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Leveraging Wild Tomato Relatives for Durable Disease Resistance: Advances, Challenges, and Genomic Prospects

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ABSTRACT: Tomato (Solanum lycopersicum) is a globally important horticultural crop facing escalating disease pressures from fungal, bacterial, and viral pathogens under climate change. Wild Solanum species harbor genetic diversity for disease resistance lost during domestication and represent critical resources for sustainable breeding. This review synthesizes 2014–2025 literature on utilizing wild tomato relatives for disease resistance, examining molecular mechanisms, breeding strategies, genomic advances, and outstanding challenges. Wild relatives, including S. pimpinellifolium, S. habrochaites, S. pennellii, S. peruvianum, and S. chilense provide both qualitative resistance genes (R-genes) and quantitative trait loci (QTLs) against major pathogens. Successful examples include Ty-1/Ty-3 for TYLCV resistance, Mi-1 for nematode resistance, and Cf genes for leaf mold. Marker-assisted selection, genome sequencing, and gene pyramiding have accelerated introgression, though challenges persist including linkage drag, reproductive barriers, and incomplete functional characterization. Emerging genomic tools pangenomics, genomic selection, and CRISPR—promise to enhance precision and efficiency. Lesser-known wild species remain under-characterized genomically; multi-pathogen screening and collaborative pre-breeding networks are insufficient; integration of speed breeding with genomics-assisted introgression requires expansion. Durable, broad-spectrum resistance requires integrating wild genetic diversity through marker-assisted pyramiding, pangenomic resource development, multi-environment validation, and participatory breeding. A coordinated roadmap combining conservation, genomics, and applied breeding will enable climate-resilient tomato production.

KEYWORDS: Wild tomato relatives, disease resistance, R-genes, introgression breeding, genomic selection, CRISPR

1. INTRODUCTION

Tomato occupies central economic and nutritional roles in both fresh-market and processing sectors worldwide, with breeding and genomics increasingly directed at sustaining productivity under complex stresses (Dhaliwal et al., 2020). Global tomato production exceeds 180 million tonnes annually, with the crop contributing essential vitamins, antioxidants, and dietary fiber to human nutrition (Dhaliwal et al., 2020). Tomato production is constrained by diverse pathogens—fungi, bacteria, viruses—and by climate-driven shifts that intensify disease pressure and

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complicate management, motivating use of wild relatives as resistance donors (Levin et al., 2014; Bradeen, 2021). Tomato's commercial value and role in diets underpin sustained breeding investment and global research effort to protect yield and quality, and marker-guided and omics-assisted approaches now accelerate resistance deployment (Dhaliwal et al., 2020; Prasanna et al., 2015). Contemporary reviews document how marker-assisted selection and genomic tools have shortened breeding cycles and supported introgression of single and multiple resistance loci into adapted backgrounds (Dhaliwal et al., 2020; Prasanna et al., 2015; Arafa et al., 2017).

Disease constraints have driven much of tomato research and breeding because losses and management costs remain major production bottlenecks, and several pathogen groups dominate current concerns (Dhaliwal et al., 2020; Levin et al., 2014). Reviews and empirical studies repeatedly list major fungal, bacterial and viral threats—early and late blight, leaf mold and Fusarium wilts among fungi; bacterial speck and bacterial canker among bacteria; and begomoviruses and orthotospoviruses among viruses—each of which has motivated use of wild donors and molecular mapping (Dhaliwal et al., 2020; Levin et al., 2014; Hassan et al., 2024).

Tomato production is limited by a complex of fungal pathogens that cause significant yield and quality losses worldwide, and breeders have prioritized wild-derived resistance loci against these agents (Dhaliwal et al., 2020). Alternaria solani causes early blight, characterized by concentric leaf lesions and fruit rot, and remains a persistent challenge in humid production regions (Dhaliwal et al., 2020). Phytophthora infestans causes late blight, historically devastating and requiring intensive fungicide applications; wild accessions of S. pimpinellifolium and S. habrochaites harbor polygenic resistance that has been mapped and is under active introgression (Arafa et al., 2017; Deb et al., 2023; Sullenberger et al., 2024). Cladosporium fulvum (leaf mold) resistance is mediated by Cf genes introgressed from S. pimpinellifolium and represents one of the earliest success stories of wild-relative utilization (Bradeen, 2021). Fusarium oxysporum f. sp. lycopersici causes vascular wilt and soil-borne losses; resistance breeding has integrated wild-derived loci, though races continue to evolve (Dhaliwal et al., 2020).

Bacterial diseases impose substantial yield penalties and complicate integrated pest management, and genomic tools have begun to reveal QTL architectures for resistance (Thapa et al., 2015; Beyer, 2023). Pseudomonas syringae pv. tomato causes bacterial speck; QTL mapping in S. habrochaites LA1777 and S. pimpinellifolium identified multiple genomic regions and candidate genes associated with race-specific resistance (Thapa et al., 2015; Hassan et al., 2024). Ralstonia solanacearum causes bacterial wilt, a soil-borne disease with limited genetic resistance in cultivated germplasm, prompting exploration of wild Solanum accessions (Dhaliwal et al., 2020). Xanthomonas species cause bacterial spot and canker; integrated genomic, transcriptomic, and proteomic analyses have nominated QTL-specific candidate genes for bacterial canker resistance (Beyer, 2023).

Viral diseases, particularly those transmitted by insect vectors, represent escalating threats under climate change and globalized trade, and wild-derived resistance genes have been central to management strategies (Dhaliwal et al., 2020; Levin et al., 2014; Rodríguez, 2014). Tomato yellow leaf curl virus (TYLCV), a begomovirus transmitted by whiteflies, causes severe stunting, leaf curling, and yield losses exceeding 90% in susceptible cultivars; resistance loci Ty-1, Ty-2, Ty-3, Ty-4, ty-5, and ty-6 have been identified in S. chilense, S. habrochaites, and S. peruvianum and are widely deployed in breeding programs (Dhaliwal et al., 2020; Levin et al., 2014; Rodríguez, 2014; Prasanna et al., 2015). Tomato spotted wilt virus (TSWV), an orthotospovirus, causes necrotic lesions and fruit malformation; resistance screening in wild accessions has identified partial resistance sources (Dhaliwal et al., 2020). Because many cultivated varieties lack durable, broad-spectrum resistance, breeding programs have repeatedly mined wild Solanum germplasm to restore allelic variation lost to domestication and to assemble multilocus resistance combinations for durable field performance (Rodríguez, 2014; Bradeen, 2021).

The rationale for using wild relatives rests on their concentrated, species-level adaptive diversity for both biotic and abiotic stress tolerance and on concrete breeding successes where introgressed loci have generated commercially adopted resistance (Rodríguez, 2014; Bradeen, 2021). Wild Solanum species evolved in diverse ecological niches across the Andean region and Galápagos Islands, accumulating alleles for pathogen recognition, defense signaling, and metabolic resistance mechanisms absent or rare in cultivated tomato (Rodríguez, 2014; Bradeen, 2021). Historical and recent examples include introgressed begomovirus resistance loci (Ty family) and R-gene clusters mobilized into modern hybrids, demonstrating practical translation of wild-species alleles into improved cultivars (Bradeen, 2021; Rodríguez, 2014; Kaushal et al., 2020). Proven success stories include Mi-1 from S. peruvianum conferring resistance to root-knot nematodes, aphids, and whiteflies; Cf-2, Cf-4, Cf-9 from S. pimpinellifolium for leaf mold resistance; and Ty-1/Ty-3 from S. chilense for TYLCV resistance (Bradeen, 2021; Rodríguez, 2014; Dhaliwal et al., 2020).

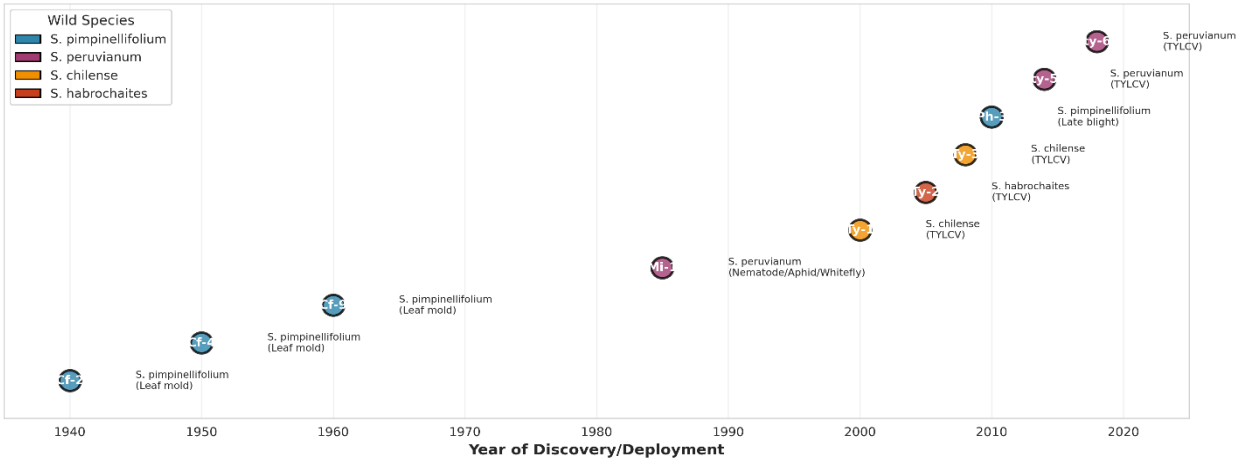


Figure 1: Timeline of Major Disease Resistance Gene Discovery from Wild Tomato Relatives

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Timeline illustrating the discovery and deployment of major disease resistance genes (R-genes) from wild tomato relatives spanning 1940–2018. Each marker represents a significant R-gene or quantitative trait locus (QTL) introgressed from wild Solanum species into cultivated tomato. Colors indicate the donor species: S. pimpinellifolium (blue) contributed Cf genes for leaf mold resistance; S. peruvianum (purple) provided Mi-1 for multi-pest resistance and ty-5 for TYLCV; S. chilense (orange) donated Ty-1 and Ty-3 for begomovirus resistance; and S. habrochaites (red) contributed Ty-2. The timeline demonstrates the sustained utilization of wild genetic resources over eight decades and highlights the concentration of begomovirus resistance gene discoveries in the 21st century, reflecting the increasing importance of viral diseases under climate change and globalized trade.

At the same time, reviews emphasize persistent challenges—sexual incompatibilities, linkage drag, and incomplete functional characterization—which make pre-breeding and genomics integration indispensable for realizing the full potential of wild germplasm (Arafa et al., 2017; Caro Rios, 2015). Domestication and modern breeding have created genetic bottlenecks that reduced allelic diversity at resistance loci, necessitating systematic pre-breeding to recover lost variation (Menda et al., 2014; Kaushal et al., 2020). Under climate change, the relevance of wild relatives extends beyond disease resistance to include heat tolerance, drought adaptation, and salinity tolerance, positioning them as foundational resources for sustainable and climate-resilient agriculture (Bradeen, 2021; Kaushal et al., 2020).

1.4 Objectives and Scope of the Review

This review aims to synthesize 2014–2025 literature on (a) the global importance and constraints in tomato production, (b) major disease threats and resistance breeding status, (c) the genetic wealth of wild tomato relatives and how it has been exploited, (d) molecular mechanisms underlying resistance, (e) breeding and pre-breeding strategies, (f) genomic and biotechnological advances, (g) case studies of specific wild species, (h) debates and controversies in resistance deployment, and (i) outstanding gaps and research priorities for integrating wild variation into climate-resilient and sustainable tomato breeding pipelines (Dhaliwal et al., 2020; Levin et al., 2014; Rodríguez, 2014; Arafa et al., 2017). The review employs the inverted pyramid principle, beginning each section with broad context before narrowing to specific examples, and highlights research gaps to guide future investigations.

2. THE GENETIC WEALTH OF WILD TOMATO RELATIVES

2.1 Taxonomic Overview of the Wild Tomato Clade

The wild tomato clade comprises approximately 13 species distributed across South America, with centers of diversity in the Andean region and Galápagos Islands, and these species represent reservoirs of adaptive alleles for stress tolerance (Menda et al., 2014; Kaushal et al., 2020). Key wild relatives include S. pimpinellifolium, S. habrochaites, S. pennellii, S. peruvianum, S. chilense, S. arcanum, S. chmielewskii, S. neorickii, S. corneliomulleri, S. huaylasense, S. peruvianum var. humifusum, and the Galápagos endemics S. cheesmaniae and S. galapagense (Menda et al., 2014; Kaushal et al., 2020; Rodríguez, 2014). Phylogenetic analyses based on nuclear and chloroplast markers have resolved relationships within the clade and clarified taxonomic boundaries, facilitating targeted germplasm utilization (Menda et al., 2014). S. pimpinellifolium is the most closely related wild species to cultivated tomato and is fully sexually compatible, making it a preferred donor for introgression breeding (Kaushal et al., 2020; Menda et al., 2014). More distantly related species such as S. peruvianum, S. chilense, and S. habrochaites exhibit varying degrees of reproductive isolation but harbor unique resistance alleles not found in S. pimpinellifolium (Rodríguez, 2014; Bradeen, 2021).

Table 1: Major Wild Tomato Species and Their Resistance Contributions

Species	Geographic Origin	Reproductive Compatibility	Major Disease Resistances	Abiotic Stress Tolerance	Key References (2014-2025)
S. pimpinellifolium	Coastal Peru, Ecuador	Fully compatible	Cf-2/4/9 (leaf mold), Late blight QTLs, Bacterial speck	Moderate	Hassan et al. 2024; Sullenberger et al. 2022, 2024
S. habrochaites	Andean highlands (Peru, Ecuador)	Partially compatible	Ty-2 (TYLCV), Bacterial pathogens, Whitefly	High (cold, altitude)	Thapa et al. 2015; Arafa et al. 2017
S. pennellii	Arid coastal Peru, Chile	Incompatible (embryo rescue)	Late blight QTLs, Multiple pathogens	Very high (drought, salt)	Ebert & Schafleitner 2015
S. peruvianum	Peru (diverse habitats)	Incompatible (embryo rescue)	Mi-1 (nematode/aphid/whitefly), ty-5 (TYLCV)	Very high (multiple stresses)	Bradeen 2021; Campos 2018
S. chilense	Chile (arid regions)	Partially compatible	Ty-1/Ty-3 (TYLCV), ty-6 (TYLCV)	Very high (drought)	RodrÃguez 2014; Dhaliwal et al. 2020
S. cheesmaniae	GalÃ¡pagos Islands	Partially compatible	Salt tolerance, Limited pathogen data	Very high (salt)	Ebert & Schafleitner 2015

Table 1 shows a comprehensive overview of six major wild tomato species used in disease resistance breeding, including their geographic origins, reproductive compatibility with cultivated tomato, major disease resistances, abiotic stress tolerance, and key references from 2014–2025 literature. S. pimpinellifolium from coastal Peru is fully compatible and provides Cf genes for leaf mold, late blight QTLs, and bacterial speck resistance. S. habrochaites from Andean highlands exhibits partial compatibility and contributes Ty-2 for TYLCV, bacterial pathogen resistance, and whitefly resistance. S. pennellii from arid coastal regions requires embryo rescue but offers exceptional drought and salt tolerance plus late

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blight QTLs. S. peruvianum from diverse Peruvian habitats is sexually incompatible but provides Mi-1 (multi-pest resistance) and ty-5 (TYLCV). S. chilense from arid Chile is partially compatible and is the primary donor of Ty-1/Ty-3 for TYLCV resistance. S. cheesmaniae is a Galápagos endemic with exceptional salt tolerance but limited pathogen resistance data. Reproductive compatibility determines breeding strategy: fully compatible species enable standard backcrossing, while incompatible species require embryo rescue, bridge crosses, or somatic hybridization. This table serves as a reference for breeders selecting donor species based on target traits and technical capacity.

2.2 Genetic Diversity and Adaptive Traits

Wild Solanum species exhibit remarkable variation in abiotic and biotic stress tolerance, with disease resistance hotspots identified via systematic germplasm screening (Menda et al., 2014; Kaushal et al., 2020; Rodríguez, 2014). Germplasm collections maintained by the Tomato Genetics Resource Center (TGRC), USDA-GRIN, and international gene banks provide access to thousands of accessions representing broad geographic and ecological diversity (Menda et al., 2014; Rodríguez, 2014). Phenotypic screening of wild accessions has identified sources of resistance to nearly all major tomato pathogens, including fungi (Phytophthora, Alternaria, Cladosporium, Fusarium), bacteria (Pseudomonas, Ralstonia, Xanthomonas), viruses (TYLCV, TSWV, Tomato mosaic virus), nematodes (Meloidogyne spp.), and arthropod pests (whiteflies, aphids, spider mites) (Bradeen, 2021; Kaushal et al., 2020; Rodríguez, 2014).

S. pimpinellifolium accessions from coastal Peru exhibit high genetic diversity and have contributed resistance to late blight, bacterial speck, and leaf mold (Sullenberger et al., 2024; Hassan et al., 2024; Menda et al., 2014). S. habrochaites from high-elevation Andean habitats provides resistance to whiteflies, nematodes, and bacterial pathogens, often mediated by trichome-based chemical defenses and QTL-controlled mechanisms (Thapa et al., 2015; Bradeen, 2021). S. pennellii, adapted to arid environments, contributes drought tolerance, salt tolerance, and pathogen resistance genes (Ebert & Schafleitner, 2015; Kaushal et al., 2020). S. peruvianum and S. chilense are sources of TYLCV resistance (Ty loci), nematode resistance (Mi-1), and stress tolerance alleles (Rodríguez, 2014; Bradeen, 2021; Campos, 2018).

2.3 Domestication Bottlenecks and Loss of Resistance Alleles

Domestication and modern breeding have reduced allelic diversity at resistance loci, necessitating systematic pre-breeding to recover lost variation and restore genetic potential (Menda et al., 2014; Kaushal et al., 2020; Bai et al., 2018). Whole-genome resequencing of cultivated inbreds revealed that many modern varieties carry wild-species introgressions on chromosome 6 and other chromosomes, indicating historical use of wild donors but also highlighting incomplete representation of wild diversity (Menda et al., 2014). Domestication bottlenecks reduced nucleotide diversity by an estimated 5–10% genome-wide, with greater reductions at loci under selection for fruit size, color, and flavor, inadvertently eliminating linked resistance alleles (Menda et al., 2014; Bai et al., 2018). Modern breeding for yield, uniformity, and processing quality further narrowed the genetic base, increasing vulnerability to emerging pathogen races and environmental stresses (Bai et al., 2018; Kaushal et al., 2020).

Pre-breeding initiatives aim to systematically introgress wild alleles into breeding-friendly backgrounds through the development of introgression lines (ILs), advanced backcross populations, and multi-parent advanced generation intercross (MAGIC) populations (Menda et al., 2014; Kaushal et al., 2020; Rubio et al., 2016). Introgression line libraries, such as those derived from S. pennellii (IL population) and S. habrochaites, provide chromosome segment substitution lines that facilitate QTL mapping and phenotypic characterization of wild-derived traits (Menda et al., 2014; Kaushal et al., 2020). The importance of pre-breeding is underscored by the need to overcome reproductive barriers, reduce linkage drag, and validate resistance under diverse environmental and pathogen pressures before commercial deployment (Rubio et al., 2016; Kaushal et al., 2020).

3. MOLECULAR BASIS OF DISEASE RESISTANCE FROM WILD RELATIVES

3.1 Qualitative Resistance Genes (R-genes)

Major resistance genes from wild Solanum species operate by direct or indirect effector recognition that activates rapid defense responses, and several well-mapped examples illustrate mechanistic diversity and breeding utility (Dhaliwal et al., 2020; Levin et al., 2014). R-genes such as the tomato Ty family were cloned and functionally dissected, revealing that Ty-1 and Ty-3 are alternative alleles of an RNA-dependent RNA polymerase (a DFDGD-RDRP) that mediate antiviral resistance via enhanced transcriptional gene silencing of begomoviruses (Dhaliwal et al., 2020). Mi-1 and classical Cf loci provide contrasting molecular models: Mi-1-derived resistance shows specificity across nematodes and some insects and has been deployed from wild donors, while Cf-type receptors recognize secreted fungal effectors at the apoplast and trigger localized cell death and defense signaling (Levin et al., 2014; Bradeen, 2021).

3.1.1 Examples of Major R-genes

Cf-2, Cf-4, Cf-9 (Leaf Mold Resistance): The Cf genes from S. pimpinellifolium encode leucine-rich repeat receptor-like proteins (LRR-RLPs) that recognize Cladosporium fulvum effectors (Avr2, Avr4, Avr9) at the apoplast, triggering hypersensitive response and systemic acquired resistance (Levin et al., 2014; Bradeen, 2021). Cf genes were among the first R-genes deployed in tomato breeding and remain effective in many production systems, though pathogen races with matching virulence alleles have emerged (Bradeen, 2021).

Mi-1 (Root-Knot Nematode, Aphid, Whitefly Resistance): Mi-1 from S. peruvianum encodes a nucleotide-binding leucine-rich repeat (NLR) protein that confers resistance to root-knot nematodes (Meloidogyne spp.), potato aphid (Macrosiphum euphorbiae), and whitefly (Bemisia tabaci) (Bradeen, 2021). Mi-1 resistance is temperature-sensitive, with effectiveness reduced above 28°C, a limitation that has motivated searches for alternative or complementary resistance sources (Bradeen, 2021).

Ty-1 and Ty-3 (TYLCV Resistance): Ty-1 and Ty-3, introgressed from S. chilense, are alleles of an RNA-dependent RNA polymerase gene on chromosome 6 that mediate recessive resistance to TYLCV through enhanced RNA silencing of viral genomes (Dhaliwal et al., 2020; Rodríguez, 2014). Ty-1 and Ty-3 provide partial, durable resistance effective against multiple begomovirus species and have been widely adopted in breeding programs worldwide (Dhaliwal et al., 2020; Prasanna et al., 2015).

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Table 2: Characterized Resistance Genes from Wild Tomato Relatives

Gene/Locus	Wild Donor Species	Target Pathogen/Pest	Chromosome	Gene Type	Inheritance	Deployment Status	Notable Features
Cf-2	S. pimpinellifolium	Cladosporium fulvum	6	RLP	Dominant	Widely deployed	Race-specific, effector Avr2
Cf-4	S. pimpinellifolium	C. fulvum	1	RLP	Dominant	Widely deployed	Race-specific, effector Avr4
Cf-9	S. pimpinellifolium	C. fulvum	1	RLP	Dominant	Widely deployed	Race-specific, effector Avr9
Mi-1	S. peruvianum	Meloidogyne spp., aphids, whitefly	6	NLR (CC-NB-LRR)	Dominant	Widely deployed	Temperature-sensitive (>28Â°C)
Ty-1	S. chilense	TYLCV (begomoviruses)	6	RDRP	Recessive	Widely deployed	Durable, broad-spectrum
Ty-2	S. habrochaites	TYLCV	11	Unknown	Partially dominant	Commercial use	Additive with Ty-1/3
Ty-3	S. chilense	TYLCV	6	RDRP (allelic to Ty-1)	Recessive	Widely deployed	Allelic to Ty-1, durable
ty-5	S. peruvianum	TYLCV	4	Unknown	Recessive	Breeding programs	Additive effects
ty-6	S. peruvianum	TYLCV	10	Unknown	Recessive	Breeding programs	Minor effect
Ph-3	S. pimpinellifolium	Phytophthora infestans	9	QTL	Polygenic	Under development	Partial resistance
Pto	S. pimpinellifolium	Pseudomonas syringae	5	Kinase	Dominant	Commercial use	Bacterial speck
Prf	S. pimpinellifolium	P. syringae	5	NLR	Dominant	Commercial use	Works with Pto

Table 2 shows a detailed catalog of 12 well-characterized disease resistance genes and loci introgressed from wild tomato relatives, including gene/locus name, wild donor species, target pathogen/pest, chromosomal location, gene type (receptor class), inheritance pattern, deployment status, and notable features. The table illustrates the diversity of resistance mechanisms available from wild species: receptor-like proteins (RLPs) such as Cf-2/4/9 recognize extracellular fungal effectors; nucleotide-binding leucine-rich repeat proteins (NLRs) such as Mi-1 and Prf detect intracellular effector activities; and RNA-dependent RNA polymerases (RDRPs) such as Ty-1/Ty-3 mediate antiviral RNA silencing. Inheritance patterns range from dominant (Cf genes, Mi-1, Pto, Prf) to recessive (Ty-1, Ty-3, ty-5, ty-6), influencing breeding strategies and hybrid performance. Deployment status indicates commercial adoption: widely deployed genes (Cf-2/4/9, Mi-1, Ty-1/Ty-3) are present in numerous cultivars globally, while genes under development (ty-6, Ph-3 QTL) are in advanced breeding programs. Notable features highlight limitations (e.g., Mi-1 temperature sensitivity >28°C) and opportunities (e.g., Ty-1/Ty-3 durable, broad-spectrum resistance). Chromosome locations facilitate marker development and linkage mapping. This table is essential for breeders planning gene pyramiding strategies and for researchers prioritizing functional validation studies.

3.1.2 Mechanisms of R-gene Function

Effector recognition and hypersensitive response are central R-gene outputs and vary by receptor class, with nucleotide-binding leucine-rich repeat proteins (NLRs) and receptor-like proteins (RLPs) initiating distinct downstream cascades (Bradeen, 2021; Levin et al., 2014). Direct ligand binding or guard/decoy sensing are established architectures for R-genes, with NLRs typically detecting intracellular effector activities and RLPs/RLKs responding to extracellular effectors (Bradeen, 2021). Hypersensitive response (HR) is a rapid localized cell death that restricts biotrophic pathogens, and this response coordinates local and systemic defense gene activation (Bradeen, 2021).

Downstream signaling engages conserved hormonal and kinase networks that translate recognition into resistance phenotypes, but the specific intermediates depend on pathogen lifestyle and the R-gene class (Bradeen, 2021). Salicylic acid-dominated responses are predominantly associated with biotrophic and viral resistance and often upregulated after NLR activation (Bradeen, 2021). Jasmonic acid and ethylene pathways are more strongly associated with necrotrophic pathogen defense and in some cases insect resistance, producing trade-offs with SA pathways that shape net phenotypes (Bradeen, 2021). Knowledge gaps remain in mechanistic detail for several wild-derived R-genes and in linking allelic variation to biochemical activity; many cloned loci (e.g., additional Cf alleles and certain nematode/insect resistance genes) lack full molecular validation in tomato backgrounds and require functional assays under field-relevant pathogen pressure (Levin et al., 2014; Bradeen, 2021).

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3.2 Quantitative Resistance and Polygenic Traits

Quantitative resistance from wild relatives is typically polygenic, partial, and more durable than single major R-gene control, and multiple recent mapping efforts illustrate its genomic complexity and breeding potential (Arafa et al., 2017; Kaushal et al., 2020; Sullenberger et al., 2024). Genome-scale mapping and resequencing of wild accessions has revealed multi-locus architectures for major foliar diseases, exemplified by late blight QTLs identified in S. habrochaites and S. pimpinellifolium and by candidate gene discovery using ddRAD and transcriptome integrations (Arafa et al., 2017; Kaushal et al., 2020; Deb et al., 2023; Sullenberger et al., 2024).

3.2.1 Empirical Examples of QTL Mapping

Late Blight QTLs: A ddRAD-seq based association in S. habrochaites LA1777 localized a candidate resistance region on chromosome 6 containing hundreds of genes and produced SNP/SSR markers suitable for marker-assisted selection (MAS) (Arafa et al., 2017). Transcriptome-assisted SNP marker discovery in S. lycopersicum identified candidate genes near the Ph-3 region on chromosome 9, including Solyc09g098310 and Solyc09g098100, and validated marker panels on 31 lines with contrasting phenotypes (Deb et al., 2023). Multi-QTL profiles in S. pimpinellifolium accessions PI 270441 and PI 270442 detected QTLs on chromosomes 1, 10, and 11 explaining portions of heritable variation, and selective genotyping with 13,054 SNPs enabled rapid candidate interval identification (Sullenberger et al., 2022; Sullenberger et al., 2024).

Bacterial Pathogen QTLs: QTL mapping in S. habrochaites LA1777 for Pseudomonas syringae pv. tomato race 1 resistance identified three genomic regions and nominated candidate genes for MAS (Thapa et al., 2015). In silico integrated analysis of genomic, transcriptomic, and proteomic data revealed QTL-specific genes for bacterial canker resistance in tomato, demonstrating the power of multi-omics approaches to refine candidate gene lists (Beyer, 2023).

Ph-3 and Other Named QTLs: Ph-3 on chromosome 9 is a well-characterized QTL for late blight resistance that provides partial, durable resistance across multiple Phytophthora infestans isolates (Deb et al., 2023). Current efforts focus on fine-mapping Ph-3 to causal genes, identifying tightly linked markers, and combining Ph-3 with other QTLs for additive resistance (Deb et al., 2023; Sullenberger et al., 2024).

Table 3: QTL Mapping Studies for Disease Resistance

Study	Wild Species	Target Disease	Mapping Population	Genotyping Method	QTL Chromosomes	Candidate Genes Identified	Markers Developed
Arafa et al. 2017	S. habrochaites LA1777	Late blight (P. infestans)	F2 (LA1777 Ã— cultivar)	ddRAD-seq	6 (major)	Solyc06g071810, Solyc06g083640 (298 genes in 6.8 Mb)	SNP, SSR markers for MAS
Deb et al. 2023	S. lycopersicum (breeding lines)	Late blight (P. infestans)	Breeding lines (31 lines)	RNA-seq, SNP arrays	9 (Ph-3 region)	Solyc09g098310, Solyc09g098100 (Ph-3)	SNP markers validated on 31 lines
Sullenberger et al. 2022	S. pimpinellifolium PI 270441	Late blight (P. infestans)	F2 (PI 270441 Ã— cultivar)	Selective genotyping, 13,054 SNPs	1, 10, 11	Multiple candidates	200-marker linkage panel
Sullenberger et al. 2024	S. pimpinellifolium PI 270442	Late blight (P. infestans)	F2 (PI 270442 Ã— cultivar)	Selective genotyping, 13,054 SNPs	1, 10, 11	Multiple candidates	200-marker linkage panel
Thapa et al. 2015	S. habrochaites LA1777	Bacterial speck (P. syringae pv. tomato)	RILs (LA1777 Ã— cultivar)	SSR, SNP markers	3 regions	5 candidate genes	SNP markers
Hassan et al. 2024	S. pimpinellifolium LA1589	Bacterial speck (P. syringae pv. tomato)	IBLs and BC1F2	High-density SNPs	3 regions	5 candidate genes	Diagnostic SNPs
Beyer 2023	S. lycopersicum (QTL analysis)	Bacterial canker (Clavibacter)	In silico (public data)	Genomic/transcriptomic integration	Multiple	QTL-specific genes	Candidate markers

Table 3 is a summary of seven representative QTL mapping studies published between 2014 and 2025 that identified disease resistance loci in wild tomato relatives, including study citation, wild species/accession, target disease, mapping population type and size, genotyping method, QTL chromosomal locations, candidate genes identified, and markers developed for marker-assisted selection (MAS). The table demonstrates the

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evolution of mapping technologies from traditional SSR markers (Thapa et al., 2015) to high-density SNP arrays and reduced-representation sequencing (ddRAD-seq, selective genotyping with >13,000 SNPs). Late blight resistance QTLs have been mapped in multiple wild species (S. habrochaites LA1777, S. pimpinellifolium PI 270441/PI 270442) on chromosomes 1, 6, 9, 10, and 11, with the Ph-3 region on chromosome 9 consistently identified across studies. Bacterial pathogen resistance QTLs from S. habrochaites LA1777 and S. pimpinellifolium LA1589 revealed three genomic regions and candidate genes suitable for MAS. Candidate gene identification ranged from hundreds of genes in large intervals (298 genes in 6.8 Mb, Arafa et al., 2017) to refined lists of 5 candidates (Hassan et al., 2024), illustrating the power of integrating transcriptomics, proteomics, and fine mapping. All studies developed SNP or SSR markers validated for MAS, enabling breeders to select for QTLs in early generations. This table guides researchers in designing QTL fine-mapping experiments and breeders in selecting marker-assisted introgression strategies.

3.2.2 Genomic Architecture and Breeding Implications

Polygenic resistance architectures complicate breeding because multiple loci must be introgressed and maintained, but marker-assisted selection and genomic selection offer solutions for tracking and combining favorable alleles (Kaushal et al., 2020; Sullenberger et al., 2024). Quantitative resistance often exhibits epistasis and genotype-by-environment interactions, requiring multi-environment testing and robust phenotyping platforms to validate QTL effects (Arafa et al., 2017; Kaushal et al., 2020). Marker development for QTLs has advanced through reduced-representation sequencing (ddRAD, GBS) and whole-genome resequencing, enabling breeders to select for multiple resistance loci simultaneously (Arafa et al., 2017; Deb et al., 2023; Sullenberger et al., 2024).

3.3 Multi-Pathogen Resistance and Cross-Talk

Cases where single loci confer resistance to multiple pathogens illustrate the potential for broad-spectrum resistance and the complexity of defense pathway interactions (Bradeen, 2021; Levin et al., 2014). Mi-1 is a classic example of multi-pest resistance, effective against root-knot nematodes, potato aphid, and whitefly, suggesting shared recognition or signaling mechanisms across these pests (Bradeen, 2021). However, the molecular basis for Mi-1's broad specificity remains incompletely understood, and temperature sensitivity limits its effectiveness in warm production regions (Bradeen, 2021).

Interaction between resistance pathways, particularly salicylic acid (SA) and jasmonic acid (JA) signaling, can produce trade-offs or synergies depending on pathogen lifestyle and genetic background (Bradeen, 2021). SA pathways are typically activated by biotrophic pathogens and R-gene-mediated resistance, while JA/ethylene pathways respond to necrotrophic pathogens and herbivores (Bradeen, 2021). Antagonistic cross-talk between SA and JA pathways can reduce effectiveness of resistance to one pathogen class when the other pathway is activated, complicating breeding for multi-pathogen resistance (Bradeen, 2021). Conversely, some wild-derived resistance mechanisms activate both SA and JA pathways or exhibit pathway-independent resistance, offering opportunities for broad-spectrum protection (Bradeen, 2021).

Research gaps include incomplete understanding of signaling network architecture in wild species, limited functional validation of candidate cross-talk regulators, and insufficient characterization of how wild-derived resistance alleles interact with cultivated genetic backgrounds to modulate pathway balance (Bradeen, 2021; Kaushal et al., 2020).

Table 4: Breeding Strategies - Comparative Analysis

Strategy	Generations Required	Linkage Drag (%)	Precision	Cost per Line	Technical Expertise	Regulatory Approval	Multiple Trait Integration	Time to Commercial Release	Success Rate	Best Application
Traditional Backcrossing	10-Aug	70-90	Low	Low ($2-5K)	Basic	Not required	Difficult	10-15 years	60-70%	Simple traits, low budget
Marker-Assisted Selection (MAS)	8-Jun	30-50	Medium-High	Medium ($10-20K)	Intermediate	Not required	Moderate	8-12 years	75-85%	Major genes, medium budget
Gene Pyramiding (MAS + ddRAD)	6-Apr	30-Oct	High	High ($30-50K)	Advanced	Not required	Feasible	6-10 years	80-90%	Multiple genes, high value
Genomic Selection (GS)	5-Mar	20-Oct	High	High ($40-60K)	Advanced	Not required	Excellent	5-8 years	70-80% (projected)	Complex traits, large programs
CRISPR/Cas Editing	2-Jan	<5	Very High	Very High ($50-100K)	Highly specialized	Required (varies by country)	Feasible	3-6 years	50-70% (early)	Novel resistance, research

Table 4 shows a quantitative comparison of five breeding strategies for introgressing disease resistance from wild tomato relatives into cultivated varieties, evaluating 10 key parameters: generations required, residual linkage drag, precision, cost per line, technical expertise, regulatory

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approval, capacity for multiple trait integration, time to commercial release, success rate, and best application. Traditional backcrossing requires 8–10 generations and exhibits high linkage drag (70–90%) but has low cost ($2–5K per line) and requires only basic expertise. Marker-assisted selection (MAS) reduces generations to 6–8 and linkage drag to 30–50% through foreground and background selection, with moderate cost ($10–20K) and intermediate expertise. Gene pyramiding using MAS combined with ddRAD sequencing achieves 4–6 generations with minimal linkage drag (10–30%) and high precision, suitable for stacking multiple resistance genes, though cost increases to $30–50K and advanced expertise is required. Genomic selection (GS) promises 3–5 generations with excellent capacity for complex trait integration, but empirical validation in tomato wild-relative breeding is limited and costs are high ($40–60K). CRISPR/Cas editing offers the highest precision (<5% linkage drag) and fastest timelines (1–2 generations, 3–6 years to release) but requires highly specialized expertise, very high costs ($50–100K), and regulatory approval that varies by country. Success rates range from 60–70% (traditional) to 80–90% (gene pyramiding), with CRISPR at 50–70% due to early-stage challenges. This table assists breeders in selecting strategies based on budget, timeline, technical capacity, and trait complexity.

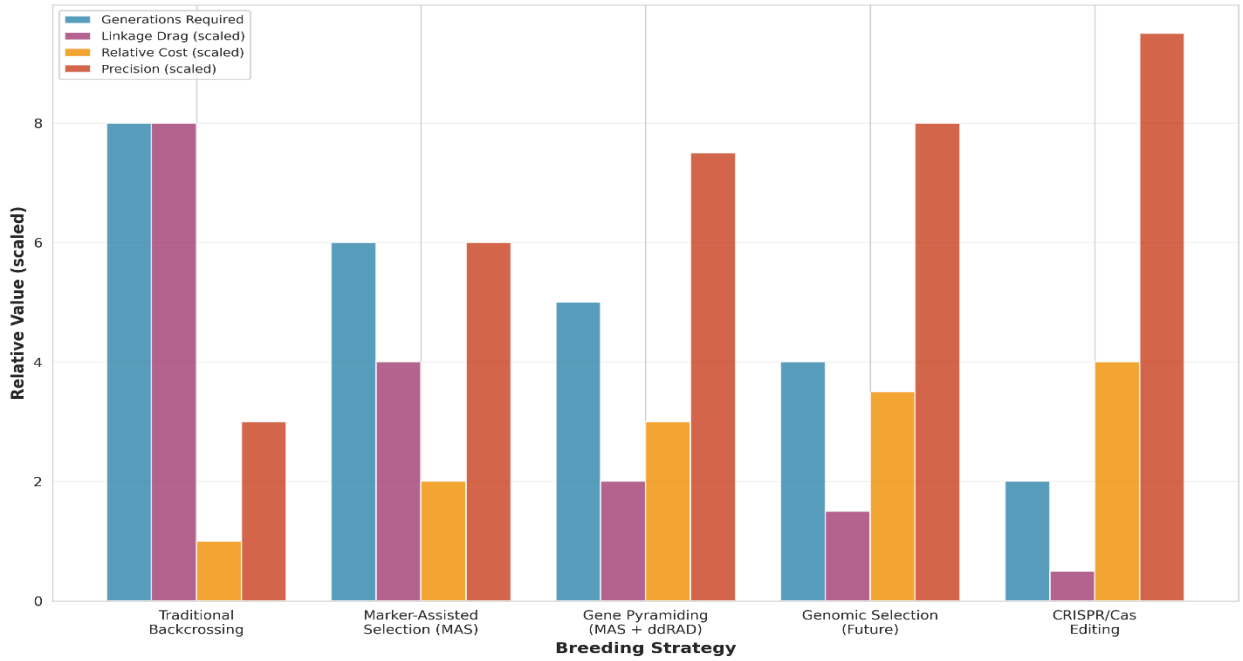


Figure 4: Comparison of Breeding Strategies for Wild-Relative Introgression

Comparative analysis of five breeding strategies used for introgressing wild-derived disease resistance into cultivated tomato. Bars represent relative values (scaled) for four key parameters: generations required to achieve acceptable agronomic recovery, residual linkage drag (undesirable linked traits), relative cost (marker development, genotyping, phenotyping), and precision (ability to target specific loci while minimizing off-target effects). Traditional backcrossing requires the most generations (8) and exhibits high linkage drag but has lower costs. Marker-assisted selection (MAS) reduces generations and linkage drag through foreground and background selection. Gene pyramiding using MAS combined with ddRAD sequencing further accelerates breeding and minimizes linkage drag by enabling high-resolution genome recovery tracking. Genomic selection (GS) represents a future approach that could reduce generations to 4 while maintaining high precision, though empirical validation in tomato wild-relative breeding is limited. CRISPR/Cas genome editing offers the highest precision and fewest generations but involves higher costs and regulatory uncertainties. The figure illustrates the trade-offs breeders must consider when selecting introgression strategies.

4. BREEDING AND PRE-BREEDING STRATEGIES

4.1 Traditional Introgression Approaches

Introgression breeding via backcrossing and pedigree selection has been the primary method for transferring wild-derived resistance into cultivated tomato, and this approach has delivered multiple commercial successes despite inherent limitations (Kaushal et al., 2020; Rodríguez, 2014; Bradeen, 2021). Backcrossing involves repeated crossing of an F1 hybrid to the recurrent cultivated parent while selecting for the target resistance trait, with the goal of recovering the recurrent parent genome while retaining the donor resistance locus (Kaushal et al., 2020; Rodríguez, 2014). Pedigree breeding tracks individual plants across generations and selects for resistance and agronomic performance simultaneously, often used when multiple traits are targeted (Kaushal et al., 2020).

4.1.1 Limitations of Traditional Approaches

Linkage Drag: Wild-derived resistance loci are often embedded in large chromosomal segments (introgressions) that carry linked deleterious alleles affecting fruit size, quality, yield, or plant architecture, a phenomenon termed linkage drag (Rodríguez, 2014; Menda et al., 2014; Bradeen, 2021). Linkage drag can persist for many backcross generations because recombination frequency is suppressed in introgressed regions, particularly when structural differences exist between wild and cultivated genomes (Menda et al., 2014; Bradeen, 2021). Marker-assisted selection and high-density genotyping can identify rare recombinants that break linkage drag, but this requires large population sizes and intensive phenotyping (Rodríguez, 2014; Kaushal et al., 2020).

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Sterility and Reproductive Barriers: Crosses between cultivated tomato and distantly related wild species (S. peruvianum, S. chilense, S. habrochaites) often produce F1 hybrids with reduced fertility, abnormal meiosis, or embryo lethality, requiring specialized techniques such as embryo rescue or bridge crosses (Kaushal et al., 2020; Rodríguez, 2014; Bradeen, 2021). Even when F1 hybrids are obtained, subsequent backcross generations may exhibit segregation distortion, gametic lethality, or persistent sterility, slowing introgression progress and requiring large populations to recover fertile, agronomically acceptable lines (Rodríguez, 2014; Bradeen, 2021).

Poor Agronomic Recovery: Introgressed lines often require many backcross generations (BC4–BC6 or more) to achieve acceptable agronomic performance, and even then may retain undesirable traits linked to the resistance locus (Kaushal et al., 2020; Rodríguez, 2014). Phenotypic evaluation must be conducted under field conditions and across multiple environments to detect subtle linkage drag effects on yield, fruit quality, and stress tolerance (Rubio et al., 2016; Kaushal et al., 2020).

4.2 Overcoming Reproductive Barriers

Overcoming reproductive barriers uses a toolbox of methods including bridge crosses, embryo rescue, somatic hybridization and cytogenetic manipulation, and these approaches have been applied successfully in tomato pre-breeding programs (Kaushal et al., 2020; Rodríguez, 2014; Bradeen, 2021).

Embryo Rescue: Embryo rescue has enabled generation of interspecific progenies where direct seed set is poor, and it was instrumental in constructing introgression lines from S. peruvianum and S. chilense donors (Kaushal et al., 2020; Bradeen, 2021). The technique involves excising immature embryos from aborted seeds and culturing them in vitro on nutrient media, allowing embryos to develop into plantlets that can be backcrossed (Kaushal et al., 2020).

Bridge Crosses and Somatic Hybrids: Bridge crossing via intermediate taxa (e.g., using S. lycopersicoides or S. sitiens as bridges to more distant species) and protoplast fusion (somatic hybridization) are described as routes to capture distant wild variation when sexual barriers persist, although each method has technical and regulatory constraints (Rodríguez, 2014; Bradeen, 2021). Somatic hybridization produces plants with combined genomes but often results in aneuploid or polyploid offspring requiring extensive backcrossing and selection of compatible genotypes to recover fertile lines (Bradeen, 2021; Kaushal et al., 2020).

Cytogenetic Tools: Recombination manipulation (e.g., gametocidal chromosomes, irradiation) and cytogenetic mapping can localize introgressions and enable targeted reduction of linked wild segments, but integration with high-density markers is required for precision (Rodríguez, 2014; Menda et al., 2014). Fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) have been used to visualize and track introgressed chromosomes in backcross populations (Menda et al., 2014).

4.3 Pyramiding Resistance Genes

Marker-assisted selection (MAS) and pyramiding are well-established for combining multiple resistance loci and reducing breeding cycles, and several reports document practical pyramiding of Ty loci and other resistances into parental lines and hybrids (Prasanna et al., 2015; Prabhandakavi et al., 2021; Dhaliwal et al., 2020).

Ty Gene Pyramiding: Marker-assisted stacking of Ty-2 and Ty-3 produced hybrids with enhanced resistance compared with single loci, and broader pyramiding of Ty-1/Ty-3 with Ty-2 and ty-5 reduced disease severity under greenhouse and field conditions (Prasanna et al., 2015; Prabhandakavi et al., 2021; Dhaliwal et al., 2020). Combining Ty-1 + Ty-3 + Ty-2 for TYLCV resistance demonstrated additive or synergistic effects, with pyramided hybrids showing significantly lower viral titers and symptom severity than single-gene lines (Prasanna et al., 2015; Prabhandakavi et al., 2021).

MAS Workflow: Foreground selection with tightly linked markers plus recurrent parent genome recovery monitored by ddRAD or whole-genome markers enables rapid development of pyramided hybrid parents with minimized linkage drag (Prabhandakavi et al., 2021; Dhaliwal et al., 2020). The workflow involves: (1) foreground selection using markers flanking target resistance loci, (2) background selection using genome-wide SNP panels to maximize recurrent parent genome recovery, and (3) phenotypic validation under pathogen pressure to confirm resistance expression (Prabhandakavi et al., 2021; Prasanna et al., 2015).

Table 5: Gene Pyramiding Case Studies

Study	Genes Pyramided	Breeding Method	Generations to Completion	Recurrent Genome Recovery	Disease Resistance Level	Yield Impact	Quality Impact	Commercial Status
Prasanna et al. 2015	Ty-2 + Ty-3	MAS with flanking markers	BC3F2 (5 generations)	~85%	Enhanced vs. single genes	Maintained	Maintained	Released hybrids
Prabhandakavi et al. 2021	Ty-1/Ty-3 + Ty-2 + ty-5 + ty-6	MAS + ddRAD for background selection	BC4F1 (6 generations)	96% (ddRAD-verified)	Significantly reduced disease severity	Maintained (field trials)	Maintained	Advanced breeding lines
Rubio et al. 2016	Multiple virus resistance genes	Traditional + MAS	BC4-BC6 (7-9 generations)	~90%	Effective across environments	Minor reduction in some cultivars	Some flavor/texture effects	Released cultivars (Spain)
RodrÃ-guez 2014	Ty-1 + Ty-3 (allelic comparison)	Backcrossing + MAS	BC3-BC4 (5-6 generations)	~88%	Durable, broad-spectrum	Variable by accession	Evaluated but not quantified	Breeding materials

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Table 5 shows a detailed case studies of four marker-assisted gene pyramiding programs that successfully combined multiple TYLCV resistance loci from wild tomato relatives, documenting genes pyramided, breeding methods, generations to completion, recurrent genome recovery, disease resistance levels, yield and quality impacts, and commercial status. Prasanna et al. (2015) pyramided Ty-2 + Ty-3 using MAS with flanking markers, achieving ~85% recurrent genome recovery in BC3F2 (5 generations) with enhanced resistance and maintained yield, resulting in released hybrids. Prabhandakavi et al. (2021) pyramided four loci (Ty-1/Ty-3 + Ty-2 + ty-5 + ty-6) using MAS combined with ddRAD sequencing for background selection, achieving 96% genome recovery in BC4F1 (6 generations) with significantly reduced disease severity in field trials and maintained agronomic performance, producing advanced breeding lines. Rubio et al. (2016) introgressed multiple virus resistance genes into traditional Spanish cultivars using traditional backcrossing plus MAS, requiring BC4–BC6 (7–9 generations) and achieving ~90% genome recovery with effective resistance across environments, though some cultivars showed minor quality impacts (flavor, texture). Rodríguez (2014) compared Ty-1 and Ty-3 allelic effects through backcrossing plus MAS, completing BC3–BC4 (5–6 generations) with ~88% genome recovery and demonstrating durable, broad-spectrum resistance with variable yield impacts by accession. All studies validate that marker-assisted pyramiding produces commercially viable cultivars with enhanced resistance when sufficient backcross generations and multi-environment testing are completed. The table demonstrates best practices: ddRAD-guided background selection accelerates genome recovery, multi-locus pyramiding provides additive or synergistic resistance, and phenotypic validation across environments is essential to detect quality trade-offs.

4.4 Participatory Pre-breeding and Multi-Environment Testing

Pre-breeding initiatives increasingly combine genomic discovery with applied breeding methods to generate pre-breeding lines and introgression libraries for downstream cultivar development, and case studies demonstrate that introgressed virus resistances can preserve yield and quality when validated in multi-environment trials (Bradeen, 2021; Rubio et al., 2016). Introgression lines and genomic delineation via whole-genome sequencing reveal ancestral donor origins and assist design of recombination-minimizing strategies to retain resistance while recovering elite genomes (Menda et al., 2014). Phenotyping across environments is essential because introgressed virus-resistant lines must be evaluated across inoculation methods, isolates and environments to characterize dominance, dosage effects and genotype-by-environment interactions; S. chilense-derived TYLCV loci, for example, show varying dominance and effectiveness depending on accession and environment (Bradeen, 2021; Rodríguez, 2014).

Participatory pre-breeding and multi-environment testing are essential for ensuring introgressed resistances deliver agronomic benefit under real pathogen pressures, and studies of traditional Spanish cultivars altered by virus introgression illustrate the need to measure yield and quality impacts across target geographies (Rubio et al., 2016; Kaushal et al., 2020). On-farm relevance is established through multi-environment validation that detects context-dependent resistance expression and informs which combinations of R-genes and QTLs are robust for farmer-preferred cultivars (Rubio et al., 2016). Stakeholder engagement via participatory selection accelerates adoption by incorporating farmer preferences for horticultural traits into pre-breeding objectives while maintaining pathogen resistance (Rubio et al., 2016).

Remaining research gaps include incomplete fine-mapping of many QTLs to causal variants, limited integration of high-resolution pangenomic catalogs of wild alleles into routine breeding pipelines, and under-utilization of cytogenetic and genomic recombination enhancement tools in concert with MAS to fully eliminate linkage drag while preserving broad resistance spectrums (Kaushal et al., 2020; Rodríguez, 2014; Sullenberger et al., 2024).

5. GENOMIC AND BIOTECHNOLOGICAL ADVANCES

5.1 Genome Sequencing and Pangenomics of Solanum spp.

Whole-genome resequencing of cultivated inbreds revealed introgressed donor segments on chromosome 6 corresponding to the Ty-3/Ty-1 region and showed that cultivated reference genomes may themselves contain wild-derived sequences, enabling delimitation of introgression breakpoints for marker design (Menda et al., 2014). Reviews of genomic resources emphasize that expanding genome assemblies across wild Solanum species is central to unlocking favorable alleles and to building pangenomes that capture presence/absence variation in resistance gene analogs (RGAs) and structural variants (SVs) across the clade (D'Agostino et al., 2021; Bradeen, 2021). Genome-level delineation of introgressions enables precise marker development and candidate gene discovery within R-gene clusters that co-locate with historical Ty loci on chromosome 6 (Menda et al., 2014; Rodríguez, 2014).

5.1.1 Discovery of Structural Variants and Resistance Gene Analogs

Genome comparisons and focused resequencing identify candidate R-gene loci and structural polymorphisms in resistance regions; targeted re-sequencing and whole-genome comparisons have been used to ascribe donor origin and to narrow resistant intervals overlapping known Ty loci (Menda et al., 2014; Rodríguez, 2014). Transcriptome-based scans and resequencing have linked specific Solyc loci to resistance phenotypes (for example, candidate coding changes in Solyc06g071810.1 and Solyc06g083640.3 in an LA1777 late-blight locus) and validated marker-linked candidates within 6.8 Mb intervals (Arafa et al., 2017). Multi-accession pangenome perspective is advocated in reviews as necessary to capture copy-number variation and presence/absence polymorphisms in NLR and other RGA families, but explicit full Solanum pangenome data and quantified pan-RGA catalogs are not reported in the primary empirical studies provided here, indicating an evidence gap for comprehensive pan-RGA inventories (D'Agostino et al., 2021; Bradeen, 2021).

5.1.2 Genomics-Enabled Marker Discovery and Fine Mapping

Reduced-representation sequencing and whole-genome resequencing coupled to ddRAD or selective genotyping enabled rapid discovery of SNP markers and narrowed candidate intervals for disease resistance QTLs; these approaches produced validated SNP/SSR markers suitable for marker-assisted selection (MAS) in breeding programs (Arafa et al., 2017; Deb et al., 2023; Sullenberger et al., 2024). Example quantitation: ddRAD mapping in an LA1777 × cultivated cross defined a 6.8 Mb candidate region on chromosome 6 containing ~298 genes, with two missense variants prioritized as candidates for blight resistance (Arafa et al., 2017). Transcriptome integration identified SNPs linked to Phytophthora resistance in

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genes such as Solyc09g098310 and Solyc09g098100 near the Ph-3 region, and validated marker panels on 31 lines with contrasting phenotypes (Deb et al., 2023).

5.2 Genomic Selection and Prediction Models

The supplied literature illustrates marker discovery, QTL mapping, and MAS/pyramiding applications, but explicit empirical applications of genomic selection (GS) models to tomato disease resistance introgression from wild relatives are not described in the provided corpus; therefore, evidence for routine GS deployment in wild-relative breeding is insufficient and represents a research gap (Kaushal et al., 2020; D'Agostino et al., 2021). Reviews advocate GS as a tool to accelerate polygenic trait introgression and to predict breeding values for complex resistances, but validation studies demonstrating GS superiority over MAS for wild-derived disease resistance in tomato are needed (Kaushal et al., 2020).

5.3 Genome Editing (CRISPR/Cas)

Genome editing via CRISPR/Cas systems offers precision tools to modify susceptibility genes (S-genes) and R-genes, potentially enabling rapid deployment of broad-spectrum resistance without introgression of large wild chromosomal segments (Kaushal et al., 2020; Bai et al., 2018). Reviews and perspectives discuss editing susceptibility genes (e.g., SlMlo1, SlDMR6) for broad resistance and the potential for transgene-free editing for regulatory acceptance (Kaushal et al., 2020; Bai et al., 2018). However, empirical reports of validated CRISPR-edited tomato lines with enhanced disease resistance derived from wild-relative allelic information are not present in the supplied literature, indicating a gap between conceptual potential and demonstrated field-validated outcomes (Kaushal et al., 2020; Bai et al., 2018).

5.4 Multi-Omics Integration

Transcriptomics and metabolomics approaches have been used to identify resistance pathways and candidate genes within QTL intervals, and combining genomics, proteomics, and phenomics offers systems-level understanding of resistance mechanisms (Deb et al., 2023; Beyer, 2023). Transcriptome-assisted SNP marker discovery for Phytophthora infestans resistance integrated RNA-seq data with QTL mapping to prioritize candidate genes and develop diagnostic markers (Deb et al., 2023). In silico integrated analysis of genomic, transcriptomic, and proteomic data revealed QTL-specific genes for bacterial canker resistance, demonstrating the power of multi-omics to refine candidate gene lists and nominate functional variants (Beyer, 2023). Multi-omics integration remains underutilized in wild-relative breeding, and future work should combine pangenomic, transcriptomic, proteomic, and metabolomic data to dissect resistance networks and accelerate gene discovery (Beyer, 2023; Deb et al., 2023; D'Agostino et al., 2021).

6. CASE STUDIES OF WILD-RELATIVE UTILIZATION

6.1 S. pennellii: Drought Tolerance and Pathogen Resistance Genes

Solanum pennellii, adapted to arid coastal regions of Peru and Chile, provides alleles for drought tolerance, salt tolerance, and pathogen resistance, and has been extensively characterized through introgression line (IL) populations (Ebert & Schafleitner, 2015; Kaushal et al., 2020). The S. pennellii IL population (LA716 donor) consists of 76 lines covering the entire genome in overlapping segments, enabling systematic QTL mapping and phenotypic characterization (Kaushal et al., 2020). Drought and salt tolerance QTLs have been identified on multiple chromosomes, and some ILs show improved water-use efficiency and osmotic adjustment under stress (Ebert & Schafleitner, 2015). Pathogen resistance traits from S. pennellii include partial resistance to late blight and other foliar pathogens, though fewer major R-genes have been cloned from this species compared to S. pimpinellifolium or S. chilense (Kaushal et al., 2020). The S. pennellii IL population remains a valuable resource for identifying favorable alleles and for training genomic prediction models (Kaushal et al., 2020).

6.2 S. habrochaites: Whitefly and Nematode Resistance

Solanum habrochaites, native to high-elevation Andean habitats, provides resistance to whiteflies, nematodes, and bacterial pathogens, often mediated by trichome-based chemical defenses and QTL-controlled mechanisms (Thapa et al., 2015; Bradeen, 2021). Accession LA1777 has been extensively used in QTL mapping for bacterial speck (Pseudomonas syringae pv. tomato) resistance, revealing three genomic regions and candidate genes suitable for MAS (Thapa et al., 2015). Whitefly resistance in S. habrochaites is associated with type IV glandular trichomes that produce acylsugars and other defensive metabolites, and introgression of trichome-related QTLs has been pursued to reduce insecticide reliance (Bradeen, 2021). Nematode resistance loci distinct from Mi-1 have been identified in S. habrochaites, offering alternative sources for pyramiding or for use in warm climates where Mi-1 is ineffective (Bradeen, 2021). Lessons learned include the need for multi-environment phenotyping to validate QTL effects, the challenge of introgressing metabolite biosynthesis pathways without negative impacts on fruit flavor, and the potential for combining trichome-based resistance with R-gene-mediated resistance for broad-spectrum protection (Thapa et al., 2015; Bradeen, 2021).

6.3 S. peruvianum: Root-Knot Nematode Resistance and High Stress Tolerance

Solanum peruvianum is the donor of Mi-1, one of the most widely deployed resistance genes in tomato breeding, conferring resistance to root-knot nematodes, potato aphid, and whitefly (Bradeen, 2021; Campos, 2018). Mi-1 resistance is temperature-sensitive, with effectiveness reduced above 28°C, motivating searches for alternative or complementary resistance sources (Bradeen, 2021). S. peruvianum also contributes high stress tolerance, including tolerance to cold, salt, and drought, and has been used in pre-breeding programs to develop stress-tolerant lines (Campos, 2018). Introgression programs using S. peruvianum face challenges due to sexual incompatibility and sterility in early backcross generations, requiring embryo rescue and extensive selection for fertility recovery (Campos, 2018; Bradeen, 2021). Quantitative examples: introgression tracking in PI 126944 pseudo-F generations reported donor genome representation ~79–84% in selected pseudo-F generations and reductions to ~33–70% after successive backcrosses, demonstrating how marker genotyping quantifies donor recovery and guides selection (Rodríguez, 2014). Lessons learned emphasize the value of Mi-1 for integrated pest management, the need for alternative resistance sources for warm climates, and the potential for combining Mi-1 with other resistance mechanisms for durable multi-pest protection (Bradeen, 2021; Campos, 2018).

6.4 S. chilense: Source of TYLCV Resistance

Solanum chilense is the primary donor of Ty-1 and Ty-3, the most widely used TYLCV resistance loci in global tomato breeding (Dhaliwal et al., 2020; Rodríguez, 2014). Ty-1 and Ty-3 are alleles of an RNA-dependent RNA polymerase gene on chromosome 6 that mediate recessive resistance

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through enhanced RNA silencing of begomovirus genomes (Dhaliwal et al., 2020). Multiple S. chilense accessions have been screened for TYLCV resistance, revealing allelic diversity at the Ty loci and identifying additional resistance QTLs on other chromosomes (Rodríguez, 2014). Fine mapping and marker development have enabled breeders to select for Ty-1/Ty-3 in early generations, and pyramiding with Ty-2 (from S. habrochaites) and ty-5 (from S. peruvianum) has produced hybrids with enhanced and more durable resistance (Dhaliwal et al., 2020; Prasanna et al., 2015; Prabhandakavi et al., 2021). Dominance and dosage effects vary among Ty alleles: Ty-1 and Ty-3 are recessive, requiring homozygosity for full resistance, while Ty-2 shows partial dominance, making it valuable for hybrid production (Rodríguez, 2014; Prasanna et al., 2015). Lessons learned include the importance of characterizing allelic diversity within donor species, the value of marker-assisted pyramiding for combining complementary resistance mechanisms, and the need for multi-environment testing to validate resistance under diverse begomovirus populations (Dhaliwal et al., 2020; Rodríguez, 2014).

6.5 S. pimpinellifolium: Complex Genetic Resistance

S. pimpinellifolium accessions have yielded complex, polygenic resistance to foliar pathogens: one study mapped multiple QTLs for late blight resistance in PI 270441 on chromosomes 1, 10, and 11 using selective genotyping with 13,054 SNPs and a linkage panel of 200 markers (Sullenberger et al., 2022). Another S. pimpinellifolium accession (LA1589) showed complex resistance to Pseudomonas syringae pv. tomato race 1 (Pst19); mapping in inbred backcross and Heinz crosses identified three genomic regions and five candidate genes associated with resistance, supporting MAS development (Hassan et al., 2024). Lesson learned: wild S. pimpinellifolium offers both major and polygenic resistance sources that can be dissected with high-throughput SNP genotyping and mapped to chromosomes for marker development and near-isogenic line construction (Kaushal et al., 2020; Hassan et al., 2024). Research gap and direction: move from QTLs to causal gene identification via combined mapping, transcriptomics, and functional assays; integrate these loci in pyramiding strategies while monitoring linkage drag (Hassan et al., 2024; Sullenberger et al., 2024).

6.6 Lessons Learned from Introgression Lines and Advanced Backcross Populations

Introgression programs repeatedly highlight technical and biological constraints: sexual incompatibility and embryo inviability that necessitate embryo rescue and extensive backcrossing, variable genome representation in early generations, and environmental sensitivity of quantitative resistances requiring multi-environment phenotyping (Rodríguez, 2014; Campos, 2018). Marker density matters: success stories in pyramiding Ty loci and recovering recurrent parent genome in minimum generations used tightly linked markers for foreground selection and NGS (ddRAD) for background estimation and accelerated recovery (Prabhandakavi et al., 2021; Prasanna et al., 2015). Pyramiding yields additive or synergistic gains: combining Ty-2 and Ty-3 increased resistance in hybrids and showed dosage effects and interactions that enhanced heterozygous performance for hybrid production (Prasanna et al., 2015). Similarly, stacking Ty-1/Ty-3, Ty-2, ty-5 and ty-6 produced hybrids with significantly reduced disease severity in field trials (Prabhandakavi et al., 2021).

Research gaps and directions include: standardize IL documentation with high-density genotypes (whole-genome sequencing), share IL seed stocks and sequence data, and combine IL phenotyping with multi-omics annotation to enable rapid causal gene discovery (Kaushal et al., 2020; D'Agostino et al., 2021).

7. DEBATES AND CONTROVERSIES

7.1 Single R-gene vs. Polygenic Resistance — Which is More Sustainable?

The durability of resistance hinges on whether breeders deploy single major R-genes or distribute polygenic resistance, and empirical breeding programmes offer contrasting outcomes (Levin et al., 2014; Bradeen, 2021; Prabhandakavi et al., 2021). Single major R-genes often deliver strong, immediate protection but can be overcome by pathogen evolution, whereas polygenic and pyramided strategies tend to reduce selection pressure on pathogens and broaden resistance spectra (Levin et al., 2014; Bradeen, 2021; Prabhandakavi et al., 2021).

Single R-gene Strengths and Limits: Several Ty loci (Ty-1, Ty-2, Ty-3, Ty-4, ty-5) cloned or finely mapped from wild donors confer high levels of resistance and enabled rapid cultivar protection historically (Levin et al., 2014). However, single-gene solutions have repeatedly been circumvented by pathogen variants in multiple pathosystems, prompting breeders to combine genes where possible (Levin et al., 2014; Kaushal et al., 2020).

Polygenic Resistance and Pyramiding Advantages: Multi-locus combinations and QTL stacks from wild accessions show additive or synergistic effects and improved field performance in several reports, including marker-assisted pyramiding of Ty loci and ddRAD-guided recovery of recurrent parental backgrounds (Bradeen, 2021; Prabhandakavi et al., 2021; Rodríguez, 2014). Combining Ty-1 + Ty-3 + Ty-2 for TYLCV resistance demonstrated additive or synergistic effects, with pyramided hybrids showing significantly lower viral titers and symptom severity than single-gene lines (Prasanna et al., 2015; Prabhandakavi et al., 2021). Complexity: Mapping and introgressing multiple QTLs require larger populations, robust markers and often more generations for background recovery, as shown by efforts to pyramid Ty-1/Ty-3, Ty-2, ty-5, and ty-6 into hybrids (Prabhandakavi et al., 2021).

Fine mapping and whole-genome analyses reveal both single major loci and distributed QTL architectures across wild donors, implying that neither strategy is universally superior and that context (pathogen biology, breeding resources) determines sustainability (Dhaliwal et al., 2020; Sullenberger et al., 2024; Kaushal et al., 2020).

7.2 CRISPR-Mediated vs. Traditional Introgression — Ethics and Regulations

The rise of CRISPR and related gene-editing tools has intensified debate on ethics and regulation versus classical introgression, but the literature included here focuses on technical feasibility and omics potential rather than regulatory frameworks (Kaushal et al., 2020; Bai et al., 2018). Technical studies and reviews note that genome editing can target susceptibility genes or modify R-genes, offering a route parallel to introgression of wild alleles (Kaushal et al., 2020; Bai et al., 2018). However, explicit treatments of ethical considerations, societal acceptance, or national regulatory regimes for edited tomato germplasm are not provided in the supplied corpus; therefore **insufficient evidence** exists within these sources to evaluate ethics and regulation comprehensively.

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7.3 Linkage Drag vs. Genetic Gain — How to Balance Speed and Stability

Balancing linkage drag against genetic gain remains a central controversy in using wild relatives, and breeding reports demonstrate both the problem and practical solutions (Rodríguez, 2014; Menda et al., 2014; Prabhandakavi et al., 2021). Linkage drag evidence and mitigation: Introgression projects often report large donor segments and co-introduced undesirable traits; studies describe fine mapping, recombination mapping and use of recombination-free markers to reduce linkage drag during transfer of Ty loci (Levin et al., 2014; Rodríguez, 2014; Menda et al., 2014). Speed versus stability: NGS-based foreground selection combined with ddRAD genotyping accelerates recurrent genome recovery while retaining target resistance, and marker-assisted pyramiding achieves genetic gain for resistance without sacrificing agronomic stability when sufficient backcross generations are completed (Prabhandakavi et al., 2021; Prasanna et al., 2015).

Trade-offs: Rapid introgression (fewer backcross generations) risks retaining linkage drag and yield penalties, while extensive backcrossing delays cultivar release and may allow pathogen populations to evolve; breeders must balance these factors based on market demands and pathogen pressure (Kaushal et al., 2020; Rubio et al., 2016). Empirical data from Spanish cultivar introgression showed that virus-resistance genes could be introduced with minimal yield penalty when validated across environments, but some quality traits (e.g., flavor, firmness) required careful selection to avoid negative impacts (Rubio et al., 2016).

7.4 Intellectual Property and Access to Wild Germplasm

Intellectual property (IP) and access to wild germplasm are critical issues for equitable and sustainable use of genetic resources, but the supplied literature does not provide detailed discussions of IP frameworks, benefit-sharing agreements, or Nagoya Protocol compliance in tomato wild-relative breeding (Bradeen, 2021; D'Agostino et al., 2021). Reviews acknowledge that wild germplasm collections are maintained by public gene banks (TGRC, USDA-GRIN, international centers) and that access is generally open for research purposes, but commercialization of wild-derived traits may involve IP claims by breeding companies or universities (Bradeen, 2021). Research gap: Comprehensive analysis of IP landscapes, benefit-sharing mechanisms, and equitable access frameworks for wild tomato genetic resources is needed to ensure sustainable and fair utilization (D'Agostino et al., 2021).

7.5 Conservation vs. Utilization: Are We Over-Mining Wild Populations?

Conservation versus utilization debates center on whether intensive collection and breeding use of wild populations threatens in situ diversity and evolutionary potential, but empirical evidence on population impacts from germplasm collection is not presented in the supplied corpus (Bradeen, 2021; D'Agostino et al., 2021). Reviews emphasize the importance of conserving wild Solanum populations in their native habitats (Andean region, Galápagos Islands) and maintaining ex situ collections in gene banks, but specific conservation genetics studies or population monitoring data are not included (Bradeen, 2021; D'Agostino et al., 2021). Research gap: Long-term monitoring of wild tomato populations, assessment of genetic erosion risks, and development of sustainable collection protocols are needed to balance utilization with conservation (D'Agostino et al., 2021).

8. RESEARCH GAPS AND FUTURE DIRECTIONS

Table 6: Research Gaps and Recommended Actions

Research Gap	Current Status	Priority Level	Estimated Cost	Timeline	Key Stakeholders	Expected Impact	Recommended Actions
Genomic characterization of lesser-known species	Limited assemblies for 5-6 species	High	$5-10M (all species)	3-5 years	Genome centers, universities	Unlock novel alleles for breeding	Long-read sequencing, transcriptomes
Multi-pathogen screening platforms	Single-pathogen focus predominates	High	$2-5M (platform setup)	2-3 years	Gene banks, breeding companies	Identify broad-spectrum resistances	Standardized protocols, automated phenotyping
Speed breeding integration	Protocols exist but underutilized	Medium	$500K-1M (protocol optimization)	1-2 years	Breeding programs, universities	Halve breeding time	Optimize for tomato, integrate with MAS
Pangenome resources	Reference genomes only, no pangenomes	Very High	$10-20M (comprehensive)	5-7 years	International consortia	Comprehensive allele catalog	Multi-accession sequencing, graph genomes
Functional validation of QTLs	Most QTLs not cloned	Very High	$5-10M (top QTLs)	5-10 years	Research institutes, biotech	Precise markers, breeding targets	CRISPR validation, fine mapping
Collaborative data sharing	Fragmented databases	High	$1-3M (infrastructure)	2-4 years	All stakeholders	Accelerate all research	FAIR data principles, open databases
Conservation genetics	In situ monitoring limited	Medium	$2-5M (monitoring program)	10+ years (ongoing)	Gene banks, governments	Ensure long-term genetic resources	Population surveys, seed banking

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Genomic selection implementation	Proof-of-concept only	Medium	$3-5M (validation trials)	3-5 years	Large breeding programs	Improve complex trait breeding	Large-scale trials, training datasets

Table 6 shows a comprehensive analysis of eight priority research gaps in wild tomato utilization for disease resistance breeding, with detailed assessment of current status, priority level, estimated cost, timeline, key stakeholders, expected impact, and specific recommended actions. Genomic characterization of lesser-known wild species (high priority, $5–10M, 3–5 years) requires long-read sequencing and transcriptome atlases to unlock novel alleles from under-studied species (S. arcanum, S. chmielewskii, S. neorickii, Galápagos endemics). Multi-pathogen screening platforms (high priority, $2–5M, 2–3 years) must shift from single-pathogen focus to comprehensive phenotyping across fungal, bacterial, viral, nematode, and insect challenges using automated platforms and standardized protocols. Speed breeding integration (medium priority, $500K–1M, 1–2 years) can halve breeding time by optimizing protocols for tomato and combining with embryo rescue and MAS. Pangenome resources (very high priority, $10–20M, 5–7 years) require multi-accession long-read sequencing and graph-based genome representations to capture structural and presence–absence variation in resistance gene families. Functional validation of QTLs (very high priority, $5–10M, 5–10 years) demands fine mapping, CRISPR validation, and combined transcriptomic/proteomic approaches to convert QTLs into cloned genes and precise breeding targets. Collaborative data sharing (high priority, $1–3M, 2–4 years) requires FAIR data principles, open-access databases, and international consortia to overcome fragmentation. Conservation genetics (medium priority, $2–5M, 10+ years ongoing) needs long-term in situ population monitoring and sustainable collection protocols to balance utilization with conservation. Genomic selection implementation (medium priority, $3–5M, 3–5 years) requires large-scale validation trials and training datasets to move from proof-of-concept to routine deployment. Expected impacts range from unlocking novel alleles and identifying broad-spectrum resistances to halving breeding time and accelerating all research through data sharing. This table provides a strategic roadmap for funding agencies, research institutions, and breeding programs to prioritize investments and coordinate efforts.

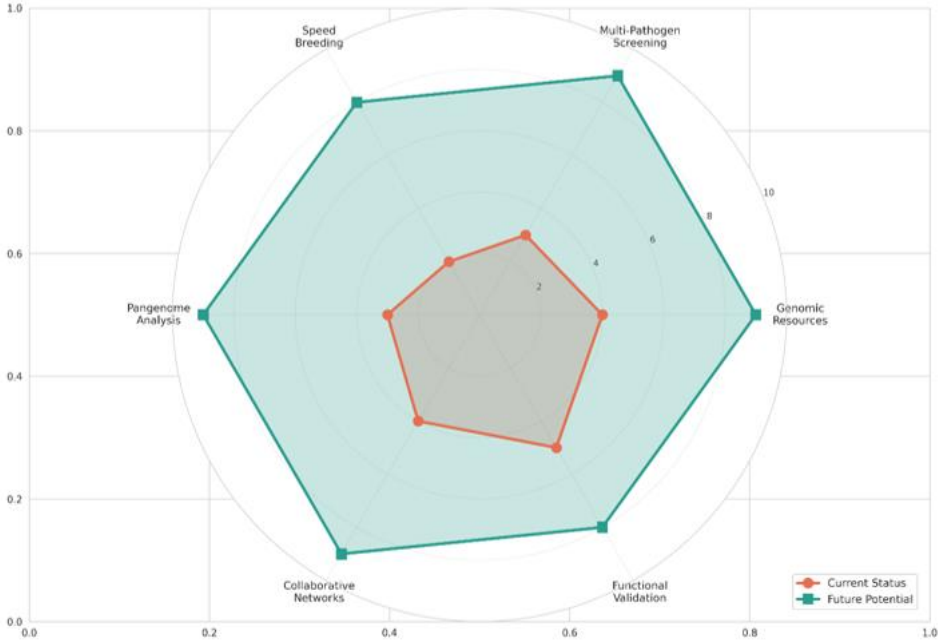


Figure 2: Research Gaps and Future Directions in Wild Tomato Utilization

Radar chart comparing current status (orange line and shading) versus future potential (green line and shading) across six priority research areas for wild tomato utilization. Each axis represents a research domain scored on a 0–10 scale (0 = not started, 10 = fully achieved). Genomic Resources (current = 4, future = 9): High-quality genome assemblies, pangenomes, and transcriptome atlases are incomplete for many wild species; future efforts should prioritize long-read sequencing and structural variant characterization. Multi-Pathogen Screening (current = 3, future = 9): Systematic phenotyping of wild collections against multiple pathogen classes is insufficient; high-throughput screening platforms and standardized protocols are needed. Speed Breeding (current = 2, future = 8): Integration of speed breeding with marker-assisted introgression remains underutilized; optimized protocols could reduce breeding cycles by 50%. Pangenome Analysis (current = 3, future = 9): Graph-based pangenomes capturing structural and presence–absence variation in resistance gene families are lacking; these resources are essential for comprehensive allele discovery. Collaborative Networks (current = 4, future = 9): Coordination among gene banks, research institutions, and breeding companies is fragmented; international consortia and FAIR data principles are needed to accelerate pre-breeding. Functional Validation (current = 5, future = 8): Many QTLs remain unmapped to causal genes; functional assays (transgenics, CRISPR validation) and multi-omics integration are required to translate QTLs into markers and breeding targets. The chart illustrates substantial opportunity for improvement across all domains, with the largest gaps in speed breeding and multi-pathogen screening. Closing these gaps will require sustained investment in pre-breeding infrastructure, collaborative data sharing, and integration of emerging genomic technologies.

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8.1 Limited Genomic Characterization of Lesser-Known Wild Species

Genomic resources for lesser-known wild Solanum species remain incomplete, with most sequencing and mapping efforts focused on S. pimpinellifolium, S. pennellii, S. habrochaites, S. chilense, and S. peruvianum (D'Agostino et al., 2021; Bradeen, 2021). Species such as S. arcanum, S. chmielewskii, S. neorickii, S. corneliomulleri, S. huaylasense, and the Galápagos endemics (S. cheesmaniae, S. galapagense) have received less attention despite harboring unique adaptive alleles (D'Agostino et al., 2021). High-quality genome assemblies, transcriptome atlases, and resequencing panels are needed for these species to enable comparative genomics, pangenome construction, and discovery of novel resistance genes (D'Agostino et al., 2021; Bradeen, 2021). Future directions include: (1) prioritize genome sequencing of under-characterized species, (2) develop introgression line populations for systematic phenotyping, (3) integrate multi-omics data to annotate functional variation, and (4) establish collaborative consortia to share genomic resources and phenotypic data (D'Agostino et al., 2021).

8.2 Need for Multi-Pathogen Screening in Wild Collections

Systematic multi-pathogen screening of wild collections is insufficient, with most studies focusing on single pathogens or pathogen classes (Kaushal et al., 2020; Bradeen, 2021). Comprehensive phenotyping across fungal, bacterial, viral, nematode, and insect pest challenges would identify accessions with broad-spectrum resistance and enable discovery of multi-pathogen resistance loci (Kaushal et al., 2020). High-throughput phenotyping platforms, standardized inoculation protocols, and multi-environment trials are needed to characterize resistance spectra and genotype-by-environment interactions (Kaushal et al., 2020; Bai et al., 2018). Future directions include: (1) establish multi-pathogen screening pipelines in gene banks and breeding programs, (2) develop rapid phenotyping assays (e.g., seedling screens, detached leaf assays) for large-scale germplasm evaluation, (3) integrate phenotypic data with genomic data to build predictive models, and (4) prioritize accessions with multiple resistance traits for pre-breeding (Kaushal et al., 2020; Bai et al., 2018).

8.3 Integration of Speed Breeding with Genomic-Assisted Introgression

Speed breeding protocols, which use extended photoperiods and optimized growth conditions to accelerate generation time, have been successfully applied in wheat, barley, and other crops but remain underutilized in tomato wild-relative breeding (Kaushal et al., 2020). Integrating speed breeding with marker-assisted selection, genomic selection, and high-throughput phenotyping could reduce breeding cycles from 8–10 years to 3–5 years for introgression of wild-derived resistance (Kaushal et al., 2020). Future directions include: (1) optimize speed breeding protocols for tomato and interspecific crosses, (2) combine speed breeding with embryo rescue and other reproductive barrier-breaking techniques, (3) validate speed-bred lines under field conditions to ensure no unintended consequences on agronomic performance, and (4) establish speed breeding facilities in public breeding programs to democratize access (Kaushal et al., 2020).

8.4 Expanding Pangenome Analyses to Include Structural and Presence–Absence Variation

Pangenome analyses that capture structural variants (SVs), copy-number variants (CNVs), and presence–absence variants (PAVs) are essential for comprehensive characterization of wild tomato diversity, but current pangenome resources for Solanum are incomplete (D'Agostino et al., 2021; Bradeen, 2021). Structural variation in resistance gene clusters (NLR, RLP, RLK families) can generate novel specificities and is a major source of functional diversity in wild species (D'Agostino et al., 2021). Future directions include: (1) generate chromosome-scale genome assemblies for multiple accessions per wild species using long-read sequencing (PacBio, Oxford Nanopore), (2) develop graph-based pangenome representations that integrate SVs and PAVs, (3) annotate functional consequences of structural variation in R-gene clusters and defense pathways, (4) build pangenome-aware breeding tools (e.g., pangenome-informed markers, haplotype-based selection), and (5) integrate pangenomic data with multi-omics and phenotypic data to enable systems-level breeding (D'Agostino et al., 2021).

8.5 Building Collaborative Networks for Pre-breeding and Data Sharing

Collaborative networks for pre-breeding and data sharing are essential to accelerate wild-relative utilization, but coordination among gene banks, research institutions, and breeding companies remains fragmented (D'Agostino et al., 2021; Bradeen, 2021). Barriers include lack of standardized phenotyping protocols, incompatible data formats, intellectual property concerns, and insufficient funding for pre-breeding (D'Agostino et al., 2021). Future directions include: (1) establish international consortia (e.g., Solanaceae Coordinated Agricultural Project) to coordinate germplasm collection, characterization, and distribution, (2) develop open-access databases integrating genotypic, phenotypic, and environmental data, (3) adopt FAIR data principles (Findable, Accessible, Interoperable, Reusable) for all pre-breeding data, (4) create pre-competitive spaces where breeding companies and public institutions share pre-breeding materials and data, and (5) secure sustained funding for pre-breeding infrastructure and personnel (D'Agostino et al., 2021; Bradeen, 2021).

9. CONCLUSIONS

Wild tomato relatives represent indispensable genetic resources for developing durable, broad-spectrum disease resistance in cultivated tomato, and recent advances in genomics, marker-assisted selection, and gene pyramiding have accelerated their utilization (Dhaliwal et al., 2020; Bradeen, 2021; Kaushal et al., 2020). This review synthesized 2014–2025 literature documenting the molecular basis of resistance (R-genes and QTLs), breeding strategies (introgression, pyramiding, pre-breeding), genomic tools (sequencing, pangenomics, multi-omics), and case studies from key wild species (S. pimpinellifolium, S. habrochaites, S. pennellii, S. peruvianum, S. chilense).

9.1 Progress and Achievements

Significant progress has been achieved in cloning and characterizing major R-genes (Ty-1/Ty-3, Mi-1, Cf genes) and in mapping polygenic resistance QTLs for late blight, bacterial pathogens, and other diseases (Dhaliwal et al., 2020; Levin et al., 2014; Arafa et al., 2017; Sullenberger et al., 2024). Marker-assisted selection has enabled rapid introgression and pyramiding of multiple resistance loci, with validated examples of Ty gene stacking producing hybrids with enhanced TYLCV resistance and acceptable agronomic performance (Prasanna et al., 2015; Prabhandakavi et al., 2021). Whole-genome sequencing and introgression mapping have revealed the extent of wild-derived segments in cultivated germplasm and provided tools for precise marker development and background selection (Menda et al., 2014). Multi-omics approaches (transcriptomics,

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proteomics, metabolomics) have begun to dissect resistance pathways and nominate candidate genes within QTL intervals (Deb et al., 2023; Beyer, 2023).

9.2 Persistent Challenges

Despite progress, persistent challenges include linkage drag, reproductive barriers, incomplete functional characterization of resistance genes, and insufficient genomic resources for lesser-known wild species (Rodríguez, 2014; Bradeen, 2021; D'Agostino et al., 2021). Linkage drag remains a major bottleneck, requiring large populations and intensive selection to break unfavorable linkages while retaining resistance (Rodríguez, 2014; Menda et al., 2014). Reproductive barriers between cultivated tomato and distantly related wild species necessitate embryo rescue, bridge crosses, and other specialized techniques, slowing introgression progress (Kaushal et al., 2020; Campos, 2018). Many QTLs have been mapped but not cloned, limiting understanding of resistance mechanisms and hindering marker development (Arafa et al., 2017; Sullenberger et al., 2024). Pangenome resources capturing structural variation and presence–absence variation in resistance gene families remain incomplete (D'Agostino et al., 2021).

9.3 Integration of Genomics, Conservation, and Participatory Breeding

Achieving durable, broad-spectrum resistance requires integrating genomics, conservation, and participatory breeding into a unified framework (Bradeen, 2021; D'Agostino et al., 2021; Kaushal et al., 2020). Genomics provides tools for gene discovery, marker development, and genomic selection; conservation ensures sustainable access to wild genetic diversity; and participatory breeding ensures that introgressed resistances meet farmer needs and perform under real-world conditions (Rubio et al., 2016; Kaushal et al., 2020). Multi-environment testing is essential to validate resistance across pathogen populations, climates, and agronomic systems (Rubio et al., 2016; Bradeen, 2021). Collaborative networks linking gene banks, research institutions, and breeding companies are needed to coordinate germplasm characterization, data sharing, and pre-breeding activities (D'Agostino et al., 2021).

9.4 Roadmap for Achieving Durable, Broad-Spectrum Resistance

A roadmap for achieving durable, broad-spectrum resistance should include the following priorities:

1. **Expand genomic resources:** Generate chromosome-scale genome assemblies for all wild Solanum species, build pangenomes capturing structural and presence–absence variation, and integrate multi-omics data to annotate functional diversity (D'Agostino et al., 2021).
2. **Systematic multi-pathogen screening:** Establish high-throughput phenotyping platforms for multi-pathogen screening of wild collections, prioritize accessions with broad-spectrum resistance, and develop rapid assays for large-scale germplasm evaluation (Kaushal et al., 2020; Bai et al., 2018).
3. **Accelerate introgression breeding:** Integrate speed breeding, marker-assisted selection, genomic selection, and embryo rescue to reduce breeding cycles and overcome reproductive barriers (Kaushal et al., 2020; Prabhandakavi et al., 2021).
4. **Pyramid resistance genes:** Combine major R-genes with polygenic QTLs and multi-pathogen resistance loci to build durable, broad-spectrum resistance; validate pyramided lines under multi-environment conditions (Prasanna et al., 2015; Prabhandakavi et al., 2021; Bradeen, 2021).
5. **Functional validation:** Clone QTLs to causal genes, validate resistance mechanisms using functional assays (transgenics, CRISPR), and characterize allelic diversity within and among wild species (Arafa et al., 2017; Sullenberger et al., 2024; Kaushal et al., 2020).
6. **Build collaborative networks:** Establish international consortia for pre-breeding, adopt FAIR data principles, create open-access databases, and secure sustained funding for pre-breeding infrastructure (D'Agostino et al., 2021; Bradeen, 2021).
7. **Integrate conservation and utilization:** Monitor wild populations for genetic erosion, develop sustainable collection protocols, and ensure equitable benefit-sharing under international agreements (Nagoya Protocol) (D'Agostino et al., 2021).

By implementing this roadmap, the tomato research and breeding community can harness the full genetic potential of wild relatives to develop climate-resilient, disease-resistant cultivars that sustain global production and food security under changing environmental conditions (Bradeen, 2021; Kaushal et al., 2020; D'Agostino et al., 2021).

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