetectable by 205–385 days), with an alkaline chlorinated-trisodium-phosphate treatment speeding up remediation [30]. Such prolonged soil persistence establishes functional soil-borne inoculum pressure, underpinning the rootstock barrier of Section 7.2. The virus also spreads mechanically and via insect vectors: Bombus terrestris carries primary inoculum during pollination [10]; the tomato leaf miner Tuta absoluta transmits it [15]; and the Hadda beetle Henosepilachna vigintioctopunctata stays transmissible for 48 h, transmitting to pepper, eggplant, black nightshade, potato, and tomato [16]. Root-released virions circulate in greenhouse drain water and infect when recycled for irrigation [14]; seed-transmitted *Solanum nigrum* weeds also aid persistence [12]; and EPPO records natural dispersal with pollinators and birds [6]. Whole-genome tracing of outbreaks confirms that seeds, fruits, and leaf inoculum each contribute to introduction and to local spread [31].

2.2. Evolutionary Dynamics and Durability

For a virus capable of breaking half-century-durable host resistance, ToBRFV is remarkably uniform. Genome-wide analyses show low diversity with strong purifying selection (genome-wide dN/dS ≈ 0.16; reported ≈ 0.088 for CP and 0.21 for MP, the MP under weaker negative selection), little evidence of recombination, three major clades in gene-region phylogenies, and an expansion phase [23,32,33]. Globally, ToBRFV isolates share >99% nucleotide identity, consistent with a short evolutionary history [23,33,34]. For breeding, one point follows: ToBRFV broke *Tm-2*^{2} not by rampant diversification but through a few MP substitutions in the emerging lineage (Section 3). It sits among host-shifting tobamovirus lineages, its emergence probably a host shift rather than in situ diversification [35], so durability is limited by a narrow resistance surface, not by viral hypervariability.

3. The Molecular Basis of Tobamovirus Resistance and How ToBRFV Escapes It

3.1. The Three-Gene Toolbox and Why ToBRFV Matters

For more than half a century, tomato breeding relied on three introgressed tobamovirus resistance genes: the semi-dominant *Tm-1* (chromosome 2) and the allelic *Tm-2*/*Tm-2*^{2}

(chromosome 9). *Tm-2*^{2} was effective against ToMV and related tobamoviruses for decades [17,18]. ToBRFV overcame the resistance conferred by all three genes: *Tm-2*^{2} and *Tm-2* through the MP, and *Tm-1* through the replicase ([3,24]). For *Tm-2*, the only direct evidence is a side-by-side challenge in which *Tm-2* homozygous lines and the control both reached near-maximal severity (disease severity index (DSI) 3.0 versus 2.8 ± 0.1, 0–3 scale) [36], so *Tm-2* confers no useful resistance on its own. Elucidating each gene's mode of action and the viral escape mechanisms underpins the breeding strategies below.

3.2. *Tm-1*: Interference with Viral Replication

Tm-1 acts biochemically rather than as an immune receptor: it binds the viral replication proteins and blocks assembly of the membrane-bound replication complex, inhibiting RNA replication [37]. In the ToMV pathosystem, LT1 resistance-breaking helicase-domain mutants abolish *Tm-1* binding, and a short, positively selected *Tm-1* region reflects antagonistic coevolution with the virus [38]; the allelic series supports this, differing by only a few residues in this region and exhibiting nested antiviral spectra. A single substitution in the positively selected region (I91T) extends its inhibitory activity to LT1-type escape mutants without losing activity against wild-type ToMV, while ToMV mutants that overcome *Tm-1* are less competitive than their parents in its absence [38]. Durability therefore remains dynamic, governed by the virus's capacity to remodel protein-interaction surfaces. On the plant side, Zois et al. pinpointed nine amino acids distinguishing ToBRFV-resistant from susceptible *Tm-1* [39], all within the N-terminal replicase-binding region (the first 201 aa suffice to inhibit replication in vitro): eight within aa 78–112 under positive selection against ToMV (positions 78, 79, 80, 85, 87, 89, 94 and 100), the ninth at position 57, plus a unique residue at 459. The family continues to yield variation: *Rug-1*, a paralogue of *Tm-1*, carries distinct residues at the virus-binding side and confers ToBRFV tolerance/resistance in an allele dosage-dependent manner and is claimed in a patent [40]. *Tm-1* is thus an inhibitory binding interface, not a receptor; its structural counterpart is the movement-protein determinant governing escape from the *Tm-2*^{2} receptor (Sections 3.3 and 3.4).

For ToBRFV specifically, that surface is a demonstrated moving target. Kubota et al. isolated spontaneous ToBRFV mutants that efficiently infect *Tm-1*-homozygous tomato, mapping the escape to substitutions in the helicase domain of the replicase (recurrently Y976H, with G983E, D1097N, or Y1104F) [24]. A minority (~7%; 16 of 229 ToBRFV sequences available in NCBI, including Tom2-Jo) already carry the H984Y substitution, and Kubota et al. found that *Tm-1* restricted systemic infection through 50 days post-inoculation (dpi) [24]; escape mutants were recovered only at ~100 dpi. *Tm-1*-mediated resistance is therefore breakable in principle, as *Tm-2*^{2}-mediated resistance is (Section 3.3), but at a far lower field frequency: escape mutants carried multiple helicase substitutions, consistent with a requirement for more than one change; only a minority of field isolates carry the pre-adapted change. This constrains the achievable durability of the two-locus resistance architecture in Section 5 and designates the replicase helicase domain (Y976H; H984Y) as a monitoring target for *Tm-1*-based deployments (Sections 8 and 9).

3.3. *Tm-2*^{2}: Movement Protein Recognition at the Plasma Membrane

Tm-2^2 is a coiled-coil–NB–LRR receptor that recognises the viral MP of tobamoviruses and triggers a hypersensitive response amounting to extreme resistance. It functions at the plasma membrane, forming a complex with the TMV movement protein [41]. Notably, *Tm-2*^{2}-mediated recognition depends on a host factor: silencing the Rubisco small subunit (RbCS) compromises *Tm-2*^{2}-mediated resistance to ToMV in *N. benthamiana*, allowing local viral proliferation while blocking systemic spread [42]. Because RbCS is an essential photosynthetic protein, knockout is not feasible in the field; nevertheless, this dependency

establishes *Tm-2²* resistance as a host factor-dependent, movement-restricting pathway (reviewed by [43]).

3.4. How ToBRFV Escapes Tm-2²: The Movement Protein Determinant

The determinant of *Tm-2²* evasion maps cleanly to the ToBRFV MP. Comparative genomics flagged the candidates first: Maayan et al. identified twelve non-conserved residues in the MP [35], and nine in the replicase, as potential resistance-breaking sites. Experimental work narrowed the MP determinant: using TMV chimeras, Yan et al. showed that transferring only ToBRFV MP residues 60–186—a central region with six critical residues (H67, N125, K129, A134, I147, I168)—suffices to let the chimera infect *Tm-2²* plants [44] (Figure 2), and Hak and Spiegelman placed the resistance-breaking elements within MP residues 1–216, showing the same MP attenuates cell-to-cell transport, so evasion carries a fitness cost of strategic value for durability [45]. Spiegelman and Dinesh-Kumar propose that *Tm-2²* recognition depends on a conserved MP residue, cysteine 68 (C68), present in all Solanaceae-infecting tobamoviruses except ToBRFV [19,46]. In TMV and ToMV, replacing C68 abolished cell-to-cell movement, so a substitution that removed this determinant would also have had to restore movement [46]. ToBRFV carries a histidine at the corresponding position (H67) and still moves [46,47]: after a host shift [35] its MP diverged from those of TMV and ToMV at multiple positions, so this residue is no longer required for its movement. In ToBRFV, H67 is not a movement defect but a recognition switch: replacing it with TMV cysteine restores *Tm-2²* activation [46], explaining the unique ability of ToBRFV to overcome decades-long *Tm-2²* resistance.

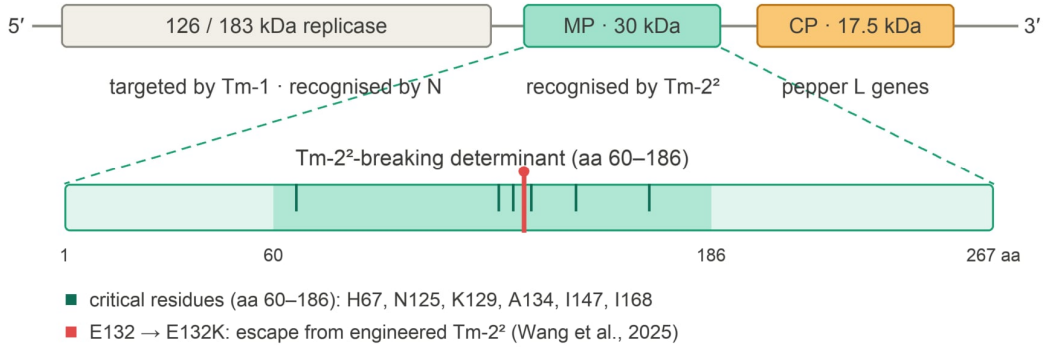


Figure 2. ToBRFV genome organisation and the movement protein determinant of *Tm-2²* breaking. The ~6.4 kb genome encodes the 126/183-kDa replicase (targeted by *Tm-1*; recognised by *N*), the 30-kDa movement protein (MP; recognised by *Tm-2²*) and the 17.5-kDa coat protein (recognised by pepper *L* genes). Dashed lines connect the MP coding region to the enlarged MP panel below. The MP zoom shows the central *Tm-2²*-breaking determinant (aa 60–186), six critical residues (H67, N125, K129, A134, I147, I168 [44]), and the E132 position at which E132K escapes engineered *Tm-2²* [16]; these residues attenuate MP–*Tm-2²* affinity so that receptor self-association and downstream immune activation are avoided [47]. The genome map and domain boundaries are schematic; only the amino acid ruler under the MP is drawn to scale.

Ma et al. resolve the mechanism at the receptor [47]: the ToBRFV MP lowers MP–*Tm-2²* affinity so that the receptor fails to self-associate and activate. They also map each residue's movement contribution: H67C and N125A delay, while K129Q and A134N enhance, cell-to-cell movement in wild-type *N. benthamiana*, and I168N delays systemic movement; in *Tm-2²*-transgenic plants, only I147M and I168N retain residual spread, the other four being arrested. ToBRFV therefore escapes not by hiding a recognised motif but by attenuating MP–*Tm-2²* affinity. Recognition is not abolished, however: the MP still triggers a *Tm-2²*-dependent hypersensitive response, merely an attenuated one [48]. Residue-level saturation

mutagenesis at MP H67 recovered only aromatic residues in *Tm-2*^{2} hosts (phenylalanine and tryptophan tolerated, synonymous codons behaving differently) [49], so the mutants' arrest reflects the activated resistance, not any intrinsic loss of MP function. Evasion therefore requires an aromatic side chain plus a permissive local RNA sequence, not merely the absence of cysteine; because the imidazole ring is aromatic, re-engineering *Tm-2*^{2} to recognise this surface has been proposed [49]. A134T also causes severe shoestring/fern symptoms even in *Tm-2*^{2} plants by rewiring auxin and MAPK signalling [50,51].

3.5. The Pepper Parallel Confirms the Fragility

The same fragility is visible in pepper. The allelic L1–L4 CC-NB-LRR genes recognise tobamovirus coat protein, but L-carrying pepper challenged with ToBRFV is not immune: the virus is not excluded, systemic infection is detected, and the plants are best described as tolerant [52]. Two qualifications matter. Transient systemic infection under artificial inoculation does not establish sustained susceptibility when only symptoms are scored, and the demonstration that pepper mild mottle virus overcomes L4 [21,53,54] shows that L4 is breakable in principle, not that ToBRFV has broken it—no ToBRFV variant selected on L4 has been reported. The parallel therefore supports a weaker but still decisive claim: single-receptor resistance to one conserved protein is fragile, and in pepper, it currently delivers tolerance rather than the extreme resistance that *Tm-2*^{2} once provided.

3.6. From Breakdown to Design: Re-Engineering Recognition

If the vulnerability is a narrow recognition surface, the solution is to expand this interface, an outcome recently demonstrated. Patent literature first claimed engineered *Tm-2*^{2} substitutions restoring recognition of ToBRFV and related tobamoviruses—at N822, S825 or C848 (optionally with F655L), a single engineered protein covering ToBRFV, ToMV and TMV [55]. The general strategy, random mutagenesis, and selection of an *R* gene to recover recognition of an escape variant (with the potato Rx/PVX system as precedent) were set out for tobamoviruses [55], proposed in silico for the *Tm-2*^{2}/ToBRFV pair [56], and flagged as a route to made-to-order NLRs [19]. Building on *Tm-2*^{2}'s MP-recognition mechanism [41], Wang et al. subjected the *Tm-2*^{2} LRR domain to in vitro random mutagenesis (error-prone PCR), screened ~8000 variants, and recovered gain-of-function receptors that recognise the ToBRFV MP while retaining TMV-MP recognition [9]. Two single-residue variants, *Tm-2*^{2}-S723Y and *Tm-2*^{2}-N744D, triggered specific hypersensitive response (HR) against ToBRFV MP. Transgenic tomato lines expressing S723Y resisted a natural ToBRFV isolate (E132); under sustained pressure, the virus acquired a single MP E132K substitution that escaped S723Y, but N744D, engaging a different interface, still recognised the K132 escape variant. The pyramided double mutant *Tm-2*^{2}-S723Y/N744D conferred broad-spectrum resistance to ToBRFV E132, ToBRFV K132 and TMV in stable transgenic tomato (escape tested under controlled conditions; field durability not yet established). This is, to our knowledge, the first peer-reviewed demonstration that artificial NLR evolution can generate broad-spectrum, reduced-escape tobamovirus resistance, although the variants are patented [55] and the evidence rests on controlled challenge, not field deployment.

4. Sources of Resistance and Tolerance

Since ToBRFV overcomes all deployed cultivated resistance, the search for new sources has turned to the wild relatives of tomato—a reservoir of resistance shaped by virus–host coevolution within the crop's centres of diversity [57]—and, more recently, to genes from heterologous species. This section catalogues available resistance sources under a consistent criterion: a "resistant" label is only as good as the assay behind it (Section 8). Many reported sources are tolerant (symptomless but infected) rather than truly resistant (symptomless

and low-titre), a distinction that determines whether a source should enter a breeding programme (Section 5.4).

4.1. The Germplasm Surveyed

An inventory has been collated by Zhang et al., who also add the distant *S. ochranthum* as a high-resistance source [17]. The underlying screening and mapping studies include large *Solanum* panels spanning sections Lycopersicon and Juglandifolia [58]; wild species and interspecific hybrids screened for resistance versus tolerance [59] (covering 44 materials including 28 accessions of eight wild species); resistant lines selected within *S. habrochaites* and *S. peruvianum* [60]; resistance screened and mapped within *S. pimpinellifolium* [61] and *S. pennellii*-derived material [39]; a genome-wide association study of 161 red-fruited Varitome accessions [4] (Section 5.3); and a screen of 52 wild and 45 cultivated accessions classifying *S. pennellii* 'LA0716' and 'LA5240' and *S. chilense* 'LA1938' as resistant (ELISA < 3× negative reference; [62]) and fine-mapping the chromosome 2 *Tm-1* factor to 89 kb in *S. pennellii*-derived lines [63].

4.2. Sources Ranked by Crossability

Close relatives (readily introgressed): *S. pimpinellifolium* is the most breeder-friendly source. It crosses freely with cultivated tomato and carries the mapped chromosome 11 resistance QTL [64], with asymptomatic accessions in broader screens. For a programme starting today, it is the highest-value donor, offering strong resistance with minimal linkage-drag penalty (Section 4.5).

Intermediate relatives (introgression feasible with effort): *S. pennellii* and *S. habrochaites* carry the key determinants of the Section 5 architecture. A resistant *Tm-1* allele (in both species; [39,65]), the chromosome 11 locus (from *S. habrochaites*-derived/cultivated material [36]), the chromosome 8 NBS-LRR (from *S. habrochaites* LYC4943 [66]), and, in *S. pennellii*, a distinct still-unmapped second recessive locus partnering *Tm-1* [39]. These require bridging crosses and careful linkage-drag management but are well within reach.

Distant relatives (strong but hard to use). *S. ochranthum* accessions (LA2160, LA2162, LA2166) are reported as highly resistant [17,58], but this species sits in section Juglandifolia and is very difficult to cross with cultivated tomato; it is best regarded as a long-term or gene isolation target rather than a direct donor.

4.3. The Tolerance Trap Made Concrete

The germplasm literature reports "tolerant" accessions across *S. lycopersicum var. cerasiforme*, *S. pimpinellifolium*, *S. habrochaites* and *S. chilense* [58], with further tolerant material in *S. pennellii* and cultivated backgrounds [36,59]. Crucially, such plants retain the capacity for viral transmission (Sections 5.4 and 8). The paired lines of Zinger et al. make the stakes explicit: VC532 is tolerant (chromosome 11 locus alone) [36], whereas VC554 is resistant (chromosome 11 and chromosome 2 together). A breeder who selects on symptoms therefore enriches for tolerance rather than resistance (Section 8.2).

4.4. Genes from Beyond Cultivated Tomato

Two routes add resistance genes not present in the tomato gene pool. The *Sm/SmRR* gene (incomplete-dominant against Stemphylium) is associated with lower ToBRFV symptom severity, but only from variety-level comparisons lacking near-isogenic-line or functional validation, so it remains a minor, preliminary trait rather than a robust solution [67]. More substantially, the tobacco *N* gene (*Nicotiana glutinosa*), a TIR-NLR recognising the tobamovirus replicase, confers resistance when expressed transgenically in tomato but fails by 30 °C [26] (Section 7.4). *N* and the separate *N′* gene—from *N. sylvestris*, a CNL that recog-

nises the coat protein rather than the replicase [19]—have so far been characterised only in *Nicotiana* [25]. These heterologous genes broaden the toolkit but carry transgenic-regulatory and, for *N*, temperature constraints.

4.5. An Inventory with Breeding Value

Table 1 consolidates these sources and ranks them by breeding value, applying the three criteria defined in the caption.

Table 1. Reported ToBRFV resistance/tolerance sources, ranked by breeding value (1–5). Three components are weighed holistically: (i) resistance strength—evidence that the accession limits virus accumulation, not just symptoms; (ii) crossability—freely crossable, bridging crosses, or effectively a gene isolation target; (iii) marker availability—locus-linked or gene-based. Anchors: 5 = titre-confirmed strong resistance, freely crossable red-fruited donor, mapped marker; 4 = strong resistance with a bridging cross or an unresolved locus; 3 = two limitations (a bridging cross and an unresolved locus), or a temperature or regulatory constraint; 2 = tolerance, or resistance unvalidated beyond symptom scoring in a hard-to-cross donor; 1 = tolerance only, unvalidated. Because source studies use non-comparable protocols (Section 8), resistance strength is a qualitative grading, not a meta-analysis, and assumes titre re-confirmation; crossability and markers do not depend on the contested severity data, so the recommendations of Section 10 build on them.

Source	Species (Crossability)	Phenotype	Gene/Locus	Breeding Value	Refs.
S. pimpinellifolium R accessions	close (easy)	R	chr11 QTL (2 cand. R genes: TNL + LRR)	5	[64]
S. pennellii Tm-1 material	intermediate	R	*Tm-1* (chr2) + 2nd recessive locus (≠chr11, unmapped)	4	[39]
S. habrochaites (Tm-1 donor)	intermediate	R	resistant *Tm-1* allele; chr8 NBS-LRR	4	[65,66]
VC554 (two-locus line)	cultivated background	R	chr2 × chr11	4	[36]
S. peruvianum selections	intermediate	R/Tol	undetermined	3	[60]
Transgenic N-gene tomato	n/a (transgenic)	R (<30 °C)	N (tobacco TIR-NLR)	3	[26]
S. ochranthum LA2160/LA2162/LA2166	distant (very hard)	R	undetermined	2	[17,58]
Sm/SmRR lines	cultivated	Tol	*Sm*	2	[67]
VC532 & other "tolerant" accessions	various	Tol	chr11 alone (partial)	1–2	[36]

Abbreviations: R, Resistant; R/Tol, Resistant to tolerant; Tol, Tolerant.

5. Genetic Architecture of ToBRFV Resistance

ToBRFV resistance genetics has converged on a coherent picture: in well-characterised materials, it comes not from a single dominant gene but from an epistatic interaction between the chromosome 2 locus *Tm-1* and a second recessive locus. In the cultivated material in which it was first mapped, this second locus is a recessive tolerance locus on chromosome 11 [36,65]; in the wild donor *S. pennellii*, by contrast, it is a different, still-unmapped locus that is not linked to chromosome 11 or 9 [39]. Two caveats frame what follows: the second-locus identity is donor-dependent rather than fixed, and the chromosome 11 signals of different groups may not be a single gene—so the true locus count behind "two-locus resistance" is probably higher than two.

5.1. A Two-Locus, Epistatic Architecture

The two-locus idea was first disclosed in the patent literature, where ToBRFV resistance in a cultivated line was attributed to *Tm-1* combined with a recessively inherited locus on chromosome 6, 9, and/or 11 ([68], the chromosome 6 and 9 QTLs were associated with fruit tolerance and the chromosome 11 QTL with foliar tolerance). Zinger et al. showed

that *Tm-1* alone does essentially nothing: over-expressing *Tm-1* conferred resistance and low titre only in a tolerant background (VC532), whereas in a susceptible background (cv. Moneymaker), it merely delayed symptoms without reducing virus [65]. Their mapping work had already shown that full resistance in line VC554 required the chromosome 11 locus together with a chromosome 2 (*Tm-1*-region) contribution, not either alone [36].

An independent donor reproduces and sharpens the two-locus logic. Zois et al. showed that the *S. pennellii Tm-1* allele is the major resistance factor: the resistant allele was homozygous in every fully resistant F2 plant, and VIGS of *Tm-1* abolished resistance [39]. Complete resistance nonetheless required a second locus: among the ~60 F2 plants homozygous for the *S. pennellii Tm-1* allele, 15 were resistant and 45 susceptible (1R:3S), the ratio expected with one additional recessive locus segregating and the other fixed. Across the whole F2, full resistance segregated 1R:15S, a robust fit to a digenic recessive model ($\chi^2 = 0.06$, $p = 0.81$ for F2 LA0716; $\chi^2 = 0.01$, $p = 0.92$ for F2 LA0750). The second locus was not chr11 or chr9, whose markers did not co-segregate with resistance. The generalisable model is therefore *Tm-1* (chr2) as an obligatory hub plus a second recessive locus whose identity varies with the donor—chromosome 11 in the *S. habrochaites*/cultivated system of Zinger, a novel unmapped locus in *S. pennellii*—rather than a fixed chr2 × chr11 pair.

Two details sharpen the model. First, the resistant line VC554 carries two tandem *Tm-1* copies (~27 kb apart), the first matching the resistant *Tm-1* allele of line GCR237 [65]; resistance depends on specific allelic identity and expression of *Tm-1*. Second, *Tm-1* is a replication inhibitor rather than an immune receptor (Section 3.2), whereas the second locus is expected to add recognition only in some donors: the distal chromosome 11 resistance QTL of the *S. pimpinellifolium* donor contains two annotation-based disease resistance gene analogs, a TIR-NBS-LRR and an LRR protein, consistent with a recognition arm [64], while the proximal chromosome 11 locus of the VC554/VC532 system has a different, unvalidated candidate, the CCA-adding enzyme gene *SlCCA1*, not an NLR (Sections 5.2 and 9). Where the pairing holds, the architecture combines replication interference with recognition, a two-layered resistance whose combined effect exceeds either mechanism alone.

5.2. Convergence on Chromosome 11

Two independent studies, using different germplasm and methods, both implicate chromosome 11—though probably not the same part. Zinger et al. mapped a single recessive tolerance locus proximally (~8.9 Mb; CAPS BstNI_8.89, explaining 91% of the tolerance variation in a VC532-derived F2) [36], with *SlCCA1* (Solyc11g018770) and *SlCCA2* as candidates [69]. Jaiswal et al. identified a chromosome 11 resistance QTL via a genome-wide association study (GWAS) in *S. pimpinellifolium* 'USVL332' (PI 390717) [64], its top SNPs delimiting a 22 kb interval (46.83–46.85 Mb, LOD ~13) with TIR-NBS-LRR (Solyc11g062150) and LRR (Solyc11g062180) candidates in the wider 327 kb region. At ~38 Mb apart, these are very unlikely to be the same gene, so the architecture is at least three loci rather than two. A third position comes from GWAS: severity-associated SNPs at 0.43 and 0.75 Mb, ~8.1–8.5 Mb proximal to the Zinger locus and ~46 Mb proximal to the Jaiswal QTL [4], too far to be described as aligning with the earlier mapping. The three signals (~0.43–0.75, ~8.9 and ~46.84 Mb) should therefore be carried forward as three candidates, and resolving them determines how many segments a breeder must pyramid (Sections 5.6 and 9.1). The chr2 partner is also well delimited, with the *Tm-1* region fine-mapped to an 89 kb, six-gene interval in *S. pennellii* LA5240-derived lines [63]; together with the 22 kb Jaiswal chr11 QTL [64], these two intervals are tightly bounded, although the other two chr11 candidates are not.

5.3. Additional and Tissue-Specific Loci

Beyond the chromosome 2 × 11 core, wild germplasm contributes further loci. A dominant NBS-LRR from *S. habrochaites* (LYC4943) on chromosome 8 was already broken in a released cultivar by a single MP substitution [66,70], underscoring how quickly a single dominant locus can fall (Section 9.4). The *S. pennellii* second locus, by contrast, remains unmapped: QTL-seq of contrasting bulks and a genome-wide Indel screen returned no linked segment [39], so this real, recessive, resistance-essential locus is defined by its segregation rather than its position—a concrete fine-mapping opportunity (Section 9.1). More consequential for phenotyping is tissue-specific behaviour: in *S. pennellii*-derived material, a strong fruit susceptibility QTL on chromosome 7 is separable from the shoot resistance signal near *Tm-1* on chromosome 2, and a locus on chromosome 3 protects shoots only in the TOP, not LEA, background [63]. A source scored resistant on foliage may therefore still support virus and symptoms in fruit—a direct threat to leaf-only screening (Section 8). Genome-wide association work reinforces the polygenic picture: screening 161 red-fruited Varitome accessions (~8.3 million SNPs plus 2.7 million INDELs; 27,000 structural variants returned no associations) recovered 14 QTLs (6 INDELs, 8 SNPs) and 5 symptom-free entries; these were three panel accessions (BGV006370, BGV007366, BGV012615) plus the green-fruited checks *S. pennellii* LA0716 and *S. habrochaites* LA1777, which corroborate the donor species of Section 4 [4]. The QTLs map to resolved positions on chromosomes 1, 2, 3, 6, 7, 10, 11 and 12, but phenotyping was severity-based with RT-qPCR on only a subset of lines, and every symptom-free line proved virus-positive (Ct 6.7–20.2), so alignment with the chr2/8/11 architecture stays provisional. Reported loci thus span several chromosomes (2, 3, 7, 8, 11), not a single cluster (Table 2; Figure 3).

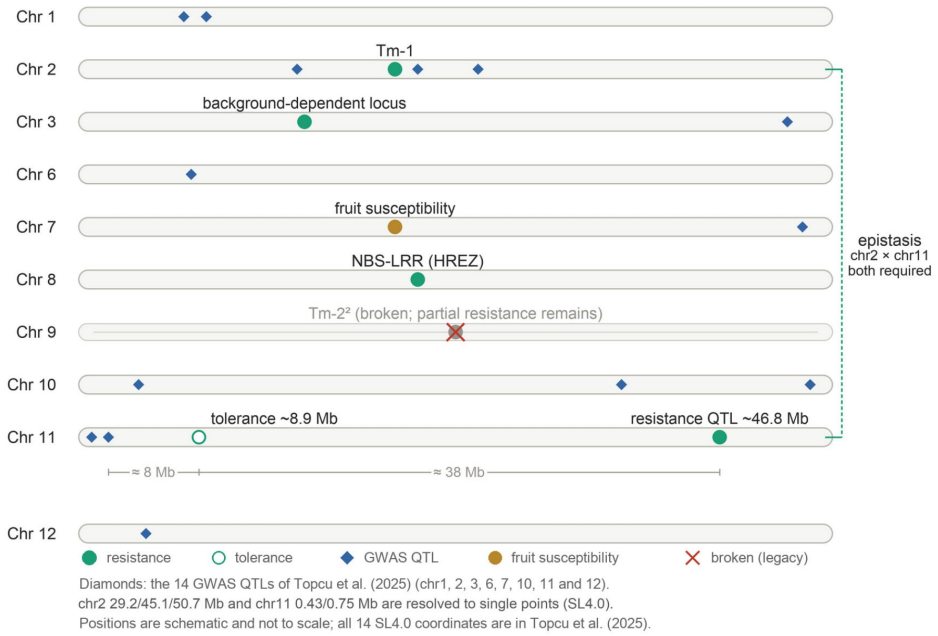


Figure 3. Genomic distribution of ToBRFV resistance and susceptibility loci in tomato. Filled circles denote resistance loci—*Tm-1* (chr2), an NBS-LRR (chr8), and the distal chr11 resistance QTL; the hollow circle is the recessive chr11 tolerance locus; diamonds mark the 14 GWAS QTLs of [4] (chr1, 2, 3, 6, 7, 10, 11 and 12), with the five listed in Table 2 given by SL4.0 coordinates (chr2 29.2/45.1/50.7 Mb; chr11 0.43/0.75 Mb). The three chr11 candidates (~0.43–0.75, ~8.9, and ~46.84 Mb) lie ~8 and ~38 Mb apart (Section 5.2). Amber marks the chr7 fruit susceptibility QTL; chr3 carries a background-dependent resistance locus [63]. The legacy *Tm-2*^{2} (chr9, marked by a red cross) no longer confers extreme resistance, though partial resistance remains (Section 7.1). The bracket marks the epistatic requirement for chr2 (*Tm-1*) and chr11 [36]. Positions are schematic; exact coordinates are given in Table 2.

Table 2. Mapped ToBRFV resistance and susceptibility. Rows move from resistance loci through the recessive tolerance locus to background-dependent and fruit-level effects; the last row summarises the GWAS panel of [4]. Loci are listed as reported in the source studies and are plotted in Figure 3. Abbreviations: BIL, backcross inbred line; CAPS, cleaved amplified polymorphic sequence; GWAS, genome-wide association study; INDEL, insertion/deletion; LD, linkage disequilibrium; LOD, logarithm of odds; QTL, quantitative trait locus; SL4.0, tomato reference assembly SL4.0; SNP, single-nucleotide polymorphism; SPET, single primer enrichment technology; TOP and LEA are the two cultivated tomato inbred backgrounds used by Rochsar et al. [63].

Locus/QTL	Chr	Interval/Peak	Marker(s)	Candidate	Population/Donor	Refs.
Resistance QTL	11	22 kb SNP peak (in 327 kb QTL; ~46.84 Mb)	SL4.0ch11 SNPs (LOD ~13)	Solyc11g062150 (TIR-NBS-LRR); Solyc11g062180 (LRR)	*S. pimpinellifolium* 'USVL332' (PI 390717; GWAS)	[64]
Tolerance locus (recessive)	11	proximal, ~8.9 Mb	CAPS BstNI_8.89 (91% of tolerance variation)	*SlCCA1*/*SlCCA2* (CCA-adding enzymes), proposed	VC532	[36,69]
Two-locus resistance	2 × 11	*Tm-1* region × chr11	BstNI_8.89 + *Tm-1*	*Tm-1* (chr2)	VC554	[36,68]
Tm-1 (major factor) + 2nd recessive locus	2 + unmapped	*Tm-1* homozygous; 2nd locus 1:15 digenic	*Tm-1* CAPS	*Tm-1* (pennellii allele); 2nd locus ≠ chr11/chr9	*S. pennellii* LA0716/LA0750	[39]
NBS-LRR (dominant, already broken)	8	—	—	dominant NBS-LRR	*S. habrochaites* LYC4943	[66,70]
Tm-1 fine-map	2	89 kb (6 genes, incl. *Tm-1*)	SNP (*Tm-1*)	*Tm-1* (Solyc02g062560)	*S. pennellii* LA5240 recombinant BILs (LEA background)	[63]
Resistance locus (background-dependent)	3	—	SPET	not identified	*S. pennellii* LA5240 (TOP bg only)	[63]
Fruit susceptibility QTL	7	—	—	—	*S. pennellii*	[63]
14 QTLs (GWAS)	1, 2, 3, 6, 7, 10, 11, 12	chr2 29.2/45.1/50.7 Mb; chr11 0.43/0.75 Mb (SL4.0)	6 INDEL + 8 SNP	proposed in LD blocks (e.g., Solyc02g091460)	Varitome panel (161 accessions)	[4]

5.4. Tolerance Versus Resistance Is a Genetics-of-Phenotyping Problem

The two-locus architecture is inseparable from a definitional issue that determines whether a mapped locus is useful. Zinger et al. operationalised the distinction with virus titre: a tolerant plant is symptomless but infected and therefore still an inoculum source, whereas a resistant plant is symptomless and carries very low or undetectable virus [36]. In their materials, the chromosome 11 locus alone confers tolerance; resistance emerges only in the two-locus combination. For mapping, this means that scoring symptoms without titre can map a "tolerance QTL" that a breeder would wrongly treat as resistance. The genetic architecture and the phenotyping standard (Section 8) are two sides of the same problem.

Resistance, tolerance, and the commercial HR/IR classes. The distinction is not only academic: the vegetable seed industry classifies varieties under ISF conventions as high resistant (HR) or intermediate resistant (IR), both defined by symptom expression under a specified inoculum pressure rather than by virus titre [71]. HR restricts pathogen growth under normal pressure with no or very limited symptoms; IR restricts it but may express symptoms under heavy pressure. A symptomless, virus-carrying genotype therefore satis-

fies the HR criterion while being tolerant in the sense used here, and it remains an inoculum source; conversely, a low-titre genotype with mild symptoms may score IR although its accumulation is agronomically negligible. Commercial "resistant rootstock" and "HR" claims should therefore be read as symptom-based statements that do not exclude silent infection, and the minimum standard of Section 8.5 should be met before an HR or IR claim for ToBRFV is accepted for registration.

5.5. Markers and the MAS Readiness Gap

The materials for marker-assisted selection exist but are not yet consolidated. The deployable markers are the chromosome 11 CAPS BstNI_8.89 [36] and the chromosome 11 GWAS SNPs of Jaiswal et al. [64], with a *Tm-1*/chromosome 2 marker completing the stack. The two chromosome 11 sets are not interchangeable: they tag loci ~38 Mb apart (Section 5.2), their functional equivalence is untested, and neither has been shown to interact with *Tm-1* in a common background [64], so a programme must decide which locus it is selecting for, or track both. Three gaps remain: the chromosome 11 gene is not cloned, so markers are locus-linked rather than gene-based; no validated two-locus KASP panel with breeder-friendly diagnostic power has been published; and tissue-specific effects (chr7) mean a complete panel should also flag fruit susceptibility risk.

5.6. The Introgression Bottleneck: Linkage Drag and the Fruit Quality Trade-Off

Sections 4 and 5 show that ToBRFV resistance can be sourced from wild *Solanum* and pyramided into a chromosome 2 × 11 architecture. For a molecular geneticist, that closes the problem; for a breeder, it opens the harder one: linkage drag, i.e., introgressing wild segments into elite backgrounds without a collapse in fruit quality and yield. This problem is largely omitted from the resistance-mapping literature. This section applies those lessons to the ToBRFV loci.

5.6.1. Wild Segments Are a Mixed Bag

The paradigm for dissecting wild tomato introgressions is the *S. pennellii* introgression-line (IL) population [72]: any wild segment carries a bundle of linked effects, mostly neutral or deleterious. Brix9-2-5 shows the exceptions—an *S. pennellii* allele increasing fruit sugar, fine-mapped to a single amino-acid change in the invertase Lin5 [73]—but capturing such benefits while shedding linked drag demands gene-level resolution that resistance introgressions rarely reach: until a locus is cloned, the breeder moves a block of unknown effects that penalise soluble solids, flavour, fruit size, and firmness in green-fruited donors.

5.6.2. The Recombination–Suppression Trap

Compounding this, many wild segments cannot be precisely truncated by recombination: the Ty-1 precedent required nearly 12,000 plants for ~70 kb resolution, of which 2000 yielded only 13 recombinants [74]. Where a locus sits in such a coldspot, marker-assisted clean-up is effectively impossible, and the drag travels with the resistance into every derived cultivar. The legacy *Tm-2*^{2} chromosome is a case in point: a recombination coldspot on chromosome 9 links resistance- and ripening-associated genes [75], and here, *Tm-2* and QTL9 markers on opposite arms co-segregated in 55 of 60 plants [39], so any resistance sourced or engineered there inherits the problem.

5.6.3. Mapping the Risk onto the ToBRFV Loci

The risk is distributed unevenly across the two-locus architecture of Section 5. The distal chromosome 11 QTL [64], from *S. pimpinellifolium*—a red-fruited, freely crossable relative—is the favourable case: introgressions from this species are associated with far

smaller quality penalties than those from green-fruited donors [72], and the QTL is tightly delimited (22 kb bracketed by the top SNPs within a 327 kb interval of only 17 genes, 2 of them disease resistance candidates), making it the locus most likely deployable without a Brix or flavour cost.

The chromosome 2 (*Tm-1*) and chromosome 8 loci are higher-risk: both the resistant *Tm-1* allele (traced via GCR237 [65]; *S. pennellii* carries an independent allele, Section 4.2) and the chromosome 8 NBS-LRR come from *S. habrochaites*, a green-fruited donor whose introgressions reduce fruit size and soluble solids and add green-note volatiles [72]—a penalty documented for *S. habrochaites* generally rather than for these loci and compounded by plausible recombination suppression at *Tm-1*.

Pyramiding is where the risk concentrates, but not in a form that can yet be quantified. Stacking two or three wild segments could impose penalties additively, but the outcome depends on the donor, the size and position of each segment, and the genetic background; where segments affect the same quality traits, the effect could equally be sub-additive. No study has measured this for ToBRFV loci (Section 5.6.5), so an additive penalty is a plausible risk, not an established outcome. Quality must be measured in pyramid lines rather than inferred from the number of segments.

5.6.4. The Escape Routes

The same body of literature also suggests the following solutions.

Segments can be resolved to genes: high-resolution recombination narrows a wild segment to a few hundred base pairs (484 bp for Brix9-2-5 [73,76]), and cloning the gene yields "perfect" markers that eliminate drag; cloning the chromosome 11 gene (Sections 5.5 and 9.1) is therefore both a marker quality and a drag elimination goal.

Screening for recombinants should be carried out aggressively, expecting cold spots: where recombination is suppressed (the Ty-1 scenario), large populations and, if needed, alternative donors are required, and the recurrent parent genome should be recovered by marker-assisted background selection.

Advanced lines should be phenotyped for quality, not only resistance: Brix, key flavour volatiles, fruit size, firmness, and yield should be measured alongside resistance screening (Section 8), since a titre-negative plant with unmarketable fruit is not a breeding success. With resistance being oligogenic (Section 5) but quality polygenic, marker-assisted selection should be combined for the chr2 × chr11 loci with genomic selection across the background; marker-assisted selection alone would require prohibitive population sizes, and genomic selection's joint accuracy on resistance and flavour remains unestablished (Section 9).

5.6.5. A ToBRFV-Specific Data Gap

No study has yet quantified the resistance-versus-fruit-quality trade-off for any ToBRFV locus. The argument above is transferred from the Ty, Mi and *S. pennellii*-IL experience; validating it for ToBRFV—Brix, flavour and yield in chromosome 2/8/11 introgression lines across environments—is an urgent need and a high-impact opportunity (Section 9).

6. Integrative Omics Analysis: Conserved Molecular Signatures and Core Research Gaps

6.1. Transcriptomic Insights into ToBRFV Infection Responses

Transcriptomic studies of tomato and pepper infected with ToBRFV (522 genes changed in susceptible tomato, cv. Moneymaker [77]; 1468 in pepper [78]) converge on a conserved programme: induction of receptor-like kinases (RLKs/LRR-RLKs)—the most robust signature across both hosts—alongside WRKY, MYB and bHLH transcription factors and the MAPK cascade [77,78]. Bulk-tissue designs are now complemented at cellular resolution:

single-cell RNA sequencing of two cultivars differing at *Tm-2*^{2} showed that ToBRFV accumulates to different levels in the two backgrounds, that entry and movement are not fully blocked early in the resistant cultivar but accumulation is restricted in specific cell types, and that brassinosteroid signalling acts in a cultivar-dependent direction, suppressing infection in one background and promoting it in the other [79]. Mechanistically, a tobamovirus RNA element (EL1, a 45-nt enhancer-like element of the coat-protein subgenomic promoter) and the replicase helicase domain suppress the host thioredoxin NbTRXh1, facilitating viral coat-protein subgenomic RNA synthesis; the equivalent EL1 motifs of other tobamoviruses, ToBRFV included, also bind NbTRXh1 [80]. The severe A134T isolate rewires auxin and MAPK signalling in *Tm-2*^{2}-carrying tomato, tightly associating symptom development with that rewiring [51].

6.2. Limitations of Conserved Transcriptomic Signatures in Resistance Inference

Interpretation of these datasets is limited by their reliance on susceptible material: differentially expressed genes in such hosts reflect the physiology of successful infection, not the determinants of resistance, so the upregulation of WRKYs, RLKs and other core signalling genes marks infection progression rather than resistance. Molecular contrasts between resistant and susceptible material remain scarce: an ionomics-led study linked iron/nickel accumulation to iron homeostasis and ABA signalling [81], and a single-cell study compared two cultivars differing at *Tm-2*^{2} [79], but the first is preliminary and the second was not built around isogenic lines; neither yields a defence transcriptome, so the resistant-versus-susceptible contrast remains the open experiment (Section 6.3).

6.3. Critical Knowledge Gaps in Current ToBRFV Omics Research

These limitations define the omics gap, though it is narrowing at its edges: integrated multi-omics—transcriptome, proteome and metabolome—has now been applied to ToBRFV-infected susceptible tomato [82], and cell-type-resolved transcriptomics has contrasted two *Tm-2*^{2} backgrounds [79]. What remains absent is a study designed around resistance: a titre-confirmed, defence-focused comparison between validated resistant lines and near-isogenic susceptible counterparts, and small-RNA profiling of that contrast (a deficiency flagged by [18]). The distinction is not semantic: infection response studies describe what the virus does to a susceptible plant, resistance studies what a resistant plant does to the virus, and only the second identifies breeding targets. Candidate genes from existing transcriptomes (Section 6.1) are therefore putative markers, not validated determinants.

7. Breeding and Deployment Strategies Against ToBRFV

No current ToBRFV mitigation integrates durable resistance, deployability, marker compatibility, and regulatory and operational freedom, so breeding programmes should combine multiple strategies hierarchically rather than pursue a single optimum. Figure 4 positions the approaches on two axes (escape resistance (mechanistic) and breeding readiness (deployability)), and the sections below evaluate each strategy and score it for breeding and deployment value (Table 3).



Figure 4. Strategy landscape for ToBRFV resistance and its deployment, positioned by escape resistance (mechanistic) (x-axis) and breeding readiness (deployability) (y-axis). The chromosome 8 HREZ locus is deployed commercially but is thermosensitive above 30 °C (Section 9.4); the *Tm-1* gene contributes partial resistance and is used mainly through rootstocks, so both occupy the ready zone; *TOM1* S-gene knockout is gene-edited rather than transgenic, and engineered *Tm-2*^{2} is transgenic; both are escape-resistant; chr11 QTL, the *N* gene (transgenic, temperature-sensitive), and dsRNA spray are lower-readiness options; *Tm-2*^{2} is broken as extreme resistance but retains partial resistance (Section 7.1). Arrows on the axes indicate increasing escape resistance (x-axis, rightward) and breeding readiness (y-axis, upward). Stars indicate evidence strength on a 1–5 scale (1 = weakest, 5 = strongest). Dashed lines divide the landscape into high/low escape resistance and breeding-readiness quadrants. Both axes are qualitative orderings, not measured quantities: position records the authors' ranking from the evidence in Sections 3–7, and no unit or interval scale is implied. Non-genetic options (RNAi topical) are shown for completeness as deployment bridges, not as breeding strategies.

Table 3. Scoring of ToBRFV resistance and deployment strategies.

Strategy	Escape Resistance †	Deploy Now	MAS-Compatible	Regulatory Ease	FTO Clarity	Note
MAS pyramid chr2 × chr11	3	3	3	5	4	non-GM; needs chr11 gene cloned
Tm-1 rootstock	1	2	4	5	4	soil-route only, not foliar
S-gene edit *TOM1*	5	2	2	3 ‡	4	durable, broad; regulation bifurcated
Transgenic *N gene*	3	2	2	2	4	fails at 30 °C
Engineered *Tm-2*^{2} stack	5	2	2	2	1	broad-spectrum; field durability untested; transgenic; core receptor patents held by few [9,55]

Table 3. *Cont.*

Strategy	Escape Resistance †	Deploy Now	MAS-Compatible	Regulatory Ease	FTO Clarity	Note
Topical dsRNA/RNAi	2	3	n/a	3	3	needs reapplication; complements resistance
chr8 HREZ	1	5	3	5	2	single MP change breaks it; thermosensitive

† Escape resistance score rates mechanistic escape probability under controlled challenge, not field-proven durability. Scoring anchors (1/3/5): Escape resistance—1 = broken by a single substitution; 3 = escaped only by a multi-residue change; 5 = mechanism-orthogonal (S-gene) or a multi-interface stack. Deploy now—1 = research-only; 3 = pilot, works with hurdles; 5 = commercially available today. MAS-compatible—1 = requires transformation; 3 = locus-linked marker only; 5 = clean gene-based marker. Regulatory ease—1 = transgenic; 3 = gene-edited (jurisdiction-dependent); 5 = conventional/non-GM. FTO clarity—1 = core patents held by few; 3 = licensing or workarounds exist; 5 = public-domain. Scores of 4 apply where the target is unpatented or public (the uncloned chromosome 11 QTL, the *Tm-1* allele, the *TOM1* knockouts, the tobacco *N* gene) but a component of the construct may fall under third-party claims; MAS-compatibility is "n/a" for topical dsRNA, which involves no marker-assisted selection. ‡ Regulatory ease for gene-edited (SDN-1) options is highly jurisdiction-dependent—effectively 5 in SDN-1-exempt countries (US, Japan, Brazil) but 1–2 where gene-edited crops are still regulated as transgenic pending NGT-type legislation; a middle value of 3 reflects this split (see Section 7.3). Disclaimer: because the underlying studies use methodologically non-comparable evaluation protocols (Section 8), this table is a qualitative grading based on best current evidence, not a quantitative meta-analysis; it guides strategic resource allocation rather than ranking studies. Scores are the authors' assessment and will shift as loci are cloned and regulations evolve.

7.1. Marker-Assisted Introgression and Pyramiding

The near-term non-transgenic route is marker-assisted selection (MAS) for the two-locus architecture described in Section 5: *Tm-1* on chromosome 2 plus a second locus (chromosome 11 in Zinger and Jaiswal; a distinct, unmapped locus in Zois's *S. pennellii*) [36,39]. Three points are decisive. First, because resistance is oligogenic and epistatic, MAS must track the marker stack (a chromosome 11 marker set plus a *Tm-1*/chromosome 2 marker), not either locus alone: selecting on *Tm-1* alone yields tolerance at best. Which chromosome 11 set to use is a donor decision, not a convenience: BstNI_8.89 tags the proximal (~8.9 Mb) tolerance locus of the *S. habrochaites*/cultivated system [36], the Jaiswal SNPs the distal *S. pimpinellifolium* QTL (~46.84 Mb) [64]. They lie ~38 Mb apart, their equivalence is untested, and combining them as one target is not justified (Section 5.5). Second, the donors differ widely in linkage drag and cross-compatibility (*S. pimpinellifolium*, *S. pennellii*, *S. habrochaites*; the strongly resistant but hard-to-cross *S. ochranthum*), so donor choice is itself a breeding value decision (Table 1, Section 4). Third, the stack need not stop at two loci: *Tm-2*^{2} retains partial resistance—its MP is still recognised, triggering an attenuated hypersensitive response, and over-expression further reduces viral accumulation [48]—so it is a dose-sensitive pyramid component rather than a spent gene. The chromosome 8 HREZ locus is a separate case: deployed at scale, but thermosensitive and broken by a single MP substitution, so it belongs in a stack for the same reason (Section 9.4).

7.2. Resistant Rootstocks: A Route-Specific Bridge

Grafting is standard practice, but rootstocks bred specifically to block soil-borne ToBRFV are new; this represents a research development, as the leading commercial rootstocks tested were susceptible. The resistance sits in the rootstock while the scion's genome is unchanged—a route-specific control measure, not genetic improvement of the variety. Controlled grafts showed this route dependency: susceptible rootstocks let root-inoculated virus reach a resistant scion, and resistant rootstocks limited soil-mediated infection of a susceptible scion, but neither prevented foliar infection. In the field, the resistant scion/re-

sistant rootstock combination lowered infection to 16.4% (N = 213) versus 92.5–94.7% for susceptible scions, and fruit symptom severity fell from 41.9% to 16.6% [62]. The resistant material consisted of tomato lines carrying the *S. pennellii*-derived *Tm-1* interval (89 kb on chromosome 2), so this was not a test of the cloned gene alone (Section 5), and the authors suggest that part of the effect may be a graft-transmissible defence signal. Rootstocks are thus a mitigation bridge lowering soil-borne inoculum pressure while durable scion resistance is bred—not a standalone solution—so commercial "resistant rootstock" claims should be read accordingly.

7.3. Susceptibility-Gene Editing: Durable but Regulated

Knocking out host susceptibility factors offers durability that recognition-based resistance cannot. CRISPR/Cas9 multiplex knockout of four of the five tomato *TOM1* homologs (*SlTOM1a–d*; the fifth, *SlTOM1e*, is essentially transcriptionally silent) abolished ToBRFV accumulation without any growth or yield penalty; the resistance was durable (viruses adapted to triple mutants failed on the quadruple mutant) and extended to ToMV, TMV, and YoMV [83]. Knockout of *SlTOM1* and *SlTOM3* also confers differential resistance to tobamovirus [84]. Because a biotrophic virus depends on host factors, removing an essential one is harder to escape than mutating a recognition surface; van Damme et al. catalogue candidate factors and favour loss-of-function alleles that abolish virus binding while retaining protein function (minimising pleiotropy) [20]. The *eIF4E* precedent—knocking out one *S*-gene raised susceptibility to a second virus recruiting a paralog—is a reminder that *S*-gene resistance is not automatically broad or cost-free [20]. The remaining trade-off is regulatory and sharply bifurcated: a transgene-free multiplex knockout is an SDN-1 edit, treated as conventional and deployable in SDN-1-exempt jurisdictions (Table 3) but tightly restricted elsewhere: in the EU, SDN-1 plants stay regulated as GMOs pending the New Genomic Techniques framework, so deployment there is a matter of legislative timing, not breeding capacity. Two caveats temper this: multiplex editing should be screened for off-target changes, and the greenhouse "no-yield penalty" must be confirmed in the field, where removing several host factors may carry agronomic costs. Where transgene-free editing is permitted, *S*-gene editing remains the most durable genetic option demonstrated to date.

7.4. Transgenic Recognition Genes: The N Gene and Its Temperature Ceiling

Introducing a heterologous recognition gene is feasible but carries an environmental caveat that matters in tomato's warm production settings: transgenic tomato expressing the *Nicotiana glutinosa N* gene resisted *ToBRFV* through HR in ~90% of plants, but resistance collapsed at 30 °C [26]. This thermosensitivity disqualifies *N* for heated or hot-climate greenhouses unless the receptor is re-engineered for thermostability and serves as a general warning: NLR-based resistance must be phenotyped at production-relevant temperatures (Section 8).

7.5. Engineered Resistance Genes: Broad-Spectrum and Reduced-Escape Resistance

Section 3.6 described the key advance: Wang et al. evolved the *Tm-2*^{2} LRR domain and recovered a double mutant (*Tm-2*^{2}-S723Y/N744D) conferring broad-spectrum, reduced-escape resistance to ToBRFV (E132 and K132) and TMV in stable transgenic tomato [9]. A second, receptor-side route exists at the same target: because ToBRFV requires an aromatic side chain at MP position 67 to evade *Tm-2*^{2}—already supplied by the imidazole ring of its histidine—*Tm-2*^{2} could be re-engineered to recognise this surface [49]. Three points matter for deployment: it is transgenic, gated by the same regulatory bifurcation as *S*-gene editing (Section 7.3); escape was tested only under controlled challenge, so field durability remains

to be shown; and its distinctive breeding value is a re-engineerable, stackable scaffold with low escape probability.

7.6. Non-Genetic Adjuncts

Several approaches complement, rather than replace, host resistance. Topically applied double-stranded RNA/RNAi protects tomato without transformation, as established first against other tomato viruses (ToMV, PVY, and ToSRV; [85]) and now demonstrated for ToBRFV [86], and nanocarrier (BioCNT) leaf-spray delivery is extending its reach [87]. The limitations are environmental RNA instability, formulation and timely application needs, and the evolving regulatory status for RNA biopesticides. Cross-protection with attenuated strains has been proposed as an interim measure, but two objections apply: such strains would retain the ability to overcome the deployed resistance genes, and for a quarantine pest, the release of even a mild variant is prohibited in jurisdictions such as the EU [21]. A suspected unauthorised use of a cross-protection product has also been linked to the emergence of a new ToBRFV lineage in the Netherlands [21,88].

7.7. A Strategy-Scoring Framework

Because the options above involve trade-offs along different axes, we score each option on five breeder-relevant criteria (1 = poor, 5 = excellent): escape resistance, readiness to deploy today, MAS compatibility, regulatory ease, and freedom-to-operate (FTO; several resistance sources are patented, e.g., Lindbo [55] and the *Tm-1* paralog *Rug-1* [40]). Scores were assigned independently by the first and corresponding authors from the evidence in Sections 3–6, with disagreements resolved by discussion; the full rubric, with a justification for each cell, is Supplementary Table S1. This is a decision aid, not a ranking, and will shift as loci are cloned and regulations change.

7.8. A Context-Dependent Decision Framework

The scoring of Section 7.7 is deliberately inconclusive: no strategy wins on every axis, and reweighting flips the ranking; doubling regulatory ease favours the non-transgenic options, while weighting escape resistance heavily favours the *S*-gene edit and engineered stacks. The best strategy is therefore a property of the production context, not of the strategy, so rather than collapsing it into a single ranking, we map choice onto three parameters: regulatory regime, production system and climate, and disease pressure and market value (Table 4). The first commercial deployments already test this logic: the chromosome 8 HREZ locus [66] reached 67 varieties (93% of one company's portfolio) by 2025 [89], yet remains thermosensitive and breakable by a single MP change [70]—exactly the solo-deployment failure mode that stacking is designed to avoid. This conditional mapping, not the static scores, is the intended decision tool.

Three rules generalise across the matrix. First, climate gates the genetics: NLR/HR recognition is frequently thermosensitive [26,90,91], so recognition-based routes (*Tm*-2^2, *N*) should be verified at ≥30–32 °C for warm production and the temperature-independent *S*-gene route up-weighted. Second, regulation gates the toolkit more than biology does: the same *TOM1* edit is near-term in an SDN-1-exempt jurisdiction and effectively unavailable in a strict one, so the first question is not which resistance, but which resistances the target market permits. Third, cost scales with the toolkit: MAS and rootstocks draw on existing breeding infrastructure, whereas editing and transgenics require transformation platforms and regulatory dossiers, a capability barrier as decisive as the science and the reason the matrix privileges non-GM routes wherever they suffice.

Table 4. Context-dependent strategy recommendations. Cells give a primary strategy and a bridge/backup; the framework is a reasoning aid to be localised to a programme's constraints, not a prescription.

Production Context	Regulatory Regime	Primary Strategy	Bridge/Backup	Decisive Caveat
Open-field, temperate	GM-restricted (EU-type)	MAS-pyramid chr2 × chr11 (non-GM)	*Tm-1* rootstock	quality drag; titre-confirm; no edit/transgenic route available
Protected, warm/hot	GM-restricted	MAS-pyramid + rootstock	dsRNA (where RNA biopesticides are permitted)	verify resistance at ≥30–32 °C
Protected, high-tech	GM-permissive (SDN-1-exempt: US/JP/BR)	*S*-gene edit *TOM1* (the most durable demonstrated)	MAS-pyramid backup	editing + transformation cost; confirm agronomy across environments
High disease pressure; transgenics accepted	GM-permissive	Engineered/pyramided *Tm-2*^{2} + *S*-gene stack	MAS-pyramid	highest cost; residue-level MP monitoring (aa 60–186; E132)
Any hot climate	any	Temperature-independent *S*-gene (if permitted); else MAS-pyramid	rootstock	avoid unverified thermosensitive NLR/*N*-gene routes

8. Evaluating Resistance: A Standardisation Crisis

A resistance source is only as trustworthy as the assay that identified it. For ToBRFV, assays differ so widely that "resistant" does not transfer between studies: the same accession can be scored resistant, tolerant, or intermediate depending on inoculation and detection methods, scoring scale, temperature, and tissue. This section documents that heterogeneity (Section 8.4), shows why five confounders make symptom-only screening unreliable, and proposes a minimum standard. Claims should therefore be weighed on an evidentiary ladder—symptom scoring, titre-confirmed phenotyping, and mapped genetics—and matched to the breeding decision they support.

8.1. Three Incompatible Scoring Scales

Across the key mapping and functional studies, disease is scored on mutually non-convertible scales. Jaiswal et al. used a 0–5 class scale (0 = asymptomatic; 5 = severe mottling, leaf deformation, and string-like leaves), defining resistance as class 0 with low or undetectable titre [64]. Zois et al. and Zinger et al. both used a 0–3 DSI, but with different category definitions, so a "2" in one study is not a "2" in the other [36,39]. Ishikawa et al. recorded only qualitative symptom presence/absence and coat-protein accumulation [83]. Because scales cannot be inter-converted, meta-analysis of resistance levels is impossible without returning to raw data; it is solvable only by convention.

8.2. Symptoms Are Not Enough: Titre and the Tolerance/Resistance Boundary

Symptoms alone cannot distinguish tolerance from resistance (Section 5.4) and can create silent inoculum reservoirs—tobamovirus infections are frequently latent, symptomless yet fully transmissible [28]. Titre confirmation by ELISA or RT-qPCR is thus a definitional requirement, and studies that report it (e.g., [26,36]) are more reliable than those that do not.

8.3. Five Confounders That Break Symptom-Only Screening

Of the five confounders, the first is mixed infection; high-throughput sequencing routinely shows that field tomatoes carry complex viromes [92], and in a survey of retail

tomatoes, ToBRFV co-occurred with pepino mosaic virus in 73% of samples [93], an interaction that can be synergistic in an isolate-dependent way. Field or natural-infection screening without virus-specific RT-qPCR can therefore attribute to ToBRFV a phenotype partly caused by a co-infecting virus.

Temperature is the second. Both major resistance mechanisms are thermosensitive. NLR/HR-based resistance fails when warm: transgenic *N*-gene tomato resisted ToBRFV at 22 °C but not at 30 °C [26]. Independently, *Tm-1*-type replication inhibition is temperature-dependent: in heterozygotes, it inhibited TMV multiplication by over 95% at 20 °C but only 20% at 33 °C, and the resistance-breaking strain 1 was inhibited at 25 °C but not at 33 °C, although symptom suppression against strain 0 persisted to 35 °C [94], so a *Tm-1*-based source scored only in cool conditions may not remain resistant in production. Because commercial tomatoes are often grown in warm temperatures, temperature must be reported and, ideally, resistance verified at production-relevant temperatures.

Tissue difference is the third. Shoot resistance and fruit susceptibility are genetically separable, with a fruit susceptibility QTL on chromosome 7 that is independent of the chromosome 2 shoot resistance signal [63]. Leaf-only screening can allow a source that remains susceptible in the marketable organ.

Inoculation route is the fourth. Root- and foliar-mediated infection routes give different outcomes: resistant rootstocks blocked the soil route but not foliar infection [62], so the inoculation route must match the deployment question being asked.

Virus isolate is the fifth. Because *Tm-1* resistance depends on a physical *Tm-1*–replicase interaction (Section 3.2), a single replicase substitution in the challenge isolate can flip the score. This is not hypothetical: *S. pennellii* LA0716 was called susceptible, tolerant], and highly resistant —yet Topcu et al.'s own RT-qPCR shows LA0716 symptomless but virus-carrying, supporting the tolerant reading [4,39,58,59]. The most parsimonious explanation is the isolate: the culture that scored LA0716 susceptible (Tom2-Jo; MZ323110) carries the *Tm-1*-breaking replicase H984Y substitution, as do fifteen other field isolates [24]. The nomenclature invites confusion: Tom2-Jo (MZ323110) is not Tom1-Jo (KT383474, the isolate used by [83]), and its H984Y assignment rests on the sequence analysis of Kubota et al. [24], not on the database record alone. Studies naming no isolate cannot be interpreted in these terms; Table 5 records one such entry, an unnamed Israeli culture (Section 8.4). Resistance claims are therefore interpretable only against a named isolate. Reporting is otherwise uneven—most US studies share CA18-01 (MT002973), Turkish studies share TBRFV-Ant-Tom (MT107885), and Dutch works share ToBRFV-NVWA (MN882011)—but accessions are absent from two entries, one of which is an unnamed Israeli isolate (Table 5).

Table 5. Heterogeneity of ToBRFV resistance evaluation protocols across key studies. Isolate designations and GenBank accessions follow the sources; the accession printed as KXG619418 corresponds to KX619418 [36], and the name Tom2-Jo for the Jordanian isolate MZ323110 is the designation used by Kubota et al. [24] for the culture of Jewehan et al. [58]. Rows for [58,59,61] are included because Section 8.3 relies on them. The one entry that names no culture or accession is the unnamed Israeli culture (row [62]), so its phenotype cannot be tied to an isolate genotype. Abbreviations: DSI, disease severity index; dpi, days post-inoculation; wpi, weeks post-inoculation.

Refs.	Isolate	Inoculation	Detection	Scale	Temperature	Timepoint
[64]	CA18-01 (US; MT002973)	mechanical, carborundum	ELISA + symptoms	class 0–5	not stated	—
[39]	ToBRFV-NVWA (NL; MN882011)	carborundum 500-mesh + finger rub; 0.03 M Na-K phosphate pH 7.7, 1:40–50	immunostrip + stain-free gel CP	DSI 0–3	20/18 °C	3–4 wpi

Table 5. *Cont.*

Refs.	Isolate	Inoculation	Detection	Scale	Temperature	Timepoint
[36]	TBRFV-IL (Israel; KX619418)	mechanical, 0.01 M phosphate pH 7.0	ELISA + DSI (titre-based)	DSI 0–3 (different definition)	18/25 °C	30 dpi
[83]	Tom1-Jo (Jordan; KT383474)	rub, cotyledons	immunoblot CP (7 & 21 dpi)	qualitative presence/absence	not stated	7 & 21 dpi
[26]	CA18-01 (US; MT002973)	rub, carborundum; phosphate-saline pH 7.0, 1:100	DAS-ELISA + RT-qPCR	symptoms + titre	22 °C and 30 °C	4–21 dpi
[62]	unnamed Israeli culture	root-dip and foliar	ELISA + Western blot	DSI + infection %	greenhouse (not specified)	30 dpi
[4]	ToBRFV-Ant-Tom (Türkiye; MT107885)	sponge rub, 0.01 M phosphate pH 7.5	DSI at 30 dpi + RT-qPCR on selected accessions	DSI 0–6	26/22 °C (glasshouse)	30 dpi
[66]	AE050 (Saudi Arabia)	sap-rub, carborundum, finger	visual scoring + ELISA	symptom categories	not stated	2, 4, 6 wpi
[58]	Tom2-Jo (Jordan; MZ323110)	mechanical	symptoms + ELISA	symptom classes	not stated	not stated
[59]	ToBRFV-Ant-Tom (Türkiye; MT107885)	mechanical	symptoms + ELISA	symptom classes	not stated	not stated
[61]	CA18-01 (US; MT002973)	mechanical, carborundum	symptoms + ELISA + RT-qPCR (subset)	class 0–5	not stated	not stated

8.4. Heterogeneity in Practice

Table 5 arranges the same studies along these confounders and shows how little of their disagreement need be biological. The challenge isolate is the least documented variable: most studies name a culture, but not all give an accession, and one names neither, so a difference between studies may be nothing more than a difference in isolate genotype. Detection method and severity scale are coupled: symptom-only scoring cannot separate tolerance from resistance, whereas the two studies combining a severity index with ELISA or RT-qPCR could [36,39]. Temperature is not stated for six of the eleven entries, and no two of the rest used the same regime, although both mechanisms are thermosensitive (Section 8.3). Timepoints run from 4 to 42 dpi, which matters because *Tm-1* can hold ToBRFV for at least 50 days, and escape mutants have been recovered only after ~100 days [24]. "Resistant" in Table 5 is thus a statement about a protocol, which is why the minimum standard of Section 8.5 matters.

8.5. A Proposed Minimum Standard

We propose that ToBRFV resistance evaluations report, at minimum, the following, using the well-specified protocol of Zois et al. as a practical template and adding the confounder controls above [39]:

Single-virus, controlled mechanical inoculation with a defined inoculum ratio and buffer (e.g., carborundum abrasion, Na-K phosphate pH ~7.7), from a titre-confirmed, named, sequenced isolate, to exclude mixed infections and isolate-driven discrepancies.

Full isolate identity, not just a designation: report the isolate name, country and year of collection, the GenBank accession, and its genotype at the known escape positions (replicase 976/984 for *Tm-1*; MP 67 and E132 for *Tm-2*^{2}). Names are unreliable: the same designation can refer to different accessions, some accession numbers are incorrect, and several field cultures lack a name or accession (Section 8.4).

Mandatory titre confirmation (ELISA and/or RT-qPCR) reported alongside symptoms, so that tolerance and resistance are distinguished rather than conflated.

A single, defined severity scale (we suggest one 0–3 DSI with published category definitions, or reporting both a standard DSI and titre) to make cross-study meta-analysis possible.

Reported temperature, including an explicit elevated-temperature challenge: because NLR/HR resistance is frequently thermosensitive (the *N* gene fails at 30 °C; Section 8.3), resistance should be verified both at a standard temperature and at a defined high-temperature control (≥30–32 °C), reflecting summer and protected cultivation.

Both leaf and fruit assessment, given the chromosome 7 fruit susceptibility effect.

Minimum plant numbers and replication: ≥ 3 independent experiments with ≥ 10 plants per genotype and treatment, a stated inoculation route, and a two-stage time course comprising a primary window at 21–30 dpi and observation to ≥ 50–60 dpi. The extension is not optional for *Tm-1* material: *Tm-1* can hold systemic ToBRFV for at least 50 days and escape may require ~100 days [24], so a 21–30-day trial can certify a genotype that fails later. Viral load should be quantified by RT-qPCR with ELISA as a cross-check at each scoring point, in inoculated and systemic leaves, and on fruit at maturity where fruit is the deployment question.

Where a claim is to be made commercially, the ISF HR/IR class should be reported alongside titre data, because symptom-based and titre-based classifications diverge (Section 5.4). The convention is proposed rather than validated across pathosystems; its worth lies in comparability, not novelty.

9. Research Gaps and Future Directions

9.1. Genetics and Mapping

The chromosome 11 locus, i.e., the linchpin of the two-locus architecture (Section 5), which remains uncloned, should be cloned.

How many chromosome 11 loci there are should be resolved. Zinger et al.'s recessive tolerance locus sits proximally (~8.9 Mb; candidate *SlCCA1*/Solyc11g018770 and *SlCCA2* [36]), while the Jaiswal GWAS QTL sits distally (~46.84 Mb)—~38 Mb apart, so probably distinct genes [64]. The severity-associated SNPs of [4] add a third candidate at ~0.43–0.75 Mb (Section 5.2). Fine-mapping, an allelism test between the two cloned candidate regions, and a common-background comparison of the three marker sets would settle whether there are two loci or three, which then determines how many segments a breeder must pyramid.

The second locus of the novel *S. pennellii* should be mapped. Zois et al. proved a second recessive locus is required alongside *Tm-1*, but their QTL-seq and genome-wide Indel screen failed to localise it, and it is demonstrably not the chromosome 11 (Zinger/Ashkenazi/*SlCCA1**) or the chromosome 9 locus [39]. This is a genuinely new, unmapped resistance determinant awaiting a dedicated mapping population.

9.2. Mechanism

The movement–evasion trade-off should be quantified. The ToBRFV MP that overcomes *Tm-2*^{2} also moves less efficiently than TMV MP, and swapping it into TMV or ToMV attenuates systemic infection—a cost that, the authors propose, explains why *Tm-2*^{2} held for over 60 years [45]. Whether that cost can be exploited is measurable: we can compare viral load, systemic movement, and transmission efficiency between resistance-breaking variants and the parental isolate and test whether stacking recognition against a transport-limited backbone slows further escape (Section 9.4).

Temperature-stable NLRs should be engineered. The *N*-gene and, potentially, engineered *Tm-2*^{2} resistance are thermosensitive [26]; decoupling recognition from temperature would matter for warm production.

9.3. Omics and Functional Genomics

The highest-value experiment remains the one defined in Section 6.3—a titre-confirmed, defence-focused comparison between validated resistant lines and near-isogenic susceptible counterparts, including small-RNA profiling of that contrast [18]—because the existing proteomic/metabolomic [82] and cell type-resolved transcriptomic [79] datasets were not designed to identify resistance determinants.

9.4. Durability and the Arms Race

For residue-level monitoring of both escape surfaces, one must track the MP resistance-breaking determinant of *Tm-2*^{2} escape (positions 60–186; E132; [9,44,47]) and the replicase helicase determinant of *Tm-1* escape (Y976H and H984Y [24]) as paired sentinels, since both resistance genes (Section 5) have demonstrated escape routes.

The novel MP polymorphisms emerging outside the known breaking sites [33] and the single-substitution escapes observed in the field should be tracked [70].

The chromosome 8 NBS-LRR (HREZ) should be monitored in deployment: it derives from *S. habrochaites* LYC4943, mapped on chromosome 8 [66] and confirmed by VIGS [89]. Although varieties covering 93% of one company's portfolio now carry it [89], it remains temperature-sensitive above 30 °C [89] and a single MP change overcomes it [70], so its value lies in stacking with *Tm-1* or the chromosome 11 segment [89], not solo deployment.

9.5. Evaluation, Sources, and Deployment

The Section 8.5 standard should be adopted across programmes and commercial claims.

Underused sources can be utilised through wide-cross access to the strongly resistant but distant *S. ochranthum* [17,58] and characterisation of the many undisclosed commercial resistances, given the freedom-to-operate landscape of competing patents.

Do not treat eggplant as a tobamovirus-immune rootstock. Eggplant was a non-host in the most comprehensive host-range study [95], but the conflicting reports it noted remain unresolved: ToBRFV infects eggplant systemically and without symptoms in three cultivars [29], is transmitted to eggplant by beetle feeding [16], and has been reported from potato [96], although that observation has not been independently replicated and potato's status as a host remains unsettled. Until its status is settled, an eggplant rootstock is a possible symptomless reservoir, not an immune barrier.

The economic and agronomic value of tolerant (versus resistant) varieties remains unstudied [18] but directly relevant to strategy scoring (Section 7.7).

A public registry of released ToBRFV-resistant varieties should be compiled. Section 7.8 can describe the deployment landscape only through company-level totals, because the declared ISF class and locus of most releases are not public; listing both, with the evaluation standard behind each, would let breeders read that landscape directly and make "resistant" claims comparable across companies (Section 5.4).

Pepper needs resistance beyond L4: ToBRFV infects systemically, and pepper mild mottle virus has already broken L4 (Section 3.5).

10. Conclusions: A Breeding Roadmap Toward Durable ToBRFV Resistance

The trajectory of ToBRFV research over less than a decade reads as a single argument: the virus overcame *Tm-2*^{2} not through unusual variability (low diversity, strong purifying selection; Section 2) but because that resistance rested on a single narrow recognition surface (Section 3). Within a few years, the field moved from cataloguing the breakdown to understanding its mechanism, mapping alternative architecture, and re-engineering recognition. Four conclusions follow.

First, ToBRFV resistance is oligogenic and epistatic, not a single dominant gene: in the best-characterised materials, it requires a chromosome 2 (*Tm-1*) locus plus a second recessive locus, most often on chromosome 11 (Section 5). Second, single-recognition resistance is intrinsically breakable yet demonstrably engineerable: a virus escapes a lone receptor with one or two mutations, but a pyramid of two synergistic activities pre-empts that escape (Sections 3 and 7). Third, tolerance is not resistance—only titre-based, temperature-controlled, fruit-inclusive phenotyping tells them apart (Sections 5.4 and 8). Fourth, because sanitation leaks through seed, weeds, insects, contaminated water, and months-long surface survival (Sections 1 and 2), genetic resistance is the strategic linchpin and must be renewed and stacked.

These principles imply a phased roadmap rather than a single recommended method.

10.1. The Near Term (0–3 Years): Pyramid and Bridge, with Rigour

The immediately deployable, non-transgenic path is marker-assisted pyramiding of the two-locus architecture—*Tm-1*/chromosome 2 plus a donor-matched chromosome 11 locus (BstNI_8.89 for *S. habrochaites*/cultivated, the Jaiswal SNP interval for *S. pimpinellifolium*); the two sets tag different loci and are *not interchangeable* (Section 5.5); for *S. pennellii* donors, *Tm-1* markers exist, but the second locus has no linked marker (Sections 4 and 5). The choice depends on the context (Section 7.8), while *Tm-1*-based rootstocks provide a parallel bridge lowering soil-borne inoculum pressure as scion resistance is assembled (Section 7.2).

The stack need not stop at two loci. Chromosome 8 HREZ material combined with *Tm-1* or a chromosome 11 segment mitigates its high-temperature failure [89], so HREZ is an additional pyramid component, not a standalone replacement. *Tm-2*^{2} belongs on the same list: its extreme resistance is broken, but it retains measurable, dose-sensitive partial resistance (Section 7.1), a low-cost addition to a chromosome 2 × 11 stack. *Tm-1* alleles differ in spectrum and strength, and the *S. pennellii* allele is a distinct, effective source [39] that still requires its unmapped second locus. Functional and unexpressed *Tm-1* copies can be distinguished [65], and the patented paralogue *Rug-1* affects both freedom-to-operate and agronomic performance (Sections 3.2 and 7.7).

10.2. The Mid Term (3–7 Years): Clone, Standardise, and Edit Where Permitted

Two deliverables would turn the near-term tactics into routine breeding: cloning the chromosome 11 gene (resolving whether the Zinger tolerance locus and the Jaiswal QTL are one gene) and publishing a validated two-locus KASP panel. In parallel, *TOM1* susceptibility gene editing—the most durable option demonstrated, with no yield penalty (Section 7.3)—should advance wherever transgene-free crops are permitted, with topical dsRNA held in reserve. This window should also deliver the standardised evaluation protocol (Section 8.5), without which cross-programme claims remain non-comparable.

10.3. The Long Term: Layered, Reduced-Escape Resistance

The durable endpoint is a layered genotype that depends on no single recognition event: a pyramided receptor stack (e.g., the engineered *Tm-2*^{2}-S723Y/N744D double mutant, which resists ToBRFV E132, its K132 escape variant and TMV; Section 7.5), an *S*-gene edit for mechanism-orthogonal durability, and quantitative/tolerance loci for buffering. Underpinning this is monitoring is that escape determinants map to the MP (residues 60–186; E132) and the replicase helicase domain (Sections 3 and 7), so emerging field variants can be tracked as sentinels. No engineered receptor should be deployed alone, since S723Y was itself escaped by a single E132K substitution. The shift documented here is from resistance as a fixed, exhaustible resource to one that is designed, monitored, and renewable.

10.4. Precompetitive Priorities

Several needs are best resolved collectively: cloning the chromosome 11 gene and delivering breeder-ready markers; a comparative resistant-versus-susceptible transcriptome/small-RNA study (Section 6); an agreed evaluation standard (Section 8.5); characterization of undisclosed commercial resistances (Sections 7.7 and 9.5); and quantification of the resistance-versus-quality trade-off of stacked introgressions (Section 5.6).

11. Outlook

ToBRFV exposed the fragility of relying on a single durable gene, but it has also catalysed rapid maturation of tobamovirus resistance science. For the breeder, the message is neither alarmist nor complacent: durable resistance is achievable, but only if built as a stack, selected with titre-based rigour, and monitored at the residues that determine escape. The priority order is unambiguous—the *Tm-1* × chromosome 11 pyramid remains the most feasible near-term objective, with *Tm-2*^{2} residual resistance and, where available, chromosome 8 HREZ as additional components; engineered receptors and *S*-gene edits are complements, not substitutes.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy16222325/s1, Table S1: Scoring rubric and cell-by-cell justifications for the seven strategies evaluated in Table 3. The workbook contains three sheets: (i) 'Table S1', which gives the score (1–5), a justification, and the key evidence for every strategy × criterion cell; (ii) 'Anchors', which defines the 1, 3 and 5 scoring anchors (score 4 is defined separately); and (iii) 'Procedure', which describes how the scores were assigned. Scores were assigned independently by the first and corresponding authors from the evidence in Sections 3–6, with disagreements resolved by discussion.

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