

Mapping of early blight (*Alternaria solani*) disease tolerance in Tomato (*Solanum Lycopersicum* L.) through QTL-Seq and expression analysis of defense-related genes

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Abstract

Early blight disease, caused by the necrotrophic fungi *Alternaria solani*, is one of the most common and destructive foliar diseases affecting tomatoes. It initially appears as small, dark brown lesions with concentric rings on the lower leaves, which eventually yellow and die, leading to severe defoliation and reduced yield. In the present study, an F₂ segregating population derived from contrasting parents was used to identify major quantitative trait loci (QTLs) governing early blight tolerance using QTL-Seq, a bulked segregant analysis method. The analysis was based on bulked DNA pools of susceptible and resistant F₂ populations derived from a cross between the Punjab Chhuharah, a susceptible parent, and IIHR-2891, a resistant parent. Based on the sliding window analysis for SNP-index, Δ SNP-index plots, five novel QTLs across two chromosomes specifically chromosome numbers 2 and 6 were identified for early blight tolerance. Four QTL regions (*eb2.1*, *eb2.2*, *eb2.3*, and *eb2.4*) were located on chromosome 2, and another QTL region (*eb6.1*) was identified on chromosome 6. The identification and expression analysis of defense-related genes located in QTL regions like *NBS-LRR*, *DRG*, *RPG*, *ZFT*, *S1WRKY1* and *PROIHIN* during disease progression in contrasting parents suggests that these genes play a major role in early blight tolerance.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important warm-season vegetable crops cultivated worldwide originally native to South America, including Peru, and the Galapagos Islands (1). Early blight (EB) disease is the most significant of the fungal diseases affecting tomatoes, drastically reducing yield in tropical and subtropical regions. EB causes extensive defoliation, exposes the fruit to sunscald and further reduces yields. The disease typically begins on the older leaves and gradually progresses upward through the plant. To date, no tomato cultivar has shown complete resistance to EB. This is due to the polygenic nature of EB tolerance and its strong association with various undesirable traits such as physiological maturity, fruit load, and plant type, making traditional breeding for EB resistance a challenging and demanding process. Additionally, EB resistance is often higher in late-maturing, low-yielding, and indeterminate tomato lines, which has hindered the development of EB-resistant cultivars with desirable traits through phenotypic selection.

Recent marker-assisted selection breeding programs require stable QTLs with strong additive effects and minimal epistatic interactions. Keeping this in mind, efforts are being made to map quantitative trait loci (QTL) controlling EB resistance and to identify markers tightly linked to EB resistant QTLs. QTLs have been discovered in wild relatives of cultivated tomato *S. habrochaites*, *S. pimpinellifolium*, and *S. arcanum*. In the *S. habrochaites* PI126445 accession, the QTL on chromosome 9 explained the highest percentage of phenotypic variance (25.6%) for EB resistance (2, 3). In a segregation population from resistant *S. arcanum* LA2157 and a susceptible *S. lycopersicum* cv. 'Solentos', six QTLs for EB resistance were discovered, located on chromosomes 1, 2, 5, 6, 7 and 9 with phenotypic variation ranging from 7 to 16 percent for each EB resistance QTL (4). QTLs were identified using PI126445 (*S. habrochaites*) and EB susceptible tomato breeding line NC84173 population, finding seven RGAs on chromosomes 3, 4, 5 and 6 (3). EB resistance was mapped using a susceptible tomato breeding line NC84173 and a resistant *S. habrochaites* accession PI126445 (3). They found 10 resistant QTLs for EB in both BC₁ and BC₁S₁ populations. Individual QTLs explained 8.4% to 25.9% of total phenotypic variation, with the combined effect exceeding 57%. A distinctive and potentially novel form of EB foliar resistance has been discovered in a processed tomato breeding line, and it is thought to be derived from *S. pimpinellifolium* via 'Hawaii 7998'. The three most promising large-effect QTLs, EB-1.2, EB-5 and EB-9, were validated in field trials. Only two QTLs, EB-5 and EB-9 were shown to be responsible for the majority of the resistance in these lines, accounting for 49.0 and 68.7% of the

variance in defoliation and stem lesion respectively (5). However, there have been limited efforts to identify SNPs linked to QTL regions for EB.

Bulked-segregant analysis (BSA) is an efficient method for rapidly identifying molecular markers that are tightly linked to the trait of interest (6, 7) and this method involves creating high and low bulks by pooling DNAs from 10–20 individual plants that exhibit contrasting traits for a given phenotype in segregating populations. However, despite its utility, the availability of DNA markers and genotyping of each marker for the two bulked DNAs can be time-consuming and costly, which limits the efficacy of BSA (8). The combination of BSA and high-throughput whole-genome re-sequencing known as QTL-Seq has been successfully employed in the identification of QTLs for important agronomic traits, such as rice blast disease resistance and seedling vigor, using RILs and F₂ populations (8). Since this, the QTL-Seq approach has been widely applied in cucumber (9, 10, 11), tomato (12, 13), groundnut (14) and peanut (15). The current study used QTL-Seq to identify QTLs associated with resistance to EB disease in an F₂ population derived from a cross between the resistant line IIHR-2891 and the susceptible variety Punjab Chhuharah (PC). Further, the expression of genes residing in the QTLs regions was studied to examine their role.

2. Material and methods

2.1. Development of Plant Material, Phenotyping

This study utilized two contrasting tomato genotypes: IIHR-2891 (an EB -tolerance breeding line from ICAR-IIHR, Bengaluru) and PC (a high-yielding but EB-susceptible popular variety from northern India). These parental lines, selected based on previous screening (mean PDI of 11.25 & 60.83, respectively), were crossed to generate a segregating F₂ population of 298 individuals. For phenotyping, a detached leaf assay was employed. Leaf samples (three replicates per plant) were collected 30 days after transplanting. Artificial inoculation was performed using a 5 mm mycelial disc from a seven-day-old *Alternaria solani* colony, placed on leaflets incubated at 22°C under a 12/12-hour light/dark cycle with high relative humidity (90–95%). Disease severity was assessed on the seventh day using a 0–4 scale, and the Percent Disease Index (PDI) was calculated. Based on PDI values, the F₂ individuals and parents were categorized into resistance groups (Fig. 4&5). From the screened population, two extreme bulks—a resistant bulk (R-bulk) and a susceptible bulk (S-bulk)—each comprising 30 individuals, were constructed for QTL-Seq analysis. Genomic DNA was isolated from all F₂ plants using a modified CTAB method, with quality and concentration verified by agarose gel electrophoresis and spectrophotometry. Whole-genome resequencing of the parental lines and the two phenotypic bulks was then conducted to identify genomic regions associated with EB resistance. For the QTL-Seq analysis two DNA bulks the Resistant DNA bulk (R-bulk) and susceptible DNA bulk (S-bulk) were constructed. Each bulk was made by mixing equal amounts of DNA from 30 susceptible and 30 resistant F₂ plants separately. Using four sequencing libraries, R- bulk, S- bulk, and the two parental genotypes (PC and IIHR-2891) were made. These libraries were sequenced using the Illumina HiSeq-2500 platform (Illumina, CA, USA), at M/S Sandor Specialty Diagnostics Pvt. Ltd. Hyderabad facility following the manufacturer's instructions.

2.2 QTL-Seq Analysis

The QTL-Seq analysis employed bulked segregant analysis, following method described by (10). Short reads with a Phred quality score of < 20 were excluded from the analysis to obtain high-quality reads. The clean reads of resistant (IIHR-2891) parent were aligned to the tomato reference genome (SL3.0) (The Tomato Genome Consortium [www.Sol Genomics networksolgenomics.net/organism/Solanum_lycopersicum/genome](http://www.Sol_Genomics_networksolgenomics.net/organism/Solanum_lycopersicum/genome)) using BWA (Burrows-Wheeler Aligner) aligner (version 0.7.15) software (20), and alignment files were converted to SAM/BAM

files using SAM tools version 1.4.1 (21). The resulting BWA alignment of reads from resistant and susceptible bulk against IIHR-2891 imputed reference was performed to obtain alignment files (SAM). SAM files were processed for generating Variation (vcf) file to identify SNPs.

To estimate confidence intervals of the SNP-index value under the null hypothesis of no QTL, a computer simulation was done. Initially, two bulks of progeny were created with a specified number of individuals by random sampling. From each bulk, a given number of alleles corresponding to the read depth were sampled, then the SNP-index was calculated for each bulk, and Δ SNP-index was derived. An average Δ SNP index of SNPs mapped across the 12 tomato chromosomes was calculated using sliding-window analysis with 1 Mb window size and 10 kb increment. The SNP-index graphs for the resistant pool and susceptible pool and the corresponding Δ SNP index graph were plotted to generate SNP index plots (8).

2.3 The identification of genes within QTL regions relevant to EB tolerance

The QTL regions for EB tolerance were identified based on the Δ SNP-index confidence interval of 95 percent and above. Regions with a mean P-value 0.05 were considered as putative QTLs. Using the Genome browser (JBrowse; ITAG4.1 gene models) of Sol Genomics Network (SGN; <http://solgenomics.net/>), putative candidate genes were identified spanning 1 kb distance from either side of each QTL region.

2.4 Validation of selected QTLs (candidate genes) identified using QTLseq.

For gene expression analysis, plants were inoculated with *A. solani* via detached leaf assay as described above. RNA was extracted from leaf tissue surrounding the inoculation site at 0, 3, 9, 15, and 21 days post-inoculation (DPI). Each biological replicate consisted of pooled leaf tissue from three plants, and three technical replicates were performed for each qPCR reaction for control and infected plants. Subsequently, cDNA was synthesized using HicDNA synthesis kit (Mol Bio HIMEDIA: Cat No. MBT076100R). qRT-PCR was performed in Quantstudio7Flex thermal cycler (Applied biosystems) using the intercalation dye TB Green Premix Ex Taq II (TaKaRa Cat No. RR820A). The primer sequences as listed in Table 3 were used. qPCR analysis was carried out (Sorrequieta et al. 2010) with the following reaction conditions: 30 sec at 95°C; 40 cycles of 5 sec at 95°C, 40 sec at 59°C and 30 sec at 72°C followed by melting curve conditions: 95°C for 15 sec, 60°C for 1 min, and 95°C for 15 sec. The relative expression level was calculated using the $2^{-\Delta\Delta C_t}$ method (23). The tomato actin (AC1) was used as a reference gene. The $2^{-\Delta\Delta C_t}$ values of samples were compared statistically using analysis of variance (ANOVA) an online statistical software package for Agricultural Research-OPSTAT (24). In all analyses, $P < 0.01$ was considered statistically significant and Duncan's Multiple Range test was performed for multiple comparisons.

3. Results

The disease severity scored on leaves of each sample was used to calculate the PDI. A total of 298 F_2 plants derived from the cross between Punjab Chhuharah (susceptible) and IIHR-2891 (resistant) were evaluated for early blight disease reaction and grouped into five classes based on disease score. Of these, 30 plants (10.1%) were highly tolerant (0–20), 111 (37.2%) were tolerant (21–40), 109 (36.6%) were moderately susceptible (41–60), 31 (10.4%) were susceptible (61–80) and 17 (5.7%) were highly susceptible (81–100) (Fig. 2). Among the genotypes,

disease expression was highest in PC (70.50) and lowest in IIHR-2891 (16.50). The mean values of disease progression in F₂ (47.93). The heatmap of the F₂ population (Fig. 3) illustrates the early blight disease score of each of the 298 plants according to its position in the plant grid. The majority of the plants showed intermediate disease scores, whereas a small number of plants showed very low scores (highly tolerant) or very high scores (highly susceptible). Plants with contrasting reactions were distributed throughout the grid without any spatial clustering, suggesting that the variation in disease response was governed by genetic differences among the F₂ individuals rather than by environmental or positional effects. The continuous range of disease scores supports the polygenic nature of early blight tolerance and provided the basis for selecting the extreme tolerant and susceptible plants for QTL-Seq bulk construction. The continuous distribution of disease scores, with most plants in the intermediate classes and few at the extremes, indicates the quantitative, polygenic nature of early blight tolerance in tomato. The plants at the two extremes were used to constitute the tolerant and susceptible bulks for QTL-Seq analysis. Whole-genome resequencing (WGRS) data were generated for these extreme bulks Resistant bulk (RB) and Susceptible bulk (SB), in addition to parental genotypes Resistant parent IIHR-2891 and Susceptible parent- PC, using Illumina Hi-Seq platform followed by their analysis using the QTL-Seq pipeline (25). A total of 154.8 million reads for PC, 201.98 million reads from IIHR-2891, 165.95 million reads for SB and 186.70 million reads for RB was generated. A total of 106.4 GB of sequencing data was generated for IIHR-2891 (30.3 GB) followed by RB (28 GB), SB (24.9 GB) and PC (23.2 GB). The reads from the two extreme bulks were mapped to the IIHR-2891 assembly, with RB achieving 89.90 percent coverage and 24.20 mean read depth and SB achieving 89.10 percent coverage and 22.00 mean read depth. A detailed sequencing analysis discovered 86,689 genome-wide SNPs between the high (RB) and low (SB) bulks (Table 1).

Table 1
Summary sheet of genomic sequencing and alignment of the parental and bulk samples

Samples	Raw data (Gb)	Processed data (Gb)	Sequencing depth (x)	Raw-Reads (Mb)	HQ-Reads (Mb)	Aligned-reads (Mb)	Unaligned reads (Mb)	Alignment percentage (%)
PC (Susceptible)	23.2	21.7	20	154.80	147.12	138.76	8.35	94.3
IIHR-2891 (Resistant)	30.3	27.5	27	201.98	185.67	164.23	21.43	88.5
Susceptible Bulk	24.9	22.0	22	165.95	148.81	132.65	16.16	89.1
Resistant Bulk	28.0	24.2	23	186.70	165.57	148.79	16.78	89.9

3.1 Genomic regions and putative candidate genes located on chromosome regions for EB disease resistance

A genome-wide Manhattan plot of Δ SNP-index across chromosomes is provided in Figs. 4 and 5, highlighting the significant QTL regions on chromosomes 2 and 6. The five QTLs were determined based on the sliding window analysis of the Δ SNP-index with a window size of 200 kb. Regions exceeding the 95% confidence interval ($P < 0.01$) under the null hypothesis of no QTL were considered significant. Specifically, QTLs were called when the Δ SNP-index value consistently surpassed the threshold across consecutive windows. This led to the identification of four QTLs on chromosome 2 (*eb2.1*, *eb2.2*, *eb2.3*, *eb2.4*) and one on chromosome 6 (*eb6.1*). (Fig. 4, Fig. 5). The QTLs identified here harbor genes related to disease resistance, The candidate gene selection workflow starts by

mapping QTL intervals and extracting all annotated genes within them. These genes are then prioritized based on functional relevance to the trait focusing on defense-related families like NBS-LRR receptors and RLKs and finalized by identifying causal DNA variants within them that distinguish the resistant and susceptible genotypes. The genes selected for expression analysis are *Zinc finger transcription factor 41*, *WRKY transcription factor 21* (*Solyc11g042500*), *Leucine-rich repeat receptor-like protein kinase family protein* (*Solyc11g042300*), *disease resistance protein*, *Resistance protein gene* and *Protease inhibitor* (Table-2).

The expression analysis of genes located within these QTL regions resulted that nucleotide-binding-site leucine-rich repeat (NBS-LRR) genes, and was up-regulated by 2.13 and 2.57 folds on 6 and 10 DPI. (Fig. 6). A tolerant genotype IIHR-2891 showed significant up-regulation (0.95 folds and 1.28 folds) of ZFT gene at 6 DPI and 10 DPI respectively (Fig. 6). The expression of the DRG gene was up-regulated in the infected samples of resistant genotype with 1.03 and 1.23 folds on 6 and 10 DPI leading to increased disease tolerance. (Fig. 6). The highest expression of RPG gene was observed in the infected resistant genotype with 3.48 and 4.31 folds up-regulation after 6 and 10 days of post-inoculation, indicating that RPG gene responded positively to *A. solani* infection in the resistant genotype (Fig. 6). additionally resistant genotype IIHR-2891 showed significant up-regulation in gene expression of SIWRKY1 as compared to control plants, with the highest expression was observed at 6 DPI to 10 DPI (3.48 folds to 4.31 folds) (Fig. 6). The expression of the *PROINH* gene was up-regulated in the infected samples of the resistant genotype by 4.00, 4.90 and 5.58 folds at 3DPI, 6DPI and 10 DPI respectively (Fig. 6). The study successfully identified the QTL regions which are tightly linked to EB disease. The SNPs found in QTL regions provide accurate insights into disease tolerance mechanisms.

Table.2 List of genes present in each QTL and position on a chromosome

Sl. No.	QTL	Chromosome	Position on chromosome	Gene id
1	eb2.1	2	8.1Mb-9.2Mb	Solyc02g082060 Solyc02g082210 Solyc02g082250 Solyc02g082290 Solyc02g082320 Solyc02g082410 Solyc02g082470 Solyc02g082520 Solyc02g082590 Solyc02g082670 Solyc02g082710 Solyc02g082790
2	eb2.2	2	12.6Mb-13.1Mb	Solyc02g088940 Solyc04g081410 psbA ycf2 Solyc01g106880
3	eb2.3	2	17.1Mb-20.3Mb	Solyc01g099130 Solyc07g042060 Solyc03g115830 Solyc02g085010 Solyc03g118090 Solyc01g106320 Solyc02g089080 Solyc04g014170 Solyc01g105850 Solyc08g076520 Solyc07g042520 matK Solyc02g092020 Solyc01g099110
4	eb2.4	2	23.1Mb-24.4Mb	Solyc09g075230 Solyc02g082920 Solyc04g081190 Solyc01g009650 Solyc10g079160 Solyc03g006830 Solyc05g013600 Solyc06g060030 Solyc07g043640 Solyc11g071260 cox2 (mitochondrial gene) Solyc09g014910 Solyc02g093030
5	eb6.1	6	1.8Mb-3.1Mb	Solyc01g106460 Solyc10g083760 Solyc07g043630 Solyc03g115710 Solyc06g053840 Solyc02g085870 Solyc09g059410 Solyc04g081440 Solyc11g065300 Solyc08g080110

Table 3: List of primers used for qPCR in the study

	Gene Name	Sequence	Start	Tm	GC Percent	Amplicon length (bp)
1	<i>LRR</i>	F-ACACATGGTCGAACGTCATC	95	62.26	50.00	118
	<i>LRR</i>	R-CCTACCGTATTTGCTGTCTAAGG	212	62.25	47.82	
2	<i>PROINH</i>	F-CCCTTAGTGTATGCAAGGAACT	161	61.97	45.45	84
	<i>PROINH</i>	R-GCTCAGATAATCAGGACGGATG	244	62.17	50.00	
3	<i>DRG</i>	F-GGACCTCTCTTCATGCATCTTC	31	62.30	50.00	97
	<i>DRG</i>	R-CCAGCCCAACTTCTTCCTTTA	127	62.19	47.61	
4	<i>ZFT</i>	F-GCTGGGATGGCAACTATTTATG	2215	61.48	45.45	86
	<i>ZFT</i>	R-GCAGAGTGTAACGGAGAGTAAG	2300	61.81	50.00	
5	<i>RPG</i>	F-GGAAAGAGCGATGCCTACATTA	401	62.26	45.45	107
	<i>RPG</i>	R-CTCCTTGCTTCTAGGGACATTG	507	62.29	50.00	
6	<i>WRKY21</i>	F-GGTAGCCCTTCTCTGGAATTAAG	389	62.12	47.82	103
	<i>WRKY21</i>	R-GCTGTTGTTGCTGTTGAAGATAG	491	62.18	43.47	

4. Discussion

The development of tomato cultivars resistant to EB has long been a challenging objective in breeding programs due to the polygenic nature of resistance and its association with undesirable agronomic traits. Recently, molecular markers have proven instrumental in addressing the limitations of traditional breeding methods. Previous studies on QTL mapping have identified several QTLs associated with EB resistance, utilizing diverse genetic materials (2; 3; 4; 5; 26; 27; 28).

The first QTL mapping for EB resistance in tomatoes was done using backcross populations derived from a cross between susceptible *S. lycopersicum* (NC84173) and resistant *S. habrochaites* (PI126445). QTLs were identified on multiple chromosomes, with individual effects ranging from 7.9% to 25.9% (3). In this study, five novel QTL regions (*eb2.1*, *eb2.2*, *eb2.3* and *eb2.4*) on chromosome 2, and one QTL region (*eb6.1*) on chromosome 6 were identified (Fig. 4, Fig. 5). Previous studies on EB disease showed three QTLs identified on chromosomes 2 and 6 were contributed from resistant line LA 2093 with individual phenotypic effects ranging from 8% to 16%. A similar study, confirmed seven QTLs using a selective genotyping approach, four of which overlapped with the initial study, indicating their potential scope for molecular breeding (2). A mapping effort on *S. arcanum* × *S. lycopersicum* cross, identified QTLs with significant phenotypic variance, highlighting their utility in EB resistance breeding (4). Additional work on *S. pimpinellifolium* (LA2093) detected QTLs with stable effects across generations, emphasizing their importance in breeding for EB resistance (27). Despite these advances, so far, no precise location, genes residing in the QTL regions and their functions were not examined.

The present study not only provides precise localization of these QTLs but also identifies key candidate genes within these regions, shedding light on the molecular mechanisms operating for tolerance. There are seven genes in the QTL regions (*eb2.3*, *eb2.4*, and *eb6.1*) that are directly related to the plant defence system. The gene identified in *eb2.3* of chromosome 2 was Leucine-rich repeat receptor-like protein kinase family (LRR-RLK). WRKY transcription factor 35 and Zinc finger transcription factor 16 were located on *eb2.4* of chromosome 2. Zinc finger transcription factor 41 (ZFT41), WRKY transcription factor 21, a disease resistance protein and NBS-LRR resistance protein-like protein were located in *eb6.1* of chromosome 6. (Table.2)

These genes are crucial in the context of plant immunity, involved in both recognizing pathogens and activating downstream defense mechanisms. The genes identified within the QTL regions are involved in plant defense responses. For instance, in the *eb2.3* region on chromosome 2, we found a Leucine-rich repeat receptor-like protein kinase (LRR-RLK), which plays a crucial role in pathogen recognition and the initiation of defense signaling. LRR-RLKs are integral to the plant's innate immune system, facilitating PAMP-triggered immunity (PTI) by recognizing conserved microbial features and triggering a defense response. Similarly, the *eb6.1* region on chromosome 6 includes an NBS-LRR gene, a critical component of effector-triggered immunity (ETI). NBS-LRR proteins recognize specific pathogen effectors and activate robust defense responses, often leading to localized cell death (hypersensitive reaction) to contain the pathogen. The significant up-regulation of this gene in the resistant genotype (IIHR-2891) post-infection underscores its role in providing effective resistance against *Alternaria solani* (Fig. 5). This finding is consistent with studies in other crops where NBS-LRR genes have been central to conferring resistance against diverse pathogens. When leaves of late blight resistant tomato lines were infected with *P. infestans*, there was a transient over-expression of SpNBS-LRR (29). Over-expression of the ZmNBS25-OE gene (from *Zea mays*) enhanced the resistance of Arabidopsis and rice to *Pseudomonas syringae* pv. tomato DC3000 and sheath blight disease without affecting the grain yield (30)

Another key finding is the presence of WRKY transcription factors in the *eb2.4* region. WRKY factors are master regulators of plant defense, controlling the expression of a wide range of defense-related genes, including those involved in the biosynthesis of secondary metabolites and pathogenesis-related proteins. The WRKY21 and WRKY35 identified in this study are particularly noteworthy for their role in modulating the plant's response to biotic stress. Their up-regulation in response to *A. solani* infection in the resistant genotype suggests that these transcription factors are pivotal in orchestrating a defense response. The SIWRKY39 gene, a homolog of AtWRKY40, was found significantly up-regulated in tomatoes upon being challenged with *P. syringae* (31). In tomato, upon over-expression of SIWRKY39 there was enhanced resistance to *P. syringae* pathogen (32). Over-expression of WRKY genes, AtWRKY18 and AtWRKY70 led to induced expression of defense-related genes, including SA-induced PR1 (33). Overexpression of the *Solanum pimpinellifolium* allele of SIWRKY33 (called SpWRKY1) in (34) resulted in tobacco and tomato resistance to the hemi-biotrophic oomycetes *Phytophthora nicotianae* and *Phytophthora infestans*. It is reported that WRKY1 acted as a key component in improving resistance against *Alternaria solani* in wild tomato, *Solanum arcanum* (35). PROINH gene inhibits the growth of a few important phytopathogenic fungal strains namely *Claviceps*, *Curvularia*, *Helminthosporium*, *Alternaria* and *Fusarium* fungal strains. Over-expression of PROINH transgene prosystemin generates a mobile wound signal in tomato (36).

Additionally, the *eb6.1* region harbors a Zinc finger transcription factor (ZFT41), known for its role in regulating stress-responsive genes. Zinc finger proteins often mediate responses to both biotic and abiotic stresses by regulating the expression of genes involved in defense and stress tolerance. The up-regulation of ZFT41 in the resistant genotype indicates its involvement in enhancing EB resistance, likely through the modulation of stress response pathways that mitigate the effects of pathogen attack. The CAZFP1, Cys2/His2-type zinc-finger

transcription factor gene plays a major role as pathogen-induced early-defense gene in *Capsicum annuum* belonging to Solanaceae family was reported (37).

Moreover, QTL-Seq technology used in this study provides a more accurate and efficient approach to QTL identification, which is critical for accelerating the breeding of disease-resistant tomato varieties with desirable horticultural trait. Compared to earlier studies, our study offers a higher resolution of QTL mapping and identifies specific defense-related genes within these regions.

In conclusion, the identification of these QTL regions and the associated defense-related genes represents a major step forward in the quest to develop EB-resistant tomato varieties. These findings not only deepen our understanding of the genetic mechanisms underlying resistance but also offer practical tools, like markers for MAS for the advancement of tomato breeding programs. Future research need focus on fine-mapping QTL regions, and validating the candidate genes functions, to further enhance our understanding of EB tolerance and also improve the efficacy of breeding strategies aimed at developing robust, disease-resistant tomato cultivars.

Declarations

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Data Availability Statement

The raw sequence data that support the findings of this study are openly available in NCBI at www.ncbi.nlm.nih.gov reference number SRR13569536 (Bioproject PRJNA695390)

Ethics and consent to participate

Plants used in the study are the breeding material in Dr. A T Sadashiva' s lab. He was the head of the breeding program at ICAR-IIHR during the time the experiments and the study took place. Therefore, the collection of plants used in the study complies with local or national guidelines, and no further affirmation is required. The material used in this study was based upon work supported by the ICAR-Indian Institute of Horticultural Research, Bengaluru, India.

Declaration of interest statement

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

Consent to publish

Not Applicable

Contributions

Amarnath. M: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft.

Satish D: Methodology, Investigation, Resources, Data Curation.

Sriram S: Validation, Investigation, Resources.

Sadashiva A. T: Resources, Supervision, Project administration.

Shivashankara K. S: Resources, Supervision.

Rekha Chittapur: Supervision, Funding acquisition.

Kundapura V Ravishankar: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – Review & Editing.

References

1. Rick CM. The Tomato. *Sci Am.* 1978;239(2):76–87.
2. Zhang LP, Lin GY, Nino-Liu D, Foolad MR. Mapping QTLs conferring early blight (*Alternaria solani*) resistance in a *Lycopersicon esculentum* × *L. hirsutum* cross by selective genotyping. *Mol Breed.* 2003;12(1):3–19. <https://doi.org/10.1023/A:1025484620521>.
3. Foolad M, Zhang L, Khan AA, Nino-Liu D, Lin G. Identification of QTLs for early blight (*Alternaria solani*) resistance in tomato using backcross populations of a *Lycopersicon esculentum* × *Lycopersicon hirsutum* cross. *Theor Appl Genet.* 2002;104(6):945–58. <https://doi.org/10.1007/s00122-002-0870-z>.
4. Brouwer DJ, St. Clair DA. Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs. *Theor Appl Genet.* 2004;108:628–38. <https://doi.org/10.1007/s00122-003-1465-z>.
5. Anderson TA, Zitter SM, De Jong DM, Francis DM, Mutschler MA. Cryptic introgressions contribute to transgressive segregation for early blight resistance in tomato. *Theor Appl Genet.* 2021;134:2561–75. <https://doi.org/10.1007/s00122-021-03844-9>.
6. Giovannoni JJ, Wing RA, Ganai MW, Tanksley SD. Isolation of molecular markers from specific chromosomal intervals using DNA from existing mapping populations. *Nucleic Acids Res.* 1991;19:6552–8. <https://doi.org/10.1093/nar/19.23.6552>.
7. Michelmore RW, Paran I, Kesseli RV. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci U S A.* 1991;88(21):9828–32. <https://doi.org/10.1073/pnas.88.21.9828>.
8. Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S, Mitsuoka C, et al. QTL-Seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *Plant J.* 2013;74(1):174–83. <https://doi.org/10.1111/tpj.12105>.
9. Lu H, Lin T, Klein J, Wang S, Qi J, Zhou Q, et al. QTL-Seq identifies an early flowering QTL located near Flowering Locus T in cucumber. *Theor Appl Genet.* 2014;127(7):1491–9. <https://doi.org/10.1007/s00122-014-2313-z>.
10. Wei Q, Wang J, Wang W, Hu T, Wang H, Bao C. A high-quality chromosome-level genome assembly of genetics model *Lycoris radiata* (Amaryllidaceae). *Mol Ecol Resour.* 2022;22(4):1744–60. <https://doi.org/10.1111/1755-0998.13576>.
11. Win KT, Vegas J, Zhang C, Song K, Lee S. QTL mapping for downy mildew resistance in cucumber via bulked segregant analysis using next-generation sequencing and conventional methods. *Theor Appl Genet.*

- 2017;130(1):199–211. <https://doi.org/10.1007/s00122-016-2806-z>.
12. 12. Illa-Berenguer E, Van Houten J, Huang Z, van der Knaap E. Rapid and reliable identification of tomato fruit weight and locule number loci by QTL-Seq. *Theor Appl Genet*. 2015;128(7):1329–42. <https://doi.org/10.1007/s00122-015-2509-x>.
 13. 13. Wen J, Jiang F, Weng Y, Sun M, Shi X, Zhou Y, et al. Identification of heat-tolerance QTLs and high-temperature stress-responsive genes through conventional QTL mapping, QTL-Seq and RNA-seq in tomato. *BMC Plant Biol*. 2019;19:398. <https://doi.org/10.1186/s12870-019-2008-3>.
 14. 14. Kumar R, Janila P, Vishwakarma MK, Khan AW, Manohar SS, Gangurde SS, et al. Whole-genome resequencing-based QTL-Seq identified candidate genes and molecular markers for fresh seed dormancy in groundnut. *Plant Biotechnol J*. 2020;18(4):992–1003. <https://doi.org/10.1111/pbi.13266>.
 15. 15. Zhao Y, Ma J, Li M, Deng L, Li G, Xia H, et al. Whole-genome resequencing-based QTL-Seq identified AhTc1 gene encoding a R2R3-MYB transcription factor controlling peanut purple testa colour. *Plant Biotechnol J*. 2020;18(1):96–105. <https://doi.org/10.1111/pbi.13175>.
 16. 16. Amarnath M, Ravishankar KV, Satish D, Sriram S, Sadashiva AT. Screening of tomato genotypes against early blight pathogen *Alternaria solani* using detached leaf assay method. *Pest Manag Hortic Ecosyst*. 2019;25(1):93–101.
 17. 17. Devanathan M, Ramanujam K. Evaluation of fungicides for the management of early blight of tomato caused by *Alternaria solani*. *Madras Agric J*. 1995;82:228–9.
 18. 18. McKinney HH. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J Agric Res*. 1923;26:195–217.
 19. 19. Ravishankar KV, Anand L, Dinesh MR. Assessment of genetic relatedness among a few Indian mango cultivars using RAPD markers. *J Hort Sci Biotechnol*. 2000;75(2):198–201. <https://doi.org/10.1080/14620316.2000.11511224>.
 20. 20. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754–60. <https://doi.org/10.1093/bioinformatics/btp324>.
 21. 21. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25(16):2078–9. <https://doi.org/10.1093/bioinformatics/btp352>.
 22. 22. Sorrequieta A, Ferraro G, Boggio SB, Valle EM. Free amino acid production during tomato fruit ripening: A focus on L-glutamate. *Amino Acids*. 2010;38:1523–32. <https://doi.org/10.1007/s00726-009-0373-1>.
 23. 23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 – $\Delta\Delta CT$ method. *Methods*. 2001;25(4):402–8. <https://doi.org/10.1006/meth.2001.1262>.
 24. 24. Sheoran OP, Tonk DS, Kaushik LS, Hasija RC, Pannu RS. Statistical Software Package for Agricultural Research Workers. In: Hooda DS, Hasija RC, editors. *Recent Advances in information theory, statistics & computer applications*. Hisar: Department of Mathematics Statistics, CCS HAU; 1998. p. 139–43.
 25. 25. Luo H, Guo J, Ren X, Chen W, Huang L, Zhou X, et al. Chromosomes A07 and A05 associated with stable and major QTLs for pod weight and size in cultivated peanut (*Arachis hypogaea* L.). *Theor Appl Genet*. 2018;131:267–82. <https://doi.org/10.1007/s00122-017-3000-7>.
 26. 26. Foolad MR, Merk HL, Ashrafi H. Genetics, Genomics and Breeding of Late Blight and Early Blight Resistance in Tomato. *Crit Rev Plant Sci*. 2008;27(2):75–107. <https://doi.org/10.1080/07352680802147353>.
 27. 27. Foolad M, Ashrafi H. Characterization of early blight resistance in a recombinant inbred line population of tomato: I. Heritability and trait correlations. *Adv Stud Biol*. 2015;7:131–48.

28. 28. Ashrafi H, Foolad M. Characterization of early blight resistance in a recombinant inbred line population of tomato: II. Identification of QTLs and their co-localization with candidate resistance genes. *Adv Stud Biol.* 2015;7(4):149–68.
29. 29. Jiang N, Meng J, Cui J, Sun G, Luan Y. Function identification of miR482b, a negative regulator during tomato resistance to *Phytophthora infestans*. *Hortic Res.* 2018;5:9. <https://doi.org/10.1038/s41438-017-0013-y>.
30. 30. Xu Y, Liu F, Zhu S, Li X. The Maize NBS-LRR Gene *ZmNBS25* Enhances Disease Resistance in Rice and Arabidopsis. *Front Plant Sci.* 2018;9:1033. <https://doi.org/10.3389/fpls.2018.01033>.
31. 31. Huang S, Gao Y, Liu J, Peng X, Niu X, Fei Z, et al. Genome-wide analysis of WRKY transcription factors in *Solanum lycopersicum*. *Mol Genet Genomics.* 2012;287:495–513. <https://doi.org/10.1007/s00438-012-0696-6>.
32. 32. Sun XC, Gao YF, Li HR, Yang SZ, Liu YS. Over-expression of SIWRKY39 leads to enhanced resistance to multiple stress factors in tomato. *J Plant Biol.* 2015;58:52–60. <https://doi.org/10.1007/s12374-014-0325-5>.
33. 33. Li J, Brader G, Palva ET. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell.* 2004;16(2):319–31. <https://doi.org/10.1105/tpc.016980>.
34. 34. Li JB, Luan YS, Liu Z. Overexpression of SpWRKY1 promotes resistance to *Phytophthora nicotianae* and tolerance to salt and drought stress in transgenic tobacco. *Physiol Plant.* 2015;155(3):248–66. <https://doi.org/10.1111/ppl.12315>.
35. 35. Shinde BA, Dholakia BB, Hussain K, Aharoni A, Giri AP, Kamble AC. WRKY1 acts as a key component improving resistance against *Alternaria solani* in wild tomato, *Solanum arcanum* Peralta. *Plant Biotechnol J.* 2018;16(8):1502–13. <https://doi.org/10.1111/pbi.12892>.
36. 36. McGurl B, Orozco-Cardenas M, Pearce G, Ryan CA. Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proc Natl Acad Sci U S A.* 1994;91(21):9799–802. <https://doi.org/10.1073/pnas.91.21.9799>.
37. 37. Kim SH, Hong JK, Lee SC, Sohn KH, Jung HW, Hwang BK. CAZFP1, Cys2/His2-type zinc-finger transcription factor gene functions as a pathogen-induced early-defense gene in *Capsicum annuum*. *Plant Mol Biol.* 2004;55(6):883–904. <https://doi.org/10.1007/s11103-005-2151-1>.

Figures

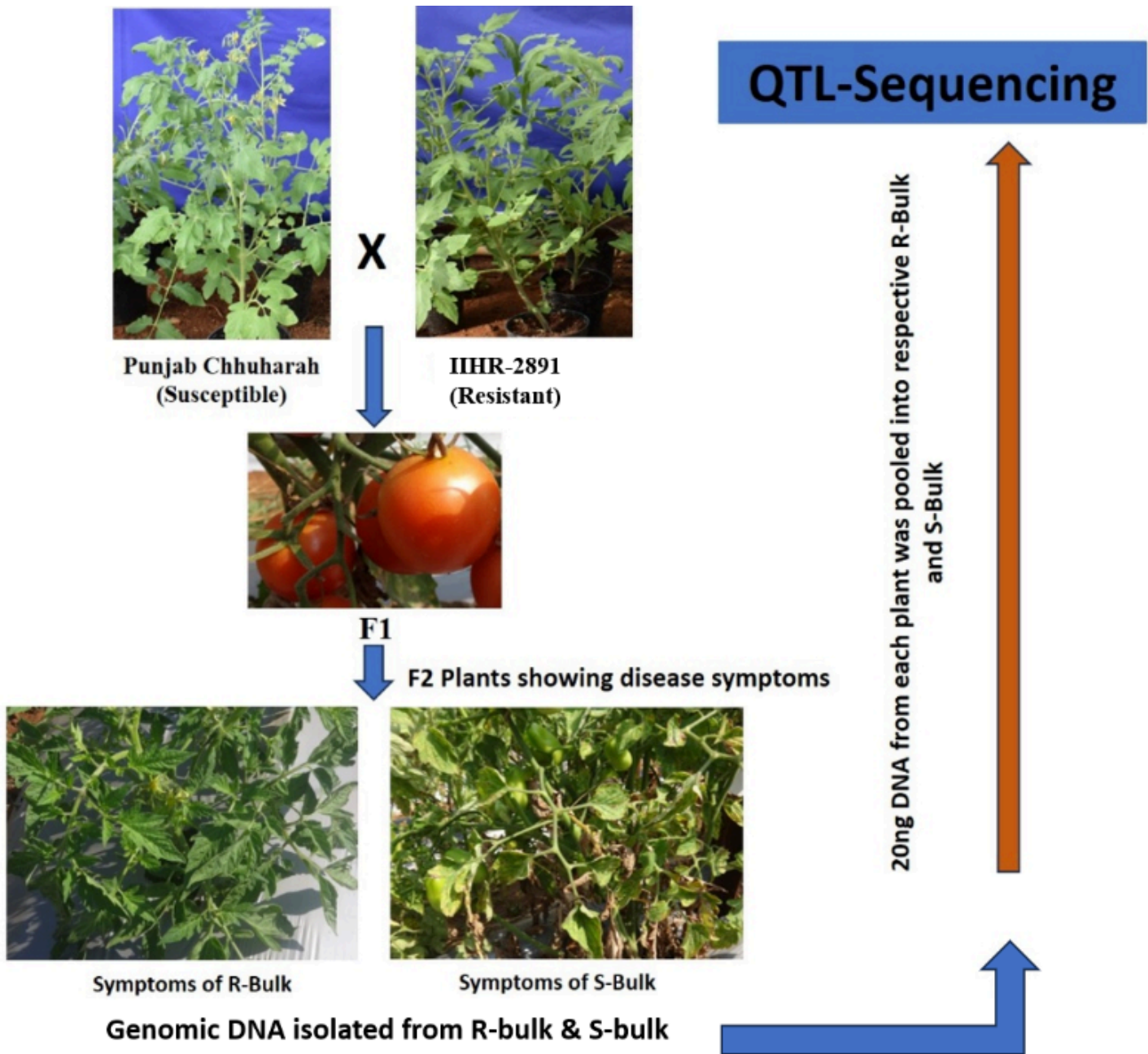


Figure 1

Schematic workflow of developing mapping population (F_2) for QTL sequencing

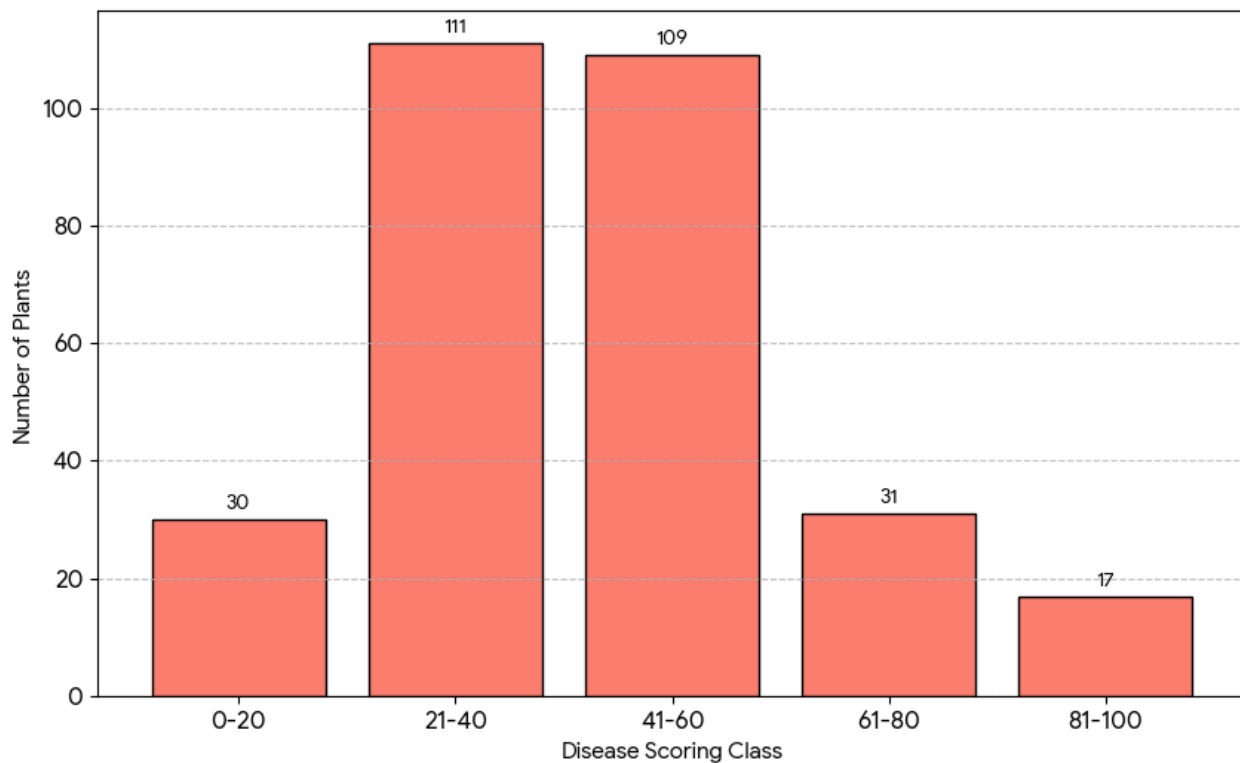


Figure 2

Distribution of disease severity (PDI) scores within the F_2 population

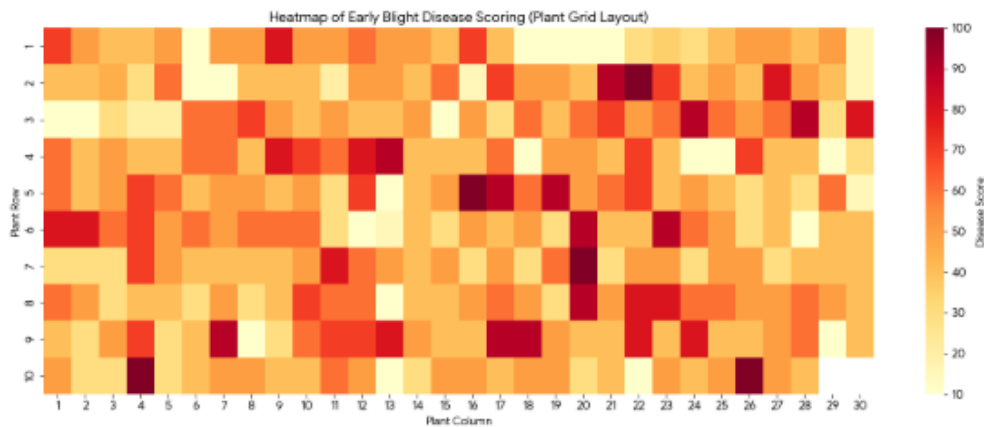


Figure 3

Heatmap of the F_2 population with disease scoring

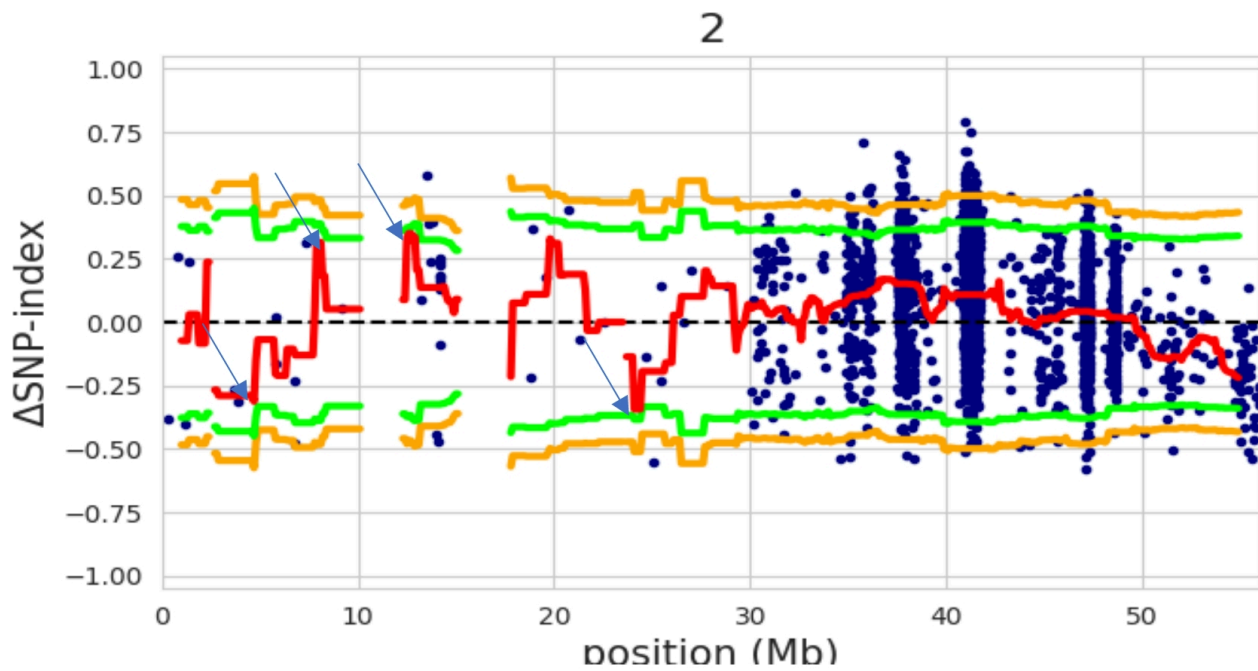


Figure 4

Single nucleotide polymorphism (SNP) index plots and QTL position on chromosome 2

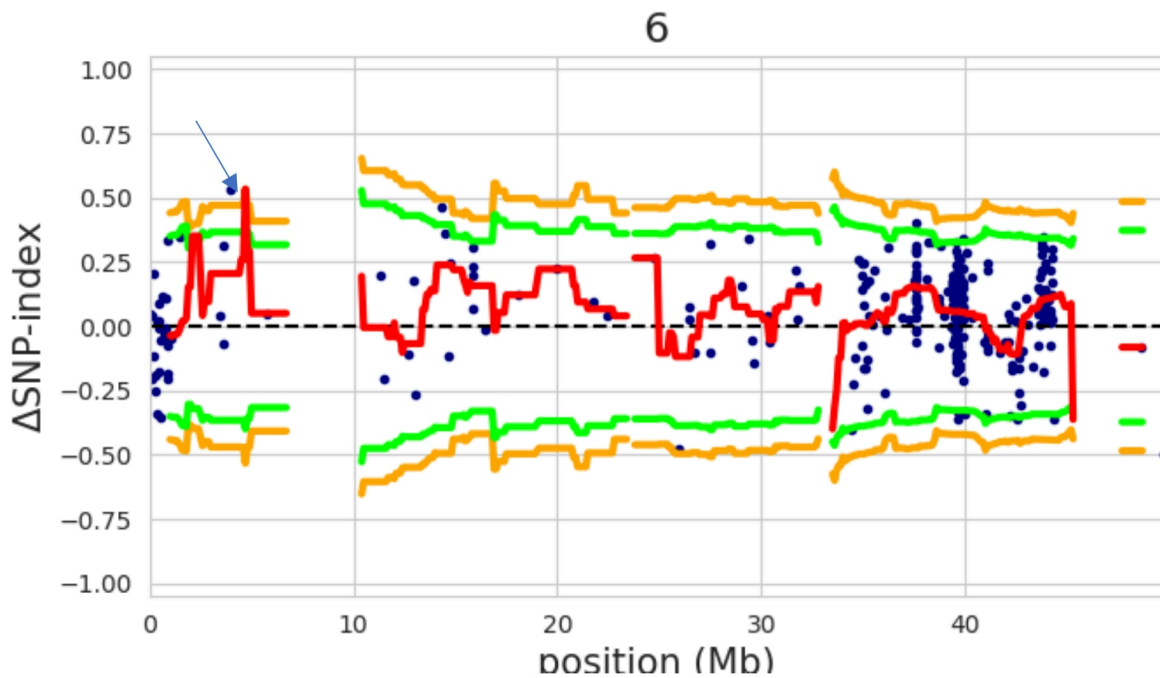


Figure 5

Single nucleotide polymorphism (SNP) index plots and QTL position on chromosome 6

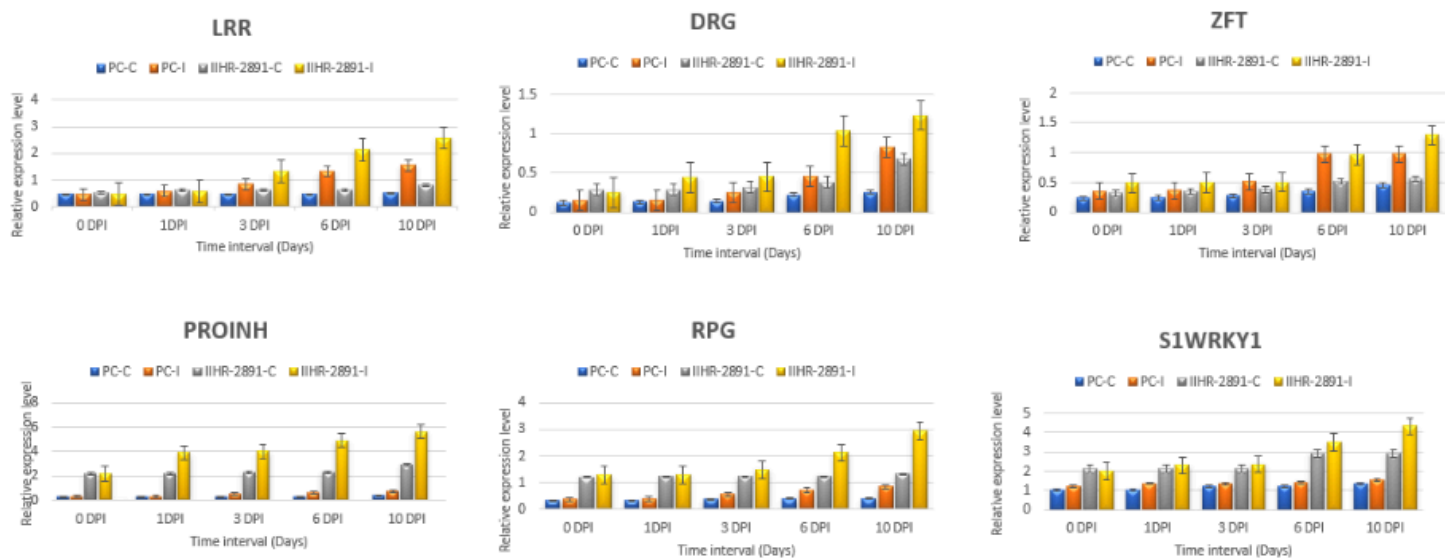


Figure 6

Gene expression pattern of different genes ($P < 0.01$) a) NBS-LRR (*Leucine-rich repeat receptor-like protein kinase family protein*) b) DRG (*disease resistance protein gene*) c) RPG (*Resistance protein gene*) d) ZFT (*Zinc finger transcription factor*) e) S1WRKY1 (*WRKY transcription factor*) and f) PROINH (*Protease inhibitor*) at different days of *A. solani* infection in contrasting tomato genotypes.