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Tomato genomic regions and candidate genes linked to responses to a resistance inducer against *Phytophthora infestans*

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Research Article

Keywords: Resistance induction, genes, GWAS, genotypic variation, tomato late blight, biocontrol

Posted Date: August 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10650999/v1

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Additional Declarations: No competing interests reported.

Abstract

Plant resistance inducers (PRIs) represent a promising and sustainable strategy for pest and disease management, but their efficacy can vary depending on plant genotype. Our objective was to identify the genetic architecture underlying tomato responses to the application of a PRI. We have established that Belvine® is an effective PRI against tomato late blight caused by *Phytophthora infestans*. Analysis of defense-related gene expression revealed that PR proteins, oxidative stress-related, and ethylene-associated genes were upregulated in Belvine®-treated plants. A Genome Wide Association Study panel of 148 tomato accessions was grown under plastic tunnel either treated with Belvine® or left untreated, then inoculated with *P. infestans*. We identified five quantitative trait loci (QTLs) associated with genotypic variation in Belvine® efficacy, located on chromosomes 2, 3, 4, and 12. Within the significant regions, we identified several candidate genes, including protein kinases, receptor-like protein kinases, transcription factors, and PR proteins. Notably, several candidate genes encoded proteins with functions analogous to those of genes upregulated under Belvine® treatment, including β-1,3-glucanases, glutathione S-transferase, and peroxidase, even though they may correspond to distinct copies of these gene families. The identified QTL regions and candidate genes constitute valuable genomic resources for defining tomato breeding strategies aimed at enhancing plant responses to PRIs.

Key message

This study identifies the first genomic regions and candidate genes associated with tomato responsiveness to the plant resistance inducer, enabling breeding for enhanced induced resistance to late blight.

Introduction

Late blight, caused by the oomycete *Phytophthora infestans*, is a devastating disease affecting both cultivated tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*), frequently leading to severe crop losses (Fry 2008; Panthee et al. 2024). Copper-based products and synthetic fungicides are widely used to control the disease. Although these compounds can effectively suppress disease development in the short term, their repeated and intensive use may promote the emergence of resistant pathogen populations (Abuley et al. 2023). Indeed, resistance of *P. infestans* to several fungicide classes has already been reported, highlighting the limitations of chemical control strategies and the need for more sustainable disease management approaches (Saville et al. 2015; Olave-Achury et al. 2022).

The cultivation of resistant varieties is an effective, economical, and environmentally friendly strategy to reduce the negative impacts of *P. infestans*. To date, more than 40 resistance genes and QTLs of resistance to *P. infestans* have been identified in different wild tomato relatives, including *Solanum pimpinellifolium*, *S. pennellii*, and *S. habrochaites* (Brouwer et al. 2004; Smart et al. 2007; Merk et al. 2012; Chen et al. 2014; Panthee et al. 2017; Sullenberger et al. 2022; Gao and Foolad 2024; Jia et al. 2025). However, only a few of these resistance loci have been introgressed into cultivated tomato. Among the introgressed genes, *Ph-1* and *Ph-3* have already been overcome by certain *P. infestans* isolates (Foolad et al. 2008; Sullenberger and Foolad 2024). Therefore, alternative solutions are needed for improving the durability of resistance.

Biosolutions that induce plant resistance are promising alternatives to conventional fungicides. These plant resistance inducers (PRIs) encompass a wide range of natural and synthetic compounds, including plant extracts, beneficial microorganisms and their derivatives, as well as phytohormones such as salicylic acid (SA) (Jamiołkowska 2020; Hönig et al. 2023). When applied to plants, PRIs activate signaling pathways involved in plant immunity, leading to the regulation of defense-related genes and the production of antimicrobial and protective compounds, including pathogenesis-related (PR) proteins, reactive oxygen species (ROS), secondary metabolites, and cell wall-strengthening components (Cohen et al. 2016; Yarullina et al. 2022; Alamri et al. 2026). These defense responses help restrict pathogen growth and spread and may result in induced systemic resistance (ISR) or systemic acquired resistance (SAR), thereby reducing plant susceptibility to subsequent pathogen attacks (Floryszak-Wieczorek et al. 2015). For instance, the application of β-aminobutyric acid (BABA) induced the expression of multiple defense-related transcripts and enhanced resistance to powdery mildew in tomato (Satková et al. 2016; Zapletalová et al. 2023). Another important mode of action of PRI is the priming phenomenon. Priming prepares plants for faster and stronger defense responses upon pathogen challenge without constitutively activating defense pathways (Hilker and Schmülling 2019; Hönig et al. 2023). Tanaka et al. (2024) demonstrated that treatment of tomato plants with L-methionine alone did not activate salicylic acid (SA)-, jasmonic acid (JA)-, or ethylene (ET)-mediated defense signaling, but SA- and JA-responsive defense genes were induced more rapidly following *Pseudomonas syringae* pv. *tomato* and *Botrytis cinerea* infection in treated plants.

The induction of resistance in plants is a complex process whose effectiveness depends on the plant genetics, as well as its physiological status, and the environmental conditions (Bruce 2014). In plant, responses to treatments with a PRI has been documented at multiple levels, including phenotypic, transcriptomic, and metabolic levels. Recently, Margaritopoulou et al. (2024) demonstrated that *Reynoutria sachalinensis* extract enhanced phospholipid production and signaling in one zucchini genotype susceptible to *Podosphaera xanthii*, whereas only limited responses were observed in one genotype with intermediate resistance, likely due to genetic resistance mechanisms. In tomato, the use of *Trichoderma spp.* against *B. cinerea* resulted in contrasting outcomes depending on the plant genotype, with no effect or even detrimental effects observed in certain genotypes (Tucci et al. 2011). Similarly, Sharma et al. (2010) reported genotype-dependent variation in BABA-induced resistance against *P. infestans*. Transcriptomic differences were also observed by Satková et al. (2016), who showed that PRI induced defense-related gene expression in the susceptible tomato cultivar *S. lycopersicum* 'Amateur', but not in the resistant accession 'LA 2128' belongings to the wild species *S. habrochaites f.*

glabratum. However, the genetic mechanisms underlying this variation remain poorly understood. The identification of genomic regions or candidate genes associated with plant responses to PRIs in tomato could provide new resources to enhance resistance defense.

The objective of this study was to decipher the genetic architecture underlying tomato resistance to *P. infestans* following the application of a biosolution that induces plant defense pathways. After demonstrating that this biosolution is capable of inducing defense pathways in the plant and that its protective efficacy depends on the tomato genotype, a Genome Wide Association Study (GWAS) panel of 148 tomato accessions was grown under plastic tunnels and either treated with the PRI or left untreated prior to *P. infestans* inoculation. GWAS identified five QTLs located on chromosomes 2, 3, 4 and 12 that were associated with genotypic variation in PRI efficacy against *P. infestans*. These findings provide valuable insights for breeding programs aimed at selecting tomato varieties with high responsiveness to PRIs.

Material and methods

Plant material, pathogen strains

Plant material

The panel of tomato genotypes used to analyze the genetic response of tomato accessions to the application of PRI was composed of 150 homozygous lines, including 116 cherry tomato accessions belonging to *S. lycopersicum* var. *cerasiforme* (SLC), 10 *S. pimpinellifolium* (SP), 18 admixte accessions of SLC and SP, 5 large-fruited tomato *S. lycopersicum* var. *lycopersicum* (SLL), and the commercial cultivar Clodano (Syngenta). Most of them have been used and characterized in previous studies (Albert et al. 2016; Bineau et al. 2021; Héreil et al. 2024). The list of accessions is provided in **Supplementary data 1**. All seeds from the accessions used in this study, with the exception of seeds from the Clodano variety, were produced by the Centre for Vegetable Germplasm (CRB-Leg) of the GAFL research unit (INRAE, Avignon, France). Five of these accessions (Clodano, Criollo, Ecu284, Marpha n2 and Vir884) were used to evaluate the expression of defense genes induced by Belvine®, and eleven accessions (Clodano, Criollo, Vir884, LA2619, Vir2759, Vir744, Ferum, Momor, Rose de Berne, St Pierre, and Vir859) were used to assess whether there is a genotypic variation among tomato accessions regarding the response to the application of the PRI.

Pathogen material

The *P. infestans* strain Pi147 was used in this study. Strain Pi147 was obtained from an infected tomato fruit collected in Bucheley (78, France) in 2021. The strain is routinely maintained by monthly subculturing on V8 agar medium in Petri dishes and incubated in a growth chamber at 18°C in the dark. Three weeks prior to inoculation, the pathogen was used to infect leaflets of the susceptible tomato accession Momor (INRAE, Avignon, France) and transferred after seven days onto fresh, healthy leaflets of the same accession to restore and maintain its aggressiveness. The pathogen inoculum was prepared by washing heavily infected tomato leaflets in tap water and using them immediately. Sporangia concentration was estimated with a Malassez counting chamber, and the suspension was adjusted to a final concentration of 5×10^3 sporangia·mL^{-1}.

Biosolution

Preliminary studies have shown that the commercial natural substance Belvine® (Cerience, https://www.cerience.fr/fr/solutions/belvine) is effective in protecting tomato against *P. infestans* (personal communication). This biosolution is approved in the European Union and has obtained national authorizations in seven-member states, including France, as a plant protection product for the management of grapevine downy mildew. The active substance of the product (ABE-IT 56) consists of components derived from a lysate of the *Saccharomyces cerevisiae* strain DDSF623 (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/1307). According to the manufacturer, the product has a preventive effect by stimulating the natural defense responses of grapevine (https://www.cerience.fr/fr/solutions/belvine). In this study, this biosolution was sprayed onto the foliage of tomato plants before inoculation with the pathogen, at a dose of 2% (V/V).

Experimentations and plant growth

This study comprised three trials. Trial 1 focused on assessing the induction of tomato defense-related genes following treatment with the biosolution. Trial 2 evaluated genotype-dependent variation in the efficacy of Belvine® against *P. infestans* across a panel of tomato accessions. Trial 3 was designed to map genomic loci associated with the plant response to the biosolution.

Seeds for the trials were directly sown into 9 x 9 cm pots containing seedling substrate (K substrates).

Assessment of tomato defense genes induced by the Belvine®

To analyze the expression of tomato defense genes induced by Belvine®, two independent experiments were conducted in 2023 in a glasshouse under semi-controlled conditions, with a two-week interval between each experiment. The experiments were conducted with five tomato accessions. Each accession × treatment combination included three biological replicates. Plants were assigned to two treatment groups: Belvine® group and

untreated control group. Four-weeks-old treated plants were sprayed with approximately 20 mL of Belvine® solution delivered per plant. The treatments were applied twice at 7 days and 2 days before sampling. Following the first treatment (Day − 7), a ring was placed near the apical meristem to mark leaf position. On day 0, for each accession, one leaf located below the ring was collected from each of the three replicates. Five leaf discs (10 mm in diameter) were excised from each leaf and pooled to form a single biological sample per accession and treatment for gene expression analysis. Samples were immediately frozen in liquid nitrogen to prevent RNA degradation.

Total RNA was extracted using the Macherey-Nagel Nucleospin RNA Plant Kit with slight modifications to the volume and reverse transcription was performed as described by Promega Corp. (M-MLV). Quantitative real-time PCR was performed as described in Gaucher et al. (2022), using a patented set of primers targeting 28 tomato defense genes and three reference genes (Brisset and Dugé de Bernonville, 2011), listed in **Table S1**. Relative changes in defense gene expression ($\log_2$ ratios) were calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak 2008) with normalization based on the three reference genes (Vandesompele et al. 2002). An untreated sample of the tomato variety 'Clodano' was used as the calibrator for the calculation of $\log_2$ expression ratios. Genes were considered differentially expressed when the absolute $\log_2$ fold change exceeded 1 and the p-value was ≤ 0.05.

Evaluation of the genotype-dependent variability in Belvine® efficacy

The first experiment to evaluate the variability in the protective efficacy of Belvine® against *P. infestans* depending on tomato genotype was carried out in autumn 2023 in an unheated 600 m² plastic tunnel. Eleven tomato accessions with comparable levels of susceptibility to tomato late blight were included. Two treatment conditions were tested: a control group of untreated plants and a group of plants treated with Belvine®. Each treatment × accession combination consisted of four biological replicates. Plants were first grown in a glasshouse for four weeks, then transplanted into the tunnel. The tunnel was organized into 18 blocks, with two blocks randomly assigned to each treatment. Each block consisted of two rows of 11 plants, with one plant per accession randomly distributed within each row. Eight-week-old plants were treated with approximately 40–50 mL per plant of a solution of Belvine®, and two applications performed 8 days and 3 days before pathogen inoculation.

GWAS of loci involved in plant response to Belvine®

The experiments were conducted from late summer (August) to late autumn (December), a period conducive to the development of tomato late blight in the Avignon region. Seeds were sown individually in 9 × 9 cm pots filled with seed-starting mix and then grown in a glasshouse under semi-controlled conditions. Six-week-old plants were then transplanted into two 600 m² plastic tunnels to mimic natural production conditions. Each tunnel contained six rows: two single rows used as borders, and two double rows. Each double row was divided into two blocks corresponding to two modalities: modalities: on one side, plants treated with Belvine®, and on the other, untreated control plants (**Fig. S1**).

The two blocks of treated plants were surrounded by one row of untreated tomato plants of the Rose de Berne variety. In each block, 150 tomato accessions were planted randomly within the double row, with a few minor adjustments made to limit the number of times the same accession appeared in the immediate neighborhood of another to a maximum of two. Plants were treated with Belvine® with approximately 20 mL per plant, and two applications performed 7 days and 3 days before pathogen inoculation. Following the last treatment, the height of each plant was recorded by marking its position on the supporting twine. The experiments were repeated over two years, in 2024 and 2025. Although 150 tomato accessions were initially included, only 148 with available genetic data were retained for genetic analysis. Data of one tunnel in 2024 was excluded from analysis due to insufficient infection.

Inoculation and disease assessment

Plants were inoculated with 15–20 ml per plant of a 5 × 10³ sporangia·mL⁻¹ suspension, using a hand-held sprayer.

Resistance to *P. infestans* was evaluated 7–10 days after inoculation. Prior to phenotyping, the first and second leaves located immediately below the plant height reference mark were selected for symptom scoring. In 2024, infestation levels were very low, especially on untreated plants seven days after inoculation. Consequently, plants intended for PRI treatment were retreated twice as previously described and inoculated 3 days after the last treatment. Only leaves that underwent both inoculations and located under the first plant height reference mark were considered for evaluation 7 days after the second inoculation. Phenotyping consisted of recording per leaf (i) the total number of leaflets, (ii) the number of infected leaflets, and (iii) the severity of infection on infected leaflets. Disease severity on infected leaflets was assessed using a 0–4 ordinal scale, where 0 = no visible symptoms; 1 = 1–25% of leaflet area infected; 2 = 26–50% of leaflet area infected; 3 = 51–75% of leaflet area infected; and 4 = more than 75% of the leaflet area infected (**Fig. S2**). The disease index (DIS) was calculated as follows:

$$\varvec{D}\varvec{I}\varvec{S} = \frac{\mathbf{NIL} * \mathbf{ILAS}}{\mathbf{NL}}$$

where NL is the total number of leaflets per leaf, NIL is the number of infested leaflets per leaf, and ILAS is the disease severity index. For each plant, DIS values from the two assessed leaves were averaged to obtain a single value per plant.

Evaluation of the efficacy of the biosolution

The efficacy of the biosolution was estimated for each plant as the percentage of protection of the plant using the formula according to (Tamm et al. 2011; Zhang et al. 2026) as:

$$\varvecP\varvecP = 100*(1 - \frac{\varvecD\varveci\varvecs\varvecp1_{(\varveci\varvecj)}}{\mathbf{DISntc_{(j)}}})$$

Where PP is the percentage of protection, DISp1(ij) is the disease index in treated condition of the ith plant of the jth genotype, and DISntc is the average of the disease index in untreated control treatment for the jth genotype. In trial 4, DISntc was calculated for each tunnel independently.

Before assessing efficacy, spatial effects on the disease index were corrected. For trial 3, the spatial correction was performed using the functions of the R package SpATS (Rodríguez-Álvarez et al. 2018). For trial 4, the spatial correction was performed using the functions of the R package statgenHTP (Millet et al., 2021).

Estimation of genotype means and heritabilities

A linear mixed model (**1**) of the Rpackage/lme4 (Bates et al. 2003), was used to estimate the variances of the random effects and to calculate the broad-sense heritability (**2**). Different mixed models were used to estimate variances for heritability and best linear unbiased predictors (BLUPs). The broad-sense heritability was calculated following formula.

$$\mathbf{Y_{ijk}} = \varvec\mu + \varvecG_{\varveci} + \varvecE_{\varvecj} + \varvecG_{\varveci}\varvec*\varvecE_{\varvecj} + \varvec\epsilon_{\varveci\varvecj\varvec k}$$

(1)

$$\varvecH^2 = \frac{\mathbf{Vg}}{\mathbf{Vg} + \frac{\varvecV\varvecg\varvece}{\varvecT} + \frac{\varvecV\varvecr}{\varvecR\varvecT}}$$

(2)

In mixed model, Y is the trait, μ is the general mean of the trait, G_i is the genetic effect of the ith genotype, following a normal distribution G ~ N(0, Vg), E_j is the effect of jth tunnel with E a fixed effect in the model, G x E is the genotype and environment interaction effect, which follows a normal distribution G x E ~ N(0, Vge), and are the residuals assumed to be independent and normally distributed with ~N(0, Vr).

In heritability formula, H^2 is the broad-sense heritability of the trait, Vg is the genetic variance, Vge is the variance of the genotype and environment interaction, and Vr is the residual variance, T is the number of tunnels, and R is the number of replicates per tunnel. The effects of tunnels (E) and GxE were used only for combined dataset.

The genomic heritability was calculated using GBLUP on GAPIT 3. It was calculated with the genetic values of the percentage of protection (BLUP + mean). All statistical analyses were performed using R software. Descriptive statistics and analysis of variance (ANOVA) were used to test the difference between accessions, tunnels and the interaction between accessions and tunnels. When the ANOVA test shows a significant difference, a post-hoc test was performed using Tukey's hsd.test function.

Genotypic data and GWAS

To detect associations between genotypic values and genomic markers, GWAS was performed using a set of 6,251,927 SNPs from genome resequencing (Héreil et al. 2024). SNP positions refer to the Heinz_1706 v.4.0.0 reference genome (SL4.0). SNPs exhibiting more than 3% heterozygosity were excluded from the dataset. All remaining heterozygous genotype calls were subsequently recoded as missing. Missing data were imputed by substituting the major allele, defined as the allele with the highest observed frequency across all samples. Following imputation, SNPs were filtered to retain only those with a minor allele frequency (MAF) greater than 10%. The final set of 82,969 SNP markers was retained and used for the identification of informative markers by GWAS. The LD parameter r^2 between pairs of SNPs was calculated using $-r^2$ command in PLINK software (Purcell et al. 2007) version based on a window size of 1000 kb, following the method described by (Wang et al. 2017).

To identify marker-trait associations to the traits, a GWAS was conducted using the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) (Huang et al. 2019) implemented in Genomic Association and Prediction Integrated Tool GAPIT 3 (Wang and Zhang 2021). Both population structure and kinship matrices were used as covariates to reduce false-positive associations due to population structure and familial relatedness. The population structure was calculated as principal component analysis (PCA) with the first three components. The kinship matrix was generated automatically in GAPIT 3 using the VanRaden method (VanRaden 2008). We used a genome-wide threshold ($P < 1.77x10^{-6}$) that was determined based on the effective number of independent markers, Me, estimated using the Gao et al. (2008) method on R software. The genome-wide threshold was calculated with the equation, 0.05/Me.

The phenotypic variance explained (PVE) by a significant marker was estimated using the equation in R (Kim et al. 2021):

$$\varvec{P}\varvec{V}\varvec{E} = \frac{\mathbf{SSsig.\,marker}}{\mathbf{SSallsig.\,marker + e}}\varvec{*}100$$

where SS is the sum of square and *e* is the residuals from the ANOVA fitted with a linear model incorporating the phenotypic data and all significant markers in decreasing order of their P-value (Knoch et al. 2020).

Candidate gene identification/detection

Genome-wide linkage disequilibrium (LD) decay was estimated by plotting the squared allele frequency correlation (r^2) against physical distance between SNPs. LD decay was defined as the chromosomal distance at which r^2 decreased to half of its maximum value (Wang et al. 2017), resulting in intervals ranging from ~ 600 kb to ~ 1 Mb. Therefore, these distances were used to define candidate genomic regions around significant SNPs. Candidate genes were investigated using the tomato reference genome assembly SL4.0 and the international tomato annotation group (ITAG) 4.0 at the Sol Genomics Network (https://solgenomics.net). From this subset, candidate genes were identified by manually searching gene descriptions for disease related genes. Candidate genes were also explored through a literature review.

Results

Defense genes induced by Belvine®

When the five tomato genotypes were analyzed together, Belvine® treatment resulted in the identification of 12 differentially expressed genes (DEGs) (Fig. 1a). Among these, nine genes were upregulated (ACCO, POX, PR1, PR2, PR4, PR5, PR8, PR15, CSL) whereas three genes (HMGR, PAL, Pect) were downregulated. Overall, the induction pattern was consistent across the five genotypes. The upregulated genes were mainly associated with PR protein and oxidative stress responses, whereas genes Involved in SA- and JA-signaling showed less induction by the treatment (Fig. 1b). Within the ET-signaling pathway, ACCO was strongly upregulated, whereas EIN3 was downregulated. Most genes involved in cell-wall reinforcement and secondary metabolite biosynthesis were downregulated, except CSL, which was upregulated in Clodano, Criollo, and Ecu284 (Fig. 1b). Differences in gene induction levels were also observed between accessions. Clodano showed the strongest defense gene induction, followed by Criollo, Marpha n2, and Vir884 (Fig. 1c), whereas Ecu284 displayed the lowest number of upregulated genes. Altogether, these results highlight substantial genotypic variation in the transcriptional response of tomato to Belvine® treatment.

Variation in Belvine®-induced resistance across genotypes

All 11 genotypes showed close levels of baseline susceptibility (scores ranging from 2 to 3) (**Fig. S3**). Treatment with Belvine® reduced late blight severity by an average of 89.1%. However, significant genotypic variation in treatment efficacy was observed among the 11 tomato accessions (ANOVA, $p < 0.001$; Fig. 2). Accessions treated with Belvine® could be assigned into two categories: those that showed no symptoms of disease after inoculation (Clodano, Ferum, and St Pierre) with 100% protection, and all other accessions, which were partially protected, with a disease severity index lower than 1 but a protection level above 70% (Fig. 2).

Phenotypic variation among 148 tomato accessions

The GWAS experiments revealed that Belvine® treatment consistently reduced the average disease index across all tomato accessions in each of the three independent experimental repetitions (tunnels). This trend was also observed for the overall disease index calculated across all replicates (Fig. 3; Fig. 4**)**. However, the reduction in disease development following treatment was not uniform among the different accessions. Differences in response were observed even among accessions displaying similar levels of susceptibility under the untreated control conditions (Fig. 3).

In terms of protection, Belvine® was the most effective in tunnel B25, with a mean protection of 65.73% and a standard deviation of 20.63 (Fig. 4). The protective efficacy observed in tunnels B24 and A25 was relatively similar, with mean protection values of 46.14% and 45.21%, respectively. The overall mean protection conferred by Belvine® was estimated at 41.71%. Histograms of the genetic values for percentage of protection (BLUP + mean) showed an approximately normal distribution of this trait in all tunnels as well as in the combined dataset (**Fig. S4**). All 148 accessions exhibited a favorable response to Belvine® application, with protection percentages ranging from 8.71% to 92.76% according to the combined phenotypic data (**Fig. S4**). The correlations between disease index values under control and Belvine® treatments were positive and statistically significant (p-value < 0.001) in all datasets (**Fig. S5a**). The Correlation coefficients were r = 0.42, r = 0.42, and r = 0.48 in tunnels A25, B24, and B25, respectively, while a higher correlation was observed for the combined dataset (r = 0.59). The correlation between disease index under control conditions and percentage of protection was negative and statistically significant in all cases except for tunnel A25 (**Fig. S5b**). Significant correlations ranged from − 0.22 to -0.32, with the strongest negative correlation observed in the combined dataset (r = -0.32).

Genetic effect on tomato accession responses to Belvine® after inoculation

Genetic effects were estimated through heritability, defined as the proportion of total phenotypic variation attributable to genetic variation. Broad-sense heritability was calculated for the disease index under both control and Belvine® treatments, as well as for the percentage of protection, which reflected PRI efficacy and plant response.

Under untreated control conditions, the broad-sense heritability of resistance to *P. infestans* ranged from 17% to 60%, with the lowest value observed in tunnel B24 and the highest obtained using the combined dataset from all tunnels (Fig. 4). Under Belvine® treatment, the broad-sense heritability of induced resistance against *P. infestans* ranged from 62% to 78%, with a broad-sense heritability of 66% in the combined dataset. The broad-sense heritability of the percentage of protection was like that observed for resistance induced by Belvine® treatment, ranging from 58% to 75% (Fig. 4). The heritability of the combined dataset reached 58%.

The data also highlighted the influence of genotype × environment interactions. These interactions contributed more strongly to traits associated with Belvine® application than to traits measured under untreated control conditions (18–22% vs. 2%) (Fig. 4). Genomic heritability estimates were 46.40% in tunnel A25, 46.14% in tunnel B24, 60.1% in tunnel B25, and 63.3% for the mean across the three tunnels (**Fig. S4**).

Population structure

Population genetic diversity analysis was conducted on 148 accessions. The PCA eigenvalue plot (**Fig. S6a**), generated using GAPIT 3, indicating that the first three principal components (PCs) explained most of the variation among the 148 accessions (40.92%). Phylogenetic trees were constructed using the neighbor-joining (NJ) method in GAPIT 3, resulting in a fan tree (**Fig. S6b**) displaying three distinct sub-populations. A 3D graphical plot of principal component analysis (PCA) (**Fig. S6c**) illustrated the three sub-populations. These plots and phylogenetic trees revealed the presence of three distinct clusters within the core-collection. The three principal components of the PCA were subsequently used as a Q-matrix in the GWAS analysis. Among the three clusters (sub-populations), G1 consisted of 18 individuals (12.2% of the entire population), G2 included 123 individuals (83.1%), and G3 comprised 7 individuals. The three subpopulations were not classed as *S. lycopersicum*, *S. pimpinellifolium* and admixes (**Supplementary data 2**).

Identification of marker-trait associations for plant responses to Belvine®

A GWAS performed using the BLUPs obtained from the overall dataset identified three significant SNPs associated with tomato response to the application of Belvine® (Fig. 5). These SNPs were located on chromosomes 2 (SNP37241), 4 (SNP77453), and 12 (SNP246258). The strongest effect was observed for the SNP located on chromosome 12, followed by those on chromosomes 4 and 2 (Table 1). The phenotypic variance explained by these SNPs was 17.06%, 13.46% and 7.56%, respectively. Together, these loci accounted for 38% of the total phenotypic variance.

Single-environment GWAS analyses identified five SNPs associated with plant response to Belvine® (Fig. 6). Two SNPs located on chromosomes 3 and 4 were detected in tunnel A25, one SNP on chromosome 4 was identified in tunnel B24, and two SNPs located on chromosomes 2 and 4 were detected in tunnel B25. These SNPs individually explained 17.48%, 10.31%, 12.86%, 21.13%, and 6.97% of the phenotypic variance in their respective environments. The two SNPs detected in tunnel A25 together explained 27% of the phenotypic variance in this tunnel, while the two SNPs identified in tunnel B25 also jointly explained 27% of the variance. The SNP detected on chromosome 4 in tunnel A25 was located very close to the SNP identified using the combined phenotypic data from all tunnels. Linkage disequilibrium (LD) analysis between SNPs located on the same chromosome showed that the two SNPs identified on chromosome 2 were strongly linked ($r^2 = 0.70$) (**Table S2**). On chromosome 4, two distinct LD groups were observed. SNP77432, SNP77453, and SNP79715 were strongly linked to each other ($r^2 \geq 0.60$), whereas their LD with SNP88909 was weak ($r^2 < 0.1$) (**Table S2**). LD analysis grouped the significant SNPs into five QTL regions: one on chromosome 2, one on chromosome 3, two on chromosome 4, and one on chromosome 12.

Table 1
Statistic of the significant SNP associated with tomato plant responses to Belvine®

Environment	SNP	Chr	Position[a]	P-value	MAF	Effect	PVE[b]
All	SNP37241	2	740 878	4.56e-07	0.17	-3.02	7.56
All	SNP77453	4	7 963 272	4.15e-08	0.30	-2.59	13.47
All	SNP246258	12	45 309 963	1.77e-07	0.11	-4.09	17.06
A25	SNP55112	3	7 461 290	5.01e-07	0.37	3.48	17.48
A25	SNP77432	4	7 955 113	1.72e-08	0.31	-3.43	10.31
B24	SNP88909	4	31 269 345	5.97e-11	0.14	-16.48	12.86
B25	SNP41286	2	11 610 659	3.34e-07	0.18	-3.95	21.13
B25	SNP79715	4	11 286 270	3.59e-07	0.30	-3.46	6.97

[a]The physical map positions of the SNP markers were determined using the tomato genome assembly SL4.0

[b]The phenotypic variance explained (PVE) by each marker

For most of the SNPs associated to tomato response to Belvine, the favorable allele corresponded to the reference allele, identical to the one of Heinz 1706 cultivar (SL4.0 genome version) (**Fig. S7, Fig. S8**). However, SNP55112, detected on chromosome 3 in tunnel A25, showed the opposite effect (Table 1; **Fig. S8**). For environments in which several significant SNPs were detected, the effects of different genotypic combinations were further analyzed. Although SNP × SNP interactions were not statistically significant in any dataset, phenotypic distributions showed that, in tunnel B25 and in the combined analysis, accessions carrying the reference genotype at all significant SNPs displayed higher levels of protection, whereas accessions carrying the alternative genotype at all significant SNPs showed the lowest levels of protection (**Fig. S7, Fig. S8**). As expected, in tunnel A25, the highest response levels were associated with the combination of the reference genotype at the chromosome 4 SNP and the alternative genotype at the chromosome 3 SNP. The other genotypic combinations showed relatively similar responses. Globally, the protection level was not linked to the allele origin.

Candidate genes surrounding significant SNPs

LD decay varied substantially among chromosomes, and candidate regions were defined based on chromosome-specific LD decay distances. LD decay was defined as the chromosomal distance at which r^2 decreased to half of its maximum value, resulting in intervals ranging from ~ 600 kb to ~ 1 Mb (Table 2**)**.

A total of 115 annotated genes were identified for the SNPs detected in the overall dataset (Table 2; **Supplementary Data 3**). In the region flanking SNP246258 on chromosome 12, 42 genes were annotated. Among these, seven were associated with plant resistance: three protein kinases or kinase regulators (Solyc12g036320.3.1, receptor-like protein kinase; Solyc12g036330.1.1, WAK-associated domain-containing protein; and Solyc12g036440.1.1, membrane-associated kinase regulator), three transcription factors (Solyc12g036430.3.1, bHLH95-like; Solyc12g036470.2.1, bHLH071; and Solyc12g036480.2.1, NAC domain protein), and one glutathione S-transferase (Solyc12g036560.2.1). For SNP37241 on chromosome 2, 21 genes were annotated, including two genes involved in transport processes, both encoding ABC transporter C family member 4 proteins (Solyc02g044050.1.1 and Solyc02g044000.3.1).

The region flanking SNP77453 on chromosome 4 contained 42 genes, among which four stress-response genes (Solyc04g016470.4.1, Solyc04g016580.1.1, Solyc04g017610.2.1, and Solyc04g017670.3.1) were considered potential candidates. The first gene was annotated as a β-1,3-glucanase, whereas the remaining three encoded F-box proteins. Because SNP77432, detected in tunnel A25, is located very close to SNP77453, the same candidate genes were considered for both SNPs. In the second region detected in tunnel A25, surrounding SNP55112 on chromosome 3, 45 genes were annotated. Four of these were associated with resistance-related functions: one peroxidase (Solyc03g044100.4.1), two protein kinases (Solyc03g044140.3.1 and Solyc03g044160.3.1), and one F-box protein (Solyc03g044220.1.1).

For SNP88909 identified on chromosome 4 in tunnel B24, 13 genes were annotated, including one gene involved in signal transduction (Solyc04g045290.1.1). In tunnel B25, 21 genes were annotated in the region surrounding SNP79715 on chromosome 4, including several genes associated with secondary metabolite biosynthesis (Solyc04g040190.1.1, Solyc04g040130.1.1, Solyc04g040120.1.1, and Solyc04g040090.4.1).

Table 2
Candidate genes related to plant defense in the interval of the significant SNP

All	SNP	Chr	Position	Regions	Number of genes [b]	Solyc number of candidate genes	Annotation
	SNP37241	**2**	**740 878**	**750 kb**	**21 (2)**	**Solyc02g044050.1.1**	**ABC transporter C family member 4**
						Solyc02g044000.3.1	ABC transporter C family member 4
	SNP77453	4	7 963 272	650 kb	42 (4)	Solyc04g016470.4.1	β-1,3-glucanases
						Solyc04g016580.1.1	F-box protein
						Solyc04g017610.2.1	F-box protein
						Solyc04g017670.3.1	F-box protein
	SNP246258	12	45 309 963	1000 kb	42 (7)	Solyc12g036320.3.1	Receptor-like protein kinase
						Solyc12g036330.1.1	WAK-associated domain-containing protein
						Solyc12g036430.3.1	Transcription factor bHLH95-like
						Solyc12g036440.1.1	Membrane-associated kinase regulator
						Solyc12g036470.2.1	bHLH transcription factor 071
						Solyc12g036480.2.1	NAC domain
						Solyc12g036560.2.1	Glutathione S-transferase
A25	SNP55112	3	7 461 290	600 kb	45 (4)	Solyc03g044100.4.1	Peroxidase
						Solyc03g044140.3.1	Protein kinase domain
						Solyc03g044160.3.1	Kinase family protein
						Solyc03g044220.1.1	F-box protein
B24	SNP88909	4	31 269 345	650 kb	13 (1)	Solyc04g045290.1.1	Transducin/WD40 repeat-like superfamily protein
B25	SNP41286	2	11 610 659	750 kb	4 (0)	-	-
	SNP79715	4	11 286 270	650 kb	21 (4)	Solyc04g040190.1.1	lycopene beta-cyclase
						Solyc04g040130.1.1	Fatty acid desaturase
						Solyc04g040120.1.1	Fatty acid desaturase
						Solyc04g040090.4.1	Strictosidine synthase-like protein

[a]Regions = interval of the candidate regions (+/-)

[b]Values in parentheses are the number of candidate genes found in the defined regions

Discussion

First report of QTL underlying tomato accession responses to PRI

Our results demonstrate that the efficacy of Belvine® against *P. infestans* is positive but strongly dependent on plant genotype, with a quantitative distribution of protection levels across accessions. This conclusion is further supported by estimates of broad-sense heritability for the percentage of protection, ranging from 60% to 78%, and estimates of narrow-sense heritability ranging from 46% to 63%, depending on the dataset used (A25, B24, B25, or combined data) (Fig. 5, Fig. 6). These heritability estimates for the percentage of protection are comparable with those reported for genetic resistance to late blight (Merk and Foolad 2012; Ohlson and Foolad 2015, 2016; Sullenberger and Foolad 2018). Overall, these results suggest that an important portion of the variation observed in the response of tomato to Belvine® treatment is genetically controlled and could therefore be amenable to genetic characterization and selection.

Sharma et al. (2010), also reported that that the protsection conferred by another PRI, the dl-3-aminobutyric acid (BABA), against *P. infestans* varied according to tomato genotype and leaf age. However, their study was conducted on only 32 accessions belonging exclusively to the species *S.*

lycopersicum, whereas our study included 148 accessions originating from several tomato species including *S. lycopersicum* var. *lycopersicum*, *S. lycopersicum* var. *cerasiforme S. pimpinellifolium*, thereby encompassing a much broader range of genetic diversity. The consistency of genotype-dependent responses across such a diverse panel strengthens the evidence that responsiveness to PRI is genetically determined and supports the existence of a quantitative genetic basis underlying the efficacy of PRI-induced resistance in tomato.

We detected a total of eight significant SNPs associated with tomato plant responses to the application of Belvine®, three SNPs with the overall dataset on chromosomes 2, 4 and 12, and four SNPs environment specifics, on chromosomes 2, 3, and 4. Based on pairwise linkage disequilibrium, the eight SNPs clustered into five QTL regions. To the best of our knowledge, this is the first time that a mapping study of loci involved in the tomato response to PRIs is reported. Our findings are consistent with previous work reporting genomic regions associated with the genotypic variability of PRI efficacy. Indeed, a few studies have identified QTL regions associated with PRI-induced resistance in other species. Soleimani et al. (2024), found 5 QTLs involved in wheat N-acyl homoserine lactone (AHL)-induced resistance against leaf rust caused by *Puccinia triticina*. Similarly, fifteen QTLs have been found to be associated with the variability of *Serendipita indica* efficacy against powdery mildew resistance in wheat (Thielmann et al. 2026). Bénéjam et al. (2021), have also detected QTLs associated with apple tree resistance following treatment with acibenzolar-S-methyl (ASM) against apple scab and fire blight. The difference with that study is that all the QTL found under PRI-treated conditions were also detected in control conditions.

In this study, we mapped loci involved in the response of tomato accessions to Belvine®, using both combined data and single-environment analyses. This approach is supported by previous studies showing that PRI efficacy is strongly influenced by environmental conditions and genotype-by-environment interactions (Walters et al. 2011; Bruce 2014; Stridh et al. 2022), suggesting that different genes may contribute to plant responses depending on the environment. Consistent with this, we identified distinct genomic regions across our three environments. Integrating detailed environmental descriptors into future genetic analyses will improve the precision of QTL interpretation. Environment-specific loci could then be strategically used in breeding programs, particularly for deployment in regions where specific environmental conditions enhance PRI efficacy.

Previous studies have reported late blight resistance QTLs, LB-qtl2 and LB-qtl12, on chromosomes 2 and 12 in the *S. pimpinellifolium* accessions PI270442, PI 163245 and the *S. lycopersicum* breeding line Fla.8059 (Ohlson et al. 2018; Sullenberger and Foolad 2024). Although these QTLs are located on the same chromosomal arms as the loci identified in the present study, they do not co-localize, suggesting that the genes underlying genetic resistance differ from those involved in the plant response to the PRI treatment.

Candidate genes associated with tomato accessions responses to Belvine®

Within the confidence intervals of the significant SNPs detected in the combined dataset, 105 genes were annotated. Among them, glutathione S-transferase (GST) and β-1,3-glucanase were upregulated under Belvine® treatment. β-1,3-glucanases, members of the PR2 family, contribute to antimicrobial defense through pathogen cell-wall degradation and callose mobilization (Balasubramanian et al. 2012; Perrot et al. 2022). In tomato, the co-expression of β-1,3-glucanase with other defense enzymes (peroxidase, chitinase, polyphenol oxidase, phenylalanine ammonia-lyase) enhances resistance to *Fusarium oxysporum f. sp. lycopersici* and *Alternaria solani* (Mohamed et al. 2007; Salim et al. 2011). Although the specific genes differ from those induced by Belvine®, they belong to the same functional family, reinforcing their relevance as candidate genes for PRI responsiveness.

Other notable candidates included protein kinases (receptor-like kinases), ABC transporters, and transcription factors such as bHLH95-like, bHLH071, and NAC (NAM, ATAF, and CUC) domain proteins. These families are widely implicated in plant immunity. For example, *SlLRR-RLK94* enhances tomato resistance to *Phytophthora infestans* ((Zhu et al. 2025), while in *Nicotiana benthamiana*, ABC transporters *NbABCG1* and *NbABCG2* limit *P. infestans* penetration (Shibata et al. 2016; Rin et al. 2017). It has been reported that NAC and bHLH transcription factors regulate SA-, JA-, ET- and ABA-dependent defense pathways (Nuruzzaman et al. 2013; Chen et al. 2023; Yan et al. 2024; Xiong et al. 2025). In tomato, the genes SlSRN1 and SlNAC1 positively modulate resistance to *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* (Huang et al. 2013; Liu et al. 2014). In rice, bHLH25 contributes to cell-wall reinforcement by repressing miR397b and activating lignin biosynthesis after infection (Liao et al. 2025).

Environment-specific candidate genes were also identified. In tunnel A25, a peroxidase gene co-localized with a significant SNP on chromosome 3. That gene is particularly relevant given that a related gene was upregulated under Belvine®. In tunnel B24, genes involved in signal transduction were detected on chromosome 4, while in tunnel B25, genes associated with alkaloid and terpenoid biosynthesis, as well as fatty-acid desaturases, were identified. These functions are consistent with previous studies showing the accumulation of alkaloids, terpenoids, and lipid-derived defenses in resistant plants or PRI-treated plants during interactions with *P. infestans* (Yogendra et al. 2017; Galeano Garcia et al. 2018; Zhao et al. 2023; Agho et al. 2023).

Together, these candidate genes highlight the diversity of molecular pathways potentially involved in Belvine® responsiveness. Further studies using RNA-seq analysis will enable evaluation of expression variation across a broader set of genes.

Conclusion

This study is the first report of chromosomal regions and candidate genes associated with tomato responses to PRI applications. We identified eight significant SNPs associated with tomato accession responses to the application of Belvine®, located on chromosomes 2, 3, 4, and 12, with some

SNPs being specific to environmental conditions. The strong linkage disequilibrium observed between some SNPs suggests the presence of five potential QTLs: one on chromosome 2, one on chromosome 3, two on chromosome 4, and one on chromosome 12. The strongest QTLs were identified on chromosome 12 for the combined dataset, and on chromosomes 2 and 3 in tunnels B25 and A25, respectively. Several candidate genes were identified within the significant SNP regions, including protein kinases, receptor-like protein kinase, transcription factors, and pathogenesis-related proteins. Some of these genes were found to be upregulated under Belvine® treatment in qPCR analysis, notably β-1,3-glucanases (PR-2), glutathione S-transferase, and peroxidase. The identified QTL regions, and candidate genes constitute a valuable genetic resource for improving tomato breeding for their responses to PRIs.

Declarations

Competing Interests

There is no conflict of interest in this research.

Funding

This work was supported by the 'CapZéroPhyto' project of the French Priority Research Programme "Cultiver et Protéger Autrement" project, the ANR (grant number ANR-20-PCPA-0003).

Author Contribution

A.S., B.C. and M.B. conceptualized and designed the study. A.S. analysed the data. A.S. and M.G. wrote the manuscript. B.C. and M.B. supervised the work and secured funding. A.S. and M.D. extracted RNA for gene expression analyses. M.G. performed the transcriptomic analysis. A.S., B.C., M.B., J.F.B., E.P., M.D. and A.M. contributed to data acquisition. M.C. provided genotypic data. B.C., M.B. and M.C. revised the manuscript critically for important intellectual content.

Acknowledgement

We are thankful to Mélanie Andrin, Octave Cabel, Alexandre Bachellez, Karine Pellegrino, and Pascale Mistral for taking care of the plants and theirs help for plant phenotyping, to the centre for vegetable germplasm (CRB-Leg) of GAFL for keeping the seeds of the CC panel available, to the experimental staff of UE A2M "Arboriculture et Maraichage Méditerranéen", and to the staff of the experimental platform PROPHYLE (Unité Pathologie Végétale). We are also grateful to Sonia Elbelt and Jacques Lagnel who helped us in bioinformatics analysis. We are highly grateful to Emilie Millet, Bénédicte Quilot, Véronique Lefebvre, Morgane Roth, and all the members of the quantitative genetic club of GAFL for their insightful and enriching discussions on GWAS analysis. We thank the 'CapZéroPhyto' project of the French Priority Research Programme "Cultiver et Protéger Autrement" project, the ANR (grant number ANR-20-PCPA-0003) for funding.

Data Availability

The datasets presented in this study are available in Tables, Figures, Supplementary Tables, Supplementary data and Supplementary Figures. The tomato genotypes used in this study belong to a diversity panel previously described in Héreil et al. (2024). Raw genomic reads for the 'Rose de Berne' accession from the core collection can be found in the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) database under accession number PRJEB123549. All data related to these genotypes are available in the corresponding publications. The phenotypic data are available https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/0ZYYBG).

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Figures

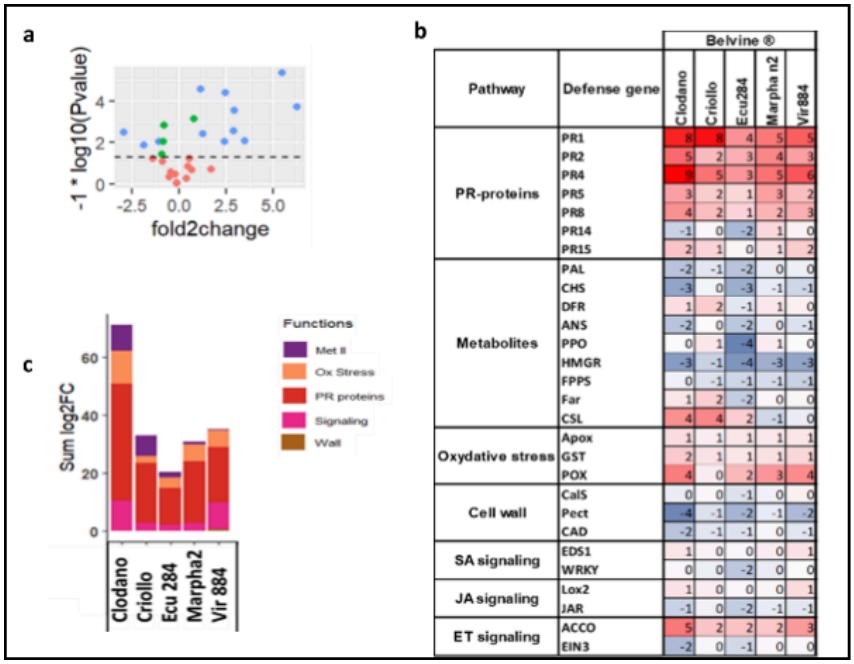

Figure 1

Defense genes induced by Belvine® three days after the second treatment. a) volcano plot of the 28 defense genes based on the data from the 5 tomato accessions. The horizontal axis represents the log2 values of the expression ratio, and the vertical axis represents the p-values (-log10). Blue dots indicate differentially expressed genes (|log2foldchange| >= 1 and p-value ≤ 0.05). Orange dots indicate genes not meeting the p-value criterion, and green dots indicate genes reaching the p-value criterion but not the log2 expression ratio criterion. b) relative gene expression in the tomato genotypes Clodano, Criollo, Ecu284, Marpha n2, and Vir884. Values represent the log2folchange values with untreated control plants used as the reference. Each value represents the mean of two independent experiments. Positive values indicate gene upregulation, whereas negative values indicate gene downregulation. c) Genetic variation in the induction of defense-related genes. The y-axis represents the cumulative $\log_2$ fold changes of upregulated genes (log2foldchange >=1). Each color corresponds to the pathways to which belongs the gene: purple = secondary metabolism, orange = oxidative stress, red = pathogenesis-related (PR) proteins, pink = hormonal signaling pathways, and brown = cell wall–related genes

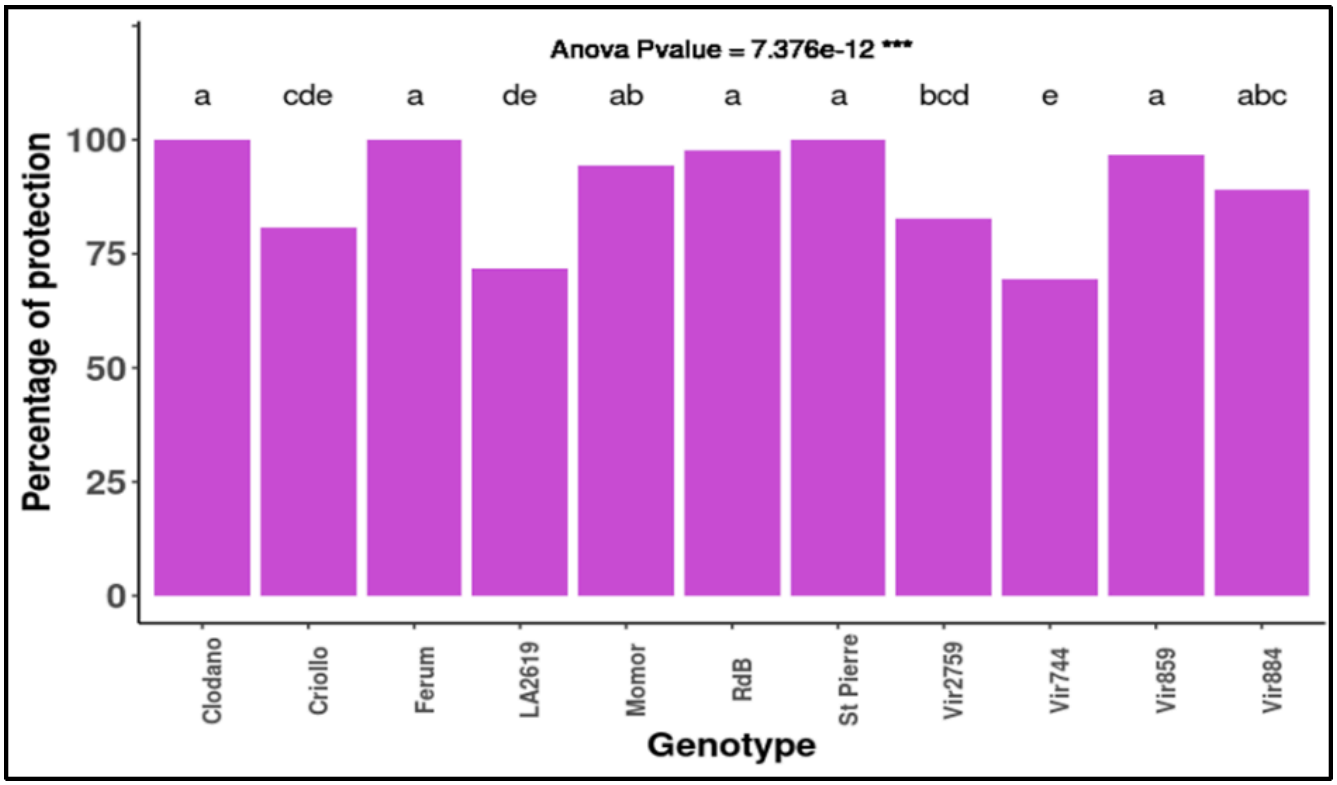

Figure 2

Genotypic variation of Belvine® efficacy. Efficacy of Belvine® against late blight across the eleven accessions. Different letters indicate statistically distinct groups among genotypes based on Tukey's post hoc test. Asterisks (*) denote significant differences between treated and control modalities according to ANOVA

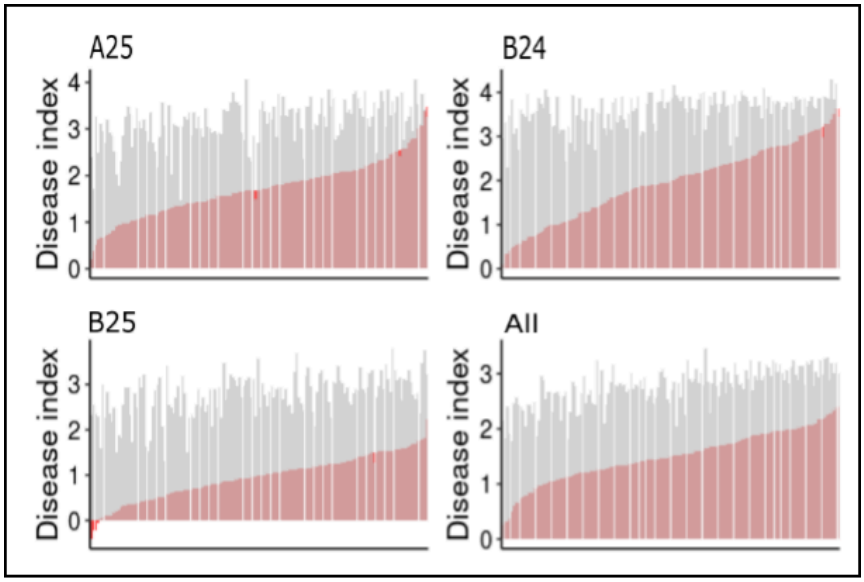

Figure 3

Variability of 148 tomato accessions resistance to *P. infestans* under control and Belvine® treatment. X axis present the accessions. Grey represents the disease index under untreated conditions and red color represents disease index under Belvine® condition. A25: Phenotypic data in tunnel A25, B24: Phenotypic data in tunnel B24, B25: Phenotypic data in tunnel B25, and All: the combined phenotypic data of the three tunnels. The order of accessions varies between plots

Environment	Trait	Mean	SE	H^2	Variance decomposition
T4B24	*Disease index in NTC*	3.58	0.03	0.17	0.17 / 0.83
	Disease index in P1	1.95	0.05	0.78	0.78 / 0.22
	Percentage of protection (PP)	46.14	1.40	0.75	0.75 / 0.25
T4B25	*Disease index in NTC*	2.75	0.03	0.53	0.53 / 0.47
	Disease index in P1	0.95	0.03	0.66	0.66 / 0.34
	Percentage of protection (PP)	65.73	1.19	0.62	0.62 / 0.38
T4A25	*Disease index in NTC*	3.13	0.04	0.34	0.34 / 0.66
	Disease index in P1	1.71	0.04	0.62	0.62 / 0.38
	Percentage of protection (PP)	45.21	1.26	0.60	0.6 / 0.4
All	*Disease index in NTC*	2.76	0.03	0.60	0.6 / 0.38
	Disease index in P1	1.49	0.02	0.66	0.66 / 0.18 / 0.16
	Percentage of protection (PP)	41.71	1.46	0.58	0.58 / 0.22 / 0.21

Figure 4

Statistics of phenotypic data collected from 148 tomato accessions. H^2: Broad-sense heritability, NTC: untreated control condition; P1: Belvine® treated condition; PP: percentage of protection. In column variance decomposition: black represents the percentage of variance explained by genetic variation, orange represents the percentage of variance explained by the interaction between genetic x environments, and green represents the percentage of variance explained by residual variation

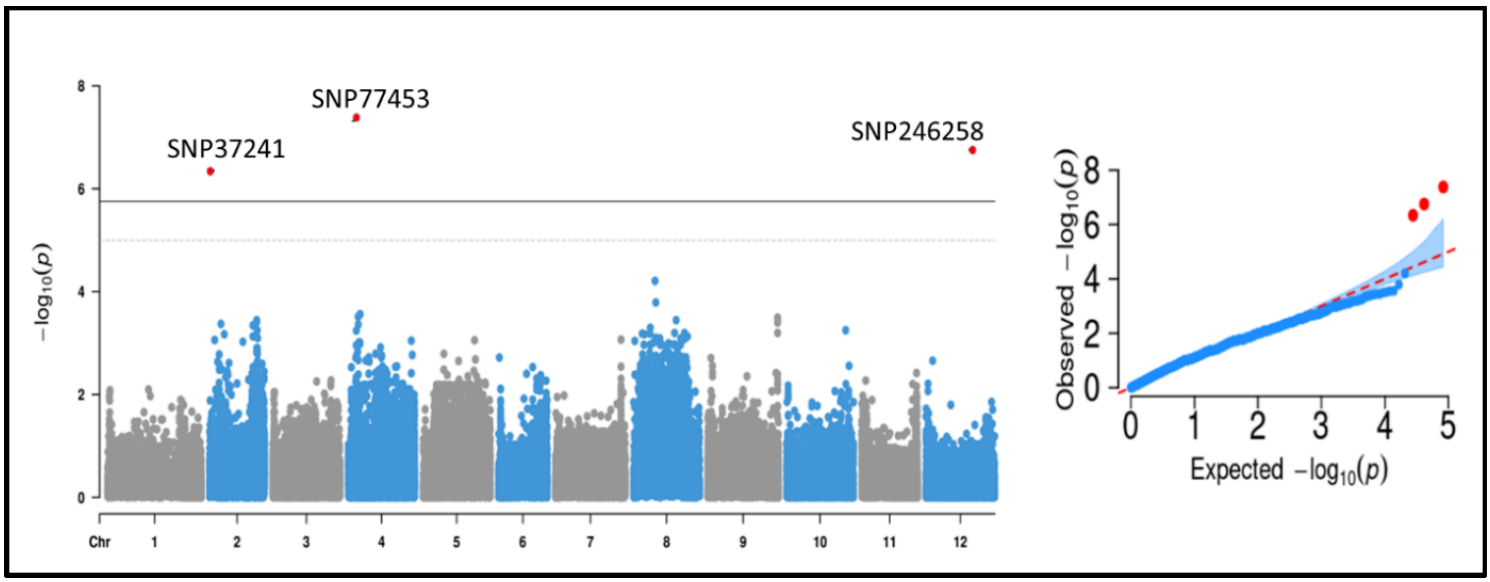

Figure 5

SNP associated with plant responses to Belvine® application. Left**:** Manhattan plot showing significant SNP associated with plant responses to Belvine®. The X-axis shows SNPs along each chromosome; the Y-axis is the -log10P for the association. The gray lines indicate the Gao significance threshold (-log10(p) = 5.75); Red points indicate the significant SNPs with their name. Right: Corresponding quantile-quantile plots (QQplot) for plant responses. The X-axis shows expected -log10 (P), and the Y-axis shows observed -log10(p). Phenotypic data used for this analysis are the combine dataset (Blups) obtained from the linear mixed model containing GxE interaction

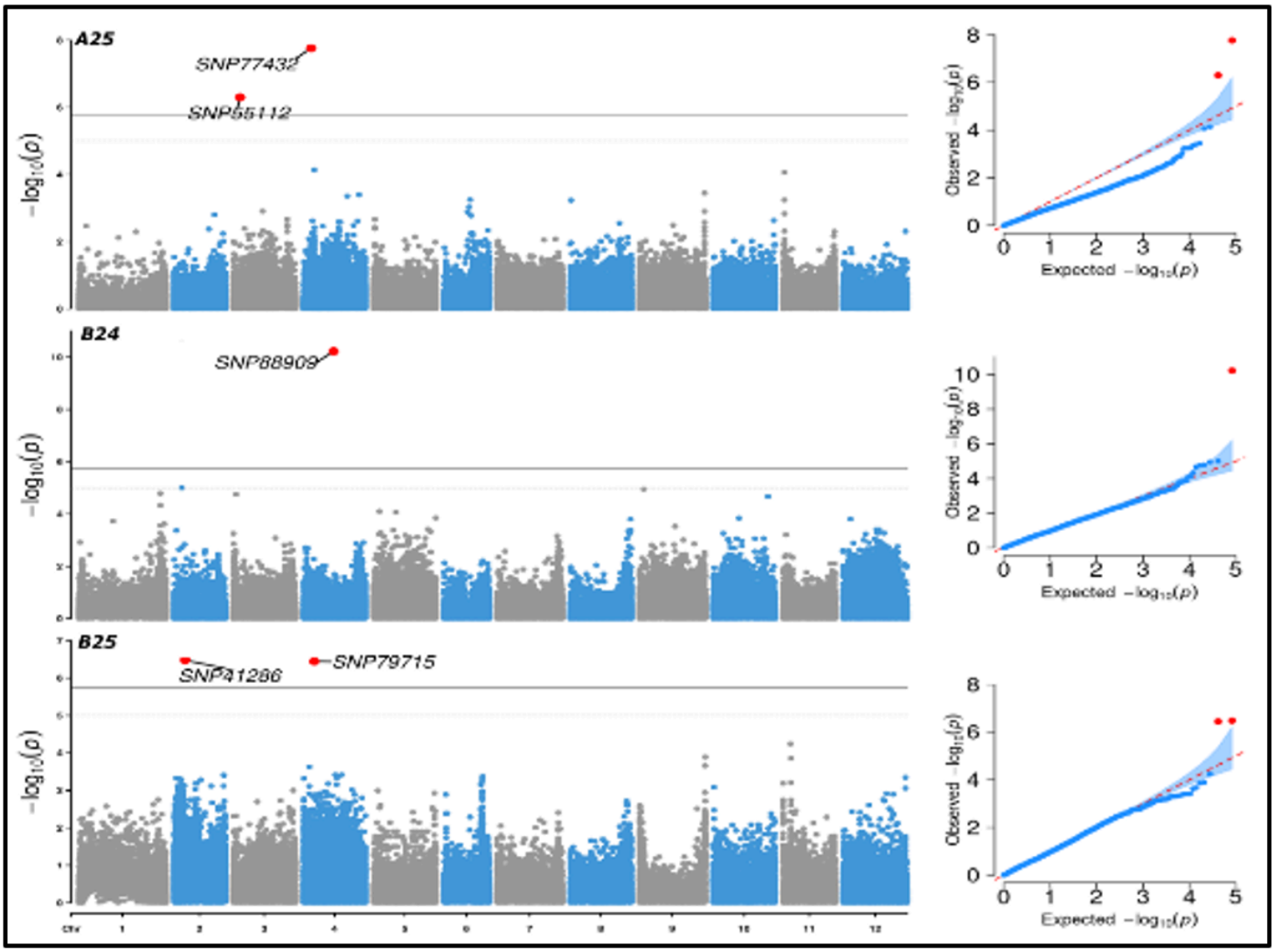

Figure 6

Left: Manhattan plot showing significant SNP associated with plant responses to Belvine® in each tunnel A25, B24 and B25, respectively. The X-axis shows SNPs along each chromosome; the Y-axis is the -log10(p-value) for the association analysis. The grey lines indicate the Gao significance threshold (-log10(p-value) = 5.75); Red points indicate the significant SNPs with their name. Right: Corresponding quantile-quantile plots (QQplot) for plant responses. The X-axis shows expected -log10 (P), and the Y-axis shows observed -log10(p)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformationGWASPi.pdf
- SupplementarydataGWASPi.xlsx