

Comparative genomic analysis of QTL for resistance to *Aphanomyces euteiches* between pea, lentil, faba bean and the model species *Medicago truncatula*

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Abstract

Aphanomyces root rot, caused by *Aphanomyces euteiches*, is a damaging disease affecting various legume species. Quantitative trait loci (QTL) for partial resistance were previously identified mainly in pea, and also in lentil and *Medicago truncatula*. This study aimed to identify novel resistance loci from available populations in lentil and faba bean and examine genomic conservation of resistance QTL across legume host species.

QTL mapping in the *Pop2* faba bean recombinant inbred line (RIL) population and genome-wide association study (GWAS) in the *AGILE* lentil diversity panel were performed for resistance to *A. euteiches* under controlled conditions, using genotyping data previously reported. A previous QTL mapping in the *LR3 M. truncatula* RIL population was updated using 1,536 new SNPs (single nucleotide polymorphisms). Synteny between resistance QTL to *A. euteiches* was analyzed based on gene orthology in the QTL regions projected onto genomes, using the OrthoLegKB graph database.

Four loci, including a major-effect QTL on chromosome 3, *Ae-Vf3.1*, were associated with resistance in faba bean. In lentil, six minor-effect GWAS-SNPs and two favorable haplotypes at *Ae-Lc1.1* and *Ae-Lc2.1* loci were identified. Updated analyses in *M. truncatula* narrowed to 8 Kb, including three candidate genes, the interval at the major-effect locus *AER1*. Synteny analysis identified 65 resistance QTL to *A. euteiches* overlapping 24 syntenic blocks defined between legume genomes. No synteny was highlighted between two major-effect QTL. These results pave the way for translational genomics approaches facilitating resistance gene discovery and for resistance QTL deployment strategies in legume rotations to preserve their durability.

Key Message

QTL mapping and GWAS detected resistance QTL to *Aphanomyces euteiches* in faba bean, lentil, and *Medicago truncatula*. Low genomic conservation between resistance QTL was identified between these legumes and pea.

1. Introduction

Grain legumes are crucial crops for sustainable agricultural systems worldwide, due to their ability to fix atmospheric nitrogen, reducing the need for fertilizers, and their high protein-rich seeds, suitable for human and animal consumption. Pea, faba bean and lentil are major cool-season legumes, with a global production of dry grains estimated at 6.14, 6.66, and 14.17 million tonnes, respectively, in 2022 (FAOSTAT 2024). However, legume crops face various biotic and abiotic stresses, leading to significant yield instabilities. Aphanomyces root rot, caused by the oomycete *Aphanomyces euteiches* Drechs., is a devastating disease representing an important threat to legume crop production worldwide (Wu et al. 2018). Symptoms of the disease are discoloration of the secondary then the main roots, resulting in leaf yellowing and plant death under high infestation conditions (Hughes and Grau 2013). *A. euteiches* displays a wide host spectrum in legumes, such as alfalfa (*Medicago sativa*), common bean (*Phaseolus vulgaris*), clover (*Trifolium repens* and *Trifolium pratense*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), pea (*Pisum sativum*), vetch (*Vicia sativa*), and the model legume *Medicago truncatula* (Wicker et al. 2001; Moussart et al. 2007, 2008). Among pea-infecting *A. euteiches* isolates, two main pathotypes, *i.e.* groups of isolates derived from a single isolation in pure culture that display the same level of pathogenicity across a differential range of plant genotypes (Agrios, 2005), were reported using a differential set of six pea genotypes (Wicker and Rouxel 2001). Pathotype I was identified in several countries worldwide, and pathotype III has been exclusively detected in the USA and Canada up to now (Sivachandra Kumar et al. 2021; Moussart et al. 2024). Among alfalfa-infecting *A. euteiches* isolates sampled from diverse regions of the USA, two pathotypes (race 1 and race 2) were identified using a differential set of three alfalfa genotypes (Malvick and Grau 2001; Malvick et al. 2009). *A. euteiches* isolates from pea can infect alfalfa, but most isolates from alfalfa were reported as nonpathogenic on pea (Malvick et al. 1998; Wicker et al. 2001). Currently, no effective method is available to control the Aphanomyces root rot disease. Therefore, farmers are advised to prevent the spread of the pathogen through crop rotations with non-host or resistant legume crops and by monitoring infestation levels using inoculum detection tests (Chatterton et al. 2023). In fields contaminated with oospores, which can remain viable in the soil for over ten years (Wu et al. 2018), these strategies are particularly valuable. Nevertheless, genetic resistance is an essential lever in combination with prophylaxis and cultural methods to manage Aphanomyces root rot. Significant advancements have been achieved over the past two decades in understanding the genetic basis of resistance to *A. euteiches* in legumes.

In pea, ten genetic regions and flanking SNP markers were consistently detected for resistance to *A. euteiches* from diverse strains in controlled conditions and/or French and American infested field nurseries. These genetic regions were identified by integrating QTL mapping study from two advanced backcross (AB) populations (Leprévost et al. 2023), along with previous QTL and GWAS analyses achieved from four RIL populations (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013) and a pea-Aphanomyces collection, respectively (Desgroux et al. 2016). In particular, genetic regions located on chromosomes 4 and 7 (named *Ae-Ps4.5* and *Ae-Ps7.6*, respectively), were detected with major effects on partial resistance to the RB84 (pathotype I) and Ae109 (pathotype III, Wisconsin, USA) pea-infecting strains of *A. euteiches*, respectively. Additionally, a QTL analysis conducted on a RIL population under greenhouse conditions using the pea strain Ae-MDCR1 (pathotype I, Manitoba, Canada), as well as in a Canadian contaminated field, resulted in the identification of minor- to moderate- effect QTL associated with resistance to *A. euteiches* (Wu et al. 2021). One of these regions, showing a moderate effect, colocalized with *Ae-Ps4.5*.

In *Medicago truncatula*, linkage analyses studies conducted in two RIL populations identified a major-effect QTL region, named *AER1* or *prAe1* depending on the population, as well as minor-effect QTL (Djébali et al. 2009; Pilet-Nayel et al. 2009; Hamon et al. 2010). The *AER1* region, located on chromosome 3, displayed a variable additive effect on resistance depending on the pea-infecting strain employed (RB84 or Ae109). It also exhibited an epistatic effect for resistance to MF-1 (race 1, Wisconsin, USA) and NC-1 (race 2, North Carolina, USA) alfalfa-infecting strains, which were reported to be pathogenic to alfalfa but not to pea (Malvick et al. 1998). Moreover, a GWAS approach using a collection of 174 *M. truncatula* accessions inoculated with the ATCC201684 pea strain (pathotype I, Denmark), identified candidate genes located near the *AER1* major-effect QTL (Bonhomme et al. 2014). The updated GWAS, using the five pea- or alfalfa- *A. euteiches* strains previously employed in genetic analyses, refined the position of the major-effect region and detected a diversity of minor-effect QTL (Bonhomme et al. 2019).

In lentil, to our knowledge, only one genetic study for resistance to *A. euteiches* has been reported. Ma et al. (2020) identified seven minor-effect resistance QTL clusters, located on six chromosomes, using a combined approach based on GWAS from a lentil diversity panel and QTL mapping analysis from a RIL population. The QTL clusters were identified for partial resistance to the Ae-16-04D pea strain (pathotype I, Washington, USA) under greenhouse conditions and/or in infested field environments in the USA.

In faba bean, no genetic analysis has been conducted for resistance to *A. euteiches* to date. Faba bean cultivars are highly resistant to *A. euteiches* and are often recommended to be integrated into crop rotations to limit the soil inoculum potential. However, under growth chamber experiment, Moussart et al. (2008) identified three susceptible faba bean genotypes to the RB84 strain (pathotype I, Riec-sur-Belon, France) of *A. euteiches*, suggesting intra-specific genetic variability in response to infection.

For sustainable agricultural and food systems, the development of grain legume crop areas represents an increased risk of *Aphanomyces* root rot spread with potential adaptation of *A. euteiches* population to the genetic resistance loci selected in legume varieties (Quillévéré-Hamard et al. 2021). Indeed, the intensification of crop cultivation sharing the same genetic resistance factors could exert pressure on the adaptation of pathogen populations and impact the durability of genetic resistance (Papaix et al. 2011). In grain legumes with highly conserved genomes that can be infected by the same pea-infecting isolates of *A. euteiches*, it can be hypothesized that resistance genetic loci are shared. A better understanding of the genomic conservation and diversity of resistance loci among grain legumes cultivated in the same climatic regions would help in better reasoning the strategies for their durable deployment at the crop rotation scale. Recent genome assemblies of legume species, such as *P. sativum* (Kreplak et al. 2019), *L. culinaris* (Ramsay et al. 2021), *V. faba* (Jayakodi et al. 2023), or *M. truncatula* (Pecrix et al. 2018), have paved the way for conducting comparative and translational genomic analyses. However, to our knowledge, no published study has reported the synteny or orthology relationships between QTL or genes identified for resistance to *A. euteiches* across closely related grain legumes.

This study aimed to analyze the synteny and orthology relationships between QTL and genes conferring resistance to *A. euteiches* in three major grain legume crops, pea, faba bean and lentil, and the model legume *M. truncatula*. The same RB84 pea-infecting strain of *A. euteiches* was consistently used in screening tests across the four species, allowing for the comparison of plant genetic loci interacting with the same *A. euteiches* genotype. First, we identified QTL for resistance to *A. euteiches* in the Silian x Hiverna RIL population of faba bean, named *Pop2* and previously reported by Carrillo-Perdomo et al. (2020). Second, we performed a GWAS to discover new genomic regions associated with resistance to *A. euteiches* in lentil, using a *L. culinaris* diversity panel named AGILE (Application of genomics to innovation in the lentil economy; Wright et al., 2021), previously genotyped using an exome capture array (Haile et al. 2020). Third, we updated the QTL mapping analysis previously reported in the *LR3 M. truncatula* RIL population by Hamon et al. (2010), using a higher density genetic map integrating new SNP markers. Finally, using the recent OrthoLegKB orthology-driven database dedicated to research on legume crop species, we examined synteny relationships between resistance QTL to *A. euteiches* identified in faba bean, lentil, pea, and *M. truncatula* in this study and/or previously reported. We additionally explored orthology relationships between genes underlying syntenic blocks involving major-effect QTL identified in the different species.

2. Materials and methods

2.1. Plant material

The *Pop2* population of 147 *V. faba* F5-derived RILs, previously described in Carrillo-Perdomo et al. (2020), was used for disease experiments (Supplementary Table 1.A). The two parental lines, Hiverna (accession ID IVIP102372) and Silian (also named Di-340, accession ID IVIP100340), provided from the genetic resource center PROTEA, based at INRAE Dijon, were used as checks. Silian is a landrace of *V. faba* spp. *minor* originating from Northern Sudan and displays susceptibility to the RB84 French strain of *A. euteiches*. Hiverna is a German winter-type cultivar with a high-level of resistance to *A. euteiches* (Moussart et al., 2008).

A total of 321 *L. culinaris* ssp. *culinaris* accessions from the lentil diversity panel AGILE, previously described in Wright et al. (2021), were also evaluated for resistance to *A. euteiches*. The panel includes cultivars, landraces, and research/breeding lines from 44 different countries across Africa (55), America (62), Asia (146), and Europe (40). Eighteen accessions from this panel display unknown origins (Supplementary Table 2.A).

A total of 176 *M. truncatula* F5:6-derived lines from the *LR3* RIL population, derived from the cross between F83005.5 and DZA045.5, susceptible and partially resistant to *A. euteiches*, respectively, were used to update the QTL mapping analyses previously reported in Hamon et al. (2010). F83005.5 and DZA045.5 are inbred line accessions originating from southern France and Algeria, respectively (Supplementary Table 3.A).

2.2. *Aphanomyces* disease experiments

Faba bean lines were challenged under growth chamber conditions (25°C 16 h / 23°C 8 h) for resistance to RB84 (Moussart et al. 2008) and Ae109 (Wicker and Rouxel 2001) *A. euteiches* strains. Lentil lines were only evaluated for resistance to the RB84 strain, under greenhouse conditions (24 ± 3°C 16 h / 19 ± 2°C 8 h).

Faba bean lines were planted in pots filled with vermiculite medium, and evaluated within a randomized complete block design. All the lines were evaluated within a two-block disease test, each block consisting of four plants per line in a pot, and the disease test was repeated three times. Each line, therefore, recorded a total of 24 observations. Inoculum solutions were prepared following the procedure presented in Moussart et al. (2008). Incubation temperatures were maintained at 25°C and 22°C for RB84 and Ae109 strain, respectively. One week after planting, 5 mL of zoospore solution, concentrated at 5.10³ zoospores/mL, was deposited at the base of each seedling. Ten days after inoculation, the plants were uprooted and scored using a disease severity (DS) scale ranging from 0 (healthy plant) to 5 (dead plant), as described by Moussart et al. (2008).

Lentil lines were individually planted in Cone-tainers™ filled with sterilized perlite medium, using a randomized complete block design with 10 inoculated replicates and 10 non-inoculated replicates with one plant per line x replicate x treatment combination. Zoospore inoculum was concentrated at 10^4 zoospores/mL and maintained at an incubation temperature of 25°C, following the procedure presented in Ma et al. (2020). Two weeks after planting, 2 mL of zoospore solution and 2 mL of sterile distilled water were pipetted at the base of each inoculated and non-inoculated seedling, respectively. After 14 days of incubation, DS was scored on each uprooted plant using the 0 (healthy plant) to 5 (dead plant) scoring scale described in Ma et al. (2020). Non-inoculated and inoculated plants were dried at 60°C in a forced-air drying room for seven days and weighed using a Mettler Toledo AT261 Delta Range analytical balance. The percentages of shoot and root dry weight loss per plant (SDL and RDL, respectively) were calculated as follows: $SDL \text{ or } RDL = (\text{dry weight per plant in non-inoculated treatment} - \text{dry weight per plant in inoculated treatment}) / \text{dry weight per plant in non-inoculated treatment}$.

Raw phenotypic data obtained from each experiment in this study, involving faba bean and lentil genotypes, are presented in Supplementary Tables 1.B and 2.B, respectively.

The phenotyping experiment for resistance to *A. euteiches* in the *LR3 M. truncatula* RIL population was achieved by Hamon et al. (2010). The RIL population was challenged in growth chamber conditions (25°C 16 h / 23°C 8 h) for resistance to four reference strains of *A. euteiches*, i.e. RB84 and Ae109 isolated from pea, and MF-1 and NC-1 isolated from alfalfa (Malvick and Grau 2001).

2.3. Statistical analysis of phenotypic data

Statistical analyses were conducted using R software version 4.3.1 (R Core Team 2023). DS traits were analyzed as ordinal variables using a cumulative link mixed model (CLMM, `clmm` function from the ordinal package, Christensen, 2019). SDL and RDL quantitative variables were analyzed using a linear mixed-effects model (LMM, `lmer` function from the lme4 package, Bates et al., 2019). For DS scores assessed in growth chamber experiments, CLMM included G (genotype) as fixed factor, B (block) and the G x B interaction as random factors. For DS, SDL, and RDL variables evaluated in greenhouse experiments, CLMM and LMM included G (genotype) as fixed factor and R (replicate) as random factor. Likelihood ratio tests ($\alpha = 5\%$, `Anova.clmm` function from the RVAideMemoire package, Hervé, 2020) and Wald tests ($\alpha = 5\%$, `Anova` function from the car package, Fox and Weisberg, 2020) were applied to evaluate the genotypic effect in CLMM and LMM, respectively. Estimated marginal means (EMMeans) were assessed for each genotype, each trait and statistical model ($\alpha = 5\%$, `emmeans` function and package; Lenth, 2020). From EMMean values obtained on each genotype, Pearson's correlation coefficients (r) were calculated between traits (`rcorr` function from the Hmisc package; Harrell and Dupont, 2020). Finally, broad-sense heritability (H^2) was computed for each variable from variance estimates obtained in a global linear model using the formula: $H^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_E^2 / r)]$, where σ_G^2 is the genetic variance, σ_E^2 the residual variance, and r the number of replicates or blocks per genotype.

EMMean values obtained on each evaluated trait and from each experiment in this study on faba bean and lentil genotypes, are presented in Supplementary Tables 1.C and 2.C, respectively. LSMeans previously assessed by Hamon et al. (2010), on each genotype and for each disease severity variable, are presented in Supplementary Table 3.B.

2.4. Linkage and QTL mapping

In faba bean, the genetic map from the Silian x Hiverna RIL population including 1,312 SNPs, previously published by Carrillo-Perdomo et al. (2020), was employed for QTL mapping (Supplementary Table 1.D). In *M. truncatula*, the previously published genotyping dataset, consisting of 201 markers (Hamon et al. 2010), including amplified fragment length polymorphism (AFLP), random-amplified polymorphic DNA (RAPD), and simple sequence repeat (SSR) markers, was supplemented with genotyping data from a 1,536 SNP- Illumina GoldenGate assay (Loridon et al. 2013). Subsequently, an updated genetic map from the F83005.5 x DZA045.5 RIL population was developed using the "group" and "annealing 100 100 0.1 0.9" commands of the CarthaGene software (de Givry et al. 2005), as detailed in Boutet et al. (2016). These commands facilitated the computation and optimization of the maximum likelihood for the order and position of markers (in cM Haldane) on each LG. For each marker, the 1:1 allelic segregation ratio was assessed using a χ^2 test ($p\text{-value} < 0.001$). The final genotyping matrix consisted of 758 markers, each displaying a cumulative heterozygosity and missing data ratio < 35%. Markers were well distributed across the eight *M. truncatula* linkage groups (LG) (Supplementary Table 3.C).

In faba bean and *M. truncatula* RIL populations, QTL mapping analyses were achieved using the composite interval mapping (CIM) model implemented in the R/qtl package (Broman et al. 2003). For each variable, a forward-backward stepwise selection of QTL covariates (window size = 5 cM) was performed employing the Haley-Knott regression method. To mitigate the number of covariates and the potential impacts of overparameterization, new LOD score thresholds were estimated for each additional covariate introduced in the CIM model. These thresholds were determined through 1,000 permutations to assess the significance of putative QTL, maintaining a genome-wide error risk of 5%. For each QTL, the percentage of phenotypic variance (R^2), additive effect, and one LOD drop-off confidence interval were computed.

2.5. Genome-wide association study

The genotyping data for the AGILE panel was acquired from KnowPulse (Sanderson et al., 2019) at the following link: https://knowpulse.usask.ca/filter_vcf?vcf_file_id=17. Genotyping of the 321 lentil accessions was performed by exome capture sequencing, as presented in Haile et al. (2020). The second assembly version of the "Redberry" lentil genome (Ramsay et al. 2021), obtained from long-read sequencing, was used to detect nucleotide variations in the captured regions. SNPs were designated by their chromosome assignment followed by their genomic position in base pairs (bp). A first set of 718,557 SNPs was obtained from the raw genotyping dataset, by removing markers that were not biallelic, and by applying the following filters: minimum Phred Quality score = 30, minimum and maximum mean depth per site = 5 and 150, respectively, minimum depth allowed for a genotype = 5, and minor allele count = 3, using VCFtools 0.1.13 (Danecek et al. 2011). Further filtering criteria were implemented to ensure quality control of the variants located on the seven lentil chromosomes: MAF > 5%, SNP heterozygosity < 15%, and missing data < 10%. A total of three accessions displaying less than five recorded phenotypic observations across all evaluated variables, were removed from the GWAS (Supplementary Table 2.B). To ensure high quality of genotyping data, a further 16

lentil genotypes were excluded from the genotyping dataset by applying the subsequent filters: missing data < 15% and heterozygosity rate < three times the standard deviation of the mean sample heterozygosity rate, as recommended by Marees et al. (2018). Missing data present in the resulting genotyping matrix were imputed using Beagle v5.4 (Browning et al. 2018). Finally, the same filters of MAF (> 5%) and SNP heterozygosity (< 15%) were once again employed on the genotypic dataset. After the whole filtering process, the genotypic matrix associated with the AGILE lentil panel included 302 accessions and 140,007 SNPs, showing high coverage across the lentil genome.

From the filtered genotyping matrix obtained from the AGILE lentil panel, overall pairwise LD (r^2) between SNPs was explored within each chromosome using PLINK v1.9b7 software (Purcell et al. 2007; Chang et al. 2015). r^2 means were computed for each bin of 100 bp, spanning a distance from 100 to 100,000 bp. A LD-decay curve was plotted by fitting a logarithmic regression model (lm function from R software) using the r^2 mean for each bin as the response variable and the natural logarithm of the physical distance between two bins as the predictor variable. The LD decay was measured for each chromosome by identifying the distance at which half of the maximum predicted r^2 value had decreased.

Kinship relationships were investigated by assessing identity-by-descent (IBD) relatedness values between accessions using the VanRaden method (VanRaden 2008). Kinship matrices were first assessed on all chromosomes. Ward's clustering of lentil accessions was performed from the computed IBD values and presented along with a heatmap of the kinship matrix (R function heatmap.2 from the gplots package). IBD values were then estimated for each chromosome according to the method presented in Rincent et al. (2014), which aims to improve the GWAS power and false positives control by estimating the kinship with all the markers that are not located on the same chromosome than the tested SNP in GWAS. The population structure analysis was performed with ADMIXTURE v1.3.0 (Alexander et al. 2009; Alexander and Lange 2011) with K values ranging from one to 15. A tenfold cross-validation error for each K was used to select the best K value. The structure matrix obtained from Admixture was displayed using the pophelper R package (Francis 2017). Finally, a principal coordinate analysis (PCoA, R function CMDSCALE), including a multidimensional scaling on the kinship matrix, was performed to project the accessions in a small number of dimensions.

GWAS was performed in the AGILE lentil panel using the mixed linear model (MLM; Lippert et al., 2011), by implementing as cofactors (i) the kinship matrix computed for each chromosome using the method presented in Rincent et al. (2014), and (ii) the population structure obtained from the three first components of the PCA. Marker-phenotype associations were considered as significant for SNPs showing p -values inferior to a 5% false discovery rate (FDR) threshold set at 5.3 (p -value = 5.0×10^{-6}) for all variables (Benjamini and Hochberg 1995). The effects of genetic variants at candidate genes were predicted using the SNPeff software with its default parameters (Cingolani et al. 2012).

Local LD analysis was conducted using the Big-LD algorithm (R function gpart, Kim et al., 2018), with r^2 values used as the measure of LD. The LD bin size was fixed to range from 15 to 500 SNPs, and the analysis employed the "LD block based only" specific algorithm, which excludes gene information. Additionally, priority was given to the largest estimated cliques using the "maximal" parameter. For each LD block, including the most significant SNP detected from each GWAS peak in MLM, groups of accessions sharing common haplotypes that were not considered as rare (frequency $\geq 5\%$), as well as a group of accessions combining different rare haplotypes (cumulated frequency $\geq 5\%$; $n \geq 2$ rare haplotypes), were defined. Phenotype-haplotype associations were analyzed using a linear model (R function lm), with the "haplotype" as fixed factor, then phenotypic means of haplotype groups were compared using a Tukey-HSD test ($\alpha = 5\%$; R functions emmeans and cld of package emmeans). For each LD block, haplotypes significantly associated with higher mean phenotypic scores compared to those associated with the other haplotypes were defined as favorable. The marginal global explanatory capacity (R^2) of each haplotype set was computed using the R function "r.squaredGLMM" from the package "MuMIn" (Bartoń 2024).

2.6. Synteny and orthology analyses

Conservation analysis of genes underlying resistance QTL to *A. euteiches* was conducted using OrthoLegKB, a legume-dedicated instance of the orthology-driven knowledge base framework for translational research, recently developed by Imbert et al. (2023). OrthoLegKB comprises the genomes published by Pecrix et al. (2018) for *M. truncatula* A17 (v.5), Ramsay et al. (2021) for *L. culinaris* Redberry (v.2), Jayakodi et al. (2023) for *V. faba* Hedin/2 (v.1), and Kreplak et al. (in prep) for *P. sativum* Cameor (v.2), for v.1, see Kreplak et al. (2019). First, resistance QTL to *A. euteiches* previously reported by Bonhomme et al. (2019), Ma et al. (2020), Wu et al. (2021), and Leprévost et al. (2023), as well as those identified in this study, in pea, faba bean, lentil, and *M. truncatula*, were integrated into OrthoLegKB. QTL were identified from diverse environments (field, greenhouse, and growth chamber), *A. euteiches* strains, and resistance variables evaluated on plant root and/or shoot parts. To overcome some vocabulary inconsistencies of the QTL descriptors, metadata were annotated using the plant trait ontology (TO), plant ontology (PO) and plant experimental conditions ontology (PECO) from Planteome (Cooper et al. 2018, 2024). A physical position was assigned to each QTL by genomic alignment of the molecular markers flanking the QTL confidence interval. Then, QTL detected for resistance to *A. euteiches* that overlapped with syntenic blocks were identified among legume genomes. Syntenic blocks were previously implemented in OrthoLegKB by Imbert et al. (2023) using MCSanX (Wang et al. 2012) with the following settings: (i) a minimum of 10 collinear orthologous genes to form a collinear block, and (ii) a maximum of 25 gene gaps allowed between collinear genes. Finally, functional orthologous genes between legume species were explored within syntenic QTL regions, using sequence homology analysis previously implemented in OrthoLegKB using OrthoFinder v2.5.5 with the ultrasensitive setting (Emms and Kelly 2015, 2019).

3. Results

3.1. Phenotypic data analysis

From all phenotypic datasets obtained on the faba bean Silian x Hiverna RIL population or the AGILE lentil panel studied, significant genotypic effects ($2.2 \times 10^{-16} \leq p\text{-value} \leq 0.01$) were detected for all variables using CLMM or LMM, except for the variable associated with root dry weight loss evaluated on the lentil panel ($p\text{-value} = 0.41$). Broad-sense heritability values for the disease severity trait evaluated in growth chamber experiments from faba bean genotypes,

using RB84 and Ae109 strains, were very high ($0.95 \leq H^2 \leq 0.98$; Fig. 1.A). However, a lower value ($H^2 = 0.46$) was computed from disease severity scores in the lentil panel evaluated in greenhouse conditions, using the RB84 strain. In addition, null and low broad-sense heritability values were obtained in the lentil panel from root and shoot dry weight loss variables ($H^2 = 0$ and 0.16), respectively (Fig. 1.B).

Frequency distributions of EMMean values computed for all evaluated variables tended to fit normal curves, except for the “Pop2_DS_Ae109” variable, which exhibited a nearly bimodal distribution. In the Silian x Hiverna RIL population, transgressive segregation was observed for both variables. The reference faba bean parents, Hiverna and Silian, showed partial resistance and susceptibility to the RB84 strain, respectively. However, both lines displayed similar levels of resistance against the Ae109 strain (Fig. 1.A). DS scores obtained for resistance to the RB84 and Ae109 strains in the faba bean experiment, were significantly ($p\text{-value} \leq 0.001$) and positively ($r = 0.42$) correlated (Supplementary Table 1.C). In the lentil experiment, RDL and SDL values were significantly ($p\text{-value} \leq 0.001$) and positively ($r = 0.55$) correlated between each other, but were less correlated ($r = 0.20$ and 0.25 , respectively) with DS scores (Supplementary Table 2.C).

3.2. QTL mapping for resistance to *A. euteiches* in faba bean

In the Silian x Hiverna faba bean RIL population, a total of four genetic regions, including one major-effect locus, were identified from DS scores obtained for the two strains of *A. euteiches* (Table 1; Fig. 2.A). The first region, *Ae-Vf2.1*, was located at the end of LGII, between 219.45 and 235.10 cM, and included two QTL for resistance to Ae109 and RB84 strains of *A. euteiches*, respectively ($R^2 = 4.9\%$ and 20.6% , respectively). A major-effect QTL, named *Ae-Vf3.1* ($R^2 = 66.5\%$), was detected in the second region, located at the top of LGIII, between 27.86 and 39.82 cM for resistance to the Ae109 strain. The third and fourth regions located on LGVI, *Ae-Vf6.1*, 61.84–68.99 cM and *Ae-Vf6.2*, 116.58–120.60 cM, respectively, each included a QTL for resistance to the RB84 strain ($R^2 = 10.2\%$ and 8.7% , respectively). In each genetic region, allelic contribution to the resistance was brought by the Hiverna parent, except at the major-effect QTL located on LGIII for which the allele contributing to the resistance to Ae109 was derived from the Silian parent. In the RIL population, the two lines (VFP-2-0601 and VFP-2-0648) showing the highest levels of partial resistance to Ae109 (EMMeans = -8.43 and -8.36 , respectively) and good levels of resistance to RB84 (EMMeans = -2.90 and -3.38 , respectively), cumulated resistance alleles at the peaks of the major-effect QTL and the four additional minor-effect QTL (Supplementary Table 1.E). A large number of genes ($49 \leq n \leq 339$) were identified within the resistance QTL intervals (Supplementary Table 1.F).

Table 1
Aphanomyces root rot resistance QTL detected from the Silian x Hiverna faba bean RIL population (*Pop2*)

Linkage group - Chromosome	QTL region	QTL ^a	Peak marker ^b	Position (cM) ^c	Position (pb) ^d	Confidence interval markers ^e	Confidence interval of position (cM) ^c	Confidence interval of position (pb) ^d	L ^f
LGII - Chr2	<i>Ae-Vf2.1</i>	Pop2_DS_Ae109	dn_rep_c3445_730	226.07	310060991	c19993_1442 - c18334_2016	219.45–230.58	278437000–374617014	4
LGII - Chr2	<i>Ae-Vf2.1</i>	Pop2_DS_RB84	c18334_2016	230.58	278437000	c16664_1657 - c16867_1064	226.51–235.10	261015098–308901178	1
LGIII - Chr3	<i>Ae-Vf3.1</i>	Pop2_DS_Ae109	dn_rep_c4180_937	31.78	39138654	dn_rep_c3365_1075 - dn_rep_c4449_1354	27.86–39.82	36009859–62879953	3
LGVI - Chr6	<i>Ae-Vf6.1</i>	Pop2_DS_RB84	c16476_1395	66.35	1103353146	dn_rep_c6482_756 - rep_c11033_898	61.84–68.99	1088818503–1122426847	6
LGVI - Chr6	<i>Ae-Vf6.2</i>	Pop2_DS_RB84	dn_rep_c3382_1319	119.82	804386074	dn_rep_c3428_771 - dn_rep_c1838_892	116.58–120.60	804331855–820584580	5

^a QTL detected from the Silian x Hiverna RIL population (*Pop2*), using the CIM model. DS: Disease Severity. RB84 and Ae109 are *A. euteiches* strains belonging to the Hiverna parent.

^b Nearest marker from the LOD score peak of the QTL.

^c Genetic position on the genetic map of the RIL population.

^d Physical position on the Hedin/2 faba bean genome v.1.

^e Markers at the LOD-1 drop-off from the QTL peak.

^f LOD (Log of likelihood ratio) value at the QTL score peak. LOD threshold values were estimated at 4.3 and 4.8 to detect significant QTL associated with the RB84 and Ae109 strains, respectively.

^g Proportion of phenotypic variance (R^2) explained by the QTL.

^h Effect of substituting Silian alleles for Hiverna alleles at the QTL. A positive sign indicates that the QTL allele increasing the resistance is contributed by the Silian parent, while a negative sign indicates that the resistant allele is brought by the resistant parent Hiverna.

ⁱ Mean effects associated with Silian and Hiverna alleles at each QTL, computed from EMMean values assessed for each variable.

^j Reference parent carrying the favorable resistance allele at each QTL.

3.3. Genome-wide association mapping for resistance to *A. euteiches* in lentil

In the AGILE lentil panel, LD decay pattern between SNPs, computed from all chromosomes, reached half of the maximum value at an average distance of 38.1 Kb (Supplementary Fig. 1). A total of six genetic subgroups were identified, according to kinship and structure analyses (Supplementary Fig. 2). The Ward's clustering computed on the kinship IBD matrix showed consistent clusters with those obtained from the Admixture analysis, with only 14 lines differing in terms of cluster assignment (Supplementary Table 2.C). The first three axes of the PCA analysis captured a total of 57.2% of the genetic variation, and separated genotypes into similar groups compared to kinship and structure analyses (Supplementary Fig. 3). The panel exhibited a significant geographical structure; (i) genetic subgroups 1 to 3 mostly gathered African, American, European, and Middle Eastern lentil accessions, and (ii) genetic subgroups 4 to 6 mainly grouped Asian and Middle Eastern lentil accessions (Supplementary Fig. 4). Accessions composing the three first genetic subgroups showed on average a slightly higher level of resistance to *A. euteiches* according to DS scores with RB84 (EMMeans = 0.95 ± 0.96), compared to the three last genetic subgroups (EMMeans = 1.41 ± 0.82).

A total of six, seven, and two GWAS-SNPs were significantly detected ($p\text{-value} < 5.0\text{e-}06$) from the DS, RDL, and SDL variables, respectively (Supplementary Table 2.D), spanning six genetic regions. For DS, the most significant GWAS-SNPs at each MLM peak were "Lcu.2RBY.Chr1_52348858", "Lcu.2RBY.Chr2_390043467", and "Lcu.2RBY.Chr5_474129221" ($p\text{-values} = 2.75\text{E-}06$, $2.46\text{E-}06$, and $3.03\text{E-}06$, respectively). In addition, (i) one most significant GWAS-SNP, "Lcu.2RBY.Chr4_317995287" ($p\text{-value} = 1.58\text{E-}06$), was detected for RDL, and (ii) two additional most significant GWAS-SNPs, "Lcu.2RBY.Chr7_515914271" and "Lcu.2RBY.Chr7_519849758" ($p\text{-values} = 1.56\text{E-}06$ and $1.02\text{E-}06$, respectively) were detected for SDL and RDL, respectively, and were separated by approximately 4 Mb at the end of chromosome 7 (Fig. 3; Table 2).

Table 2
Most significant GWAS-SNPs at each marker peak detected for resistance to *A. euteiches* from the AGILE lentil panel.

Chromosome	LD block ^a	Trait ^b	Peak GWAS-SNP ^c	Position (pb) ^d	Confidence interval of position (pb) ^e	MAF ^f	$p\text{-value}$ ^g	SNP weight ^h	Favorable allele ⁱ
1	<i>Ae-Lc1.1</i>	AGILE_DS_RB84	Lcu.2RBY.Chr1_523488581	523488581	523486933–523530125	0.13	2.75E-06	0.30	T/A
2	<i>Ae-Lc2.1</i>	AGILE_DS_RB84	Lcu.2RBY.Chr2_390043467	390043467	389924963–390072304	0.17	2.46E-06	-0.26	A/C
4	<i>Ae-Lc4.1</i>	AGILE_RDL_RB84	Lcu.2RBY.Chr4_317995287	317995287	317977994–317996793	0.49	1.58E-06	-0.11	C/T
5	<i>Ae-Lc5.1</i>	AGILE_DS_RB84	Lcu.2RBY.Chr5_474129221	474129221	473971929–474165142	0.18	3.03E-06	-0.27	T/C
7	<i>Ae-Lc7.1</i>	AGILE SDL_RB84	Lcu.2RBY.Chr7_515914271	515914271	515914044–516112687	0.27	1.56E-06	-0.07	A/C
7	<i>Ae-Lc7.2</i>	AGILE_RDL_RB84	Lcu.2RBY.Chr7_519849758	519849758	519597210–519925061	0.23	1.02E-06	0.11	T/G
^a Name of the LD block comprising the most significant SNP at each GWAS peak detected using the MLM.									
^b Traits from which GWAS-SNPs were detected from the AGILE lentil panel. DS: Disease Severity; RDL: Root Dry Weight Loss; SDL: Shoot Dry Weight Loss. RB84 is an <i>A. euteiches</i> strain belonging to the pea pathotype I.									
^c SNP showing the most significant $p\text{-value}$ for each GWAS peak, using the MLM model.									
^d Physical position on the Redberry lentil genome v.2.									
^e Confidence interval around significant GWAS-SNPs, derived from local LD analysis.									
^f Minor allele frequency (MAF) computed for each GWAS-SNP from the filtered genotyping dataset (140,007 SNPs).									
^g $P\text{-value}$ associated with presented significant GWAS-SNPs passing the $p\text{-value}$ threshold ($p\text{-value} = 5.0\text{e-}06$).									
^h Contribution of the SNP to the phenotype. A positive sign indicates that the allele increasing the resistance is in major frequency whereas a negative sign means that resistance allele is in minor frequency.									
ⁱ Favorable resistance allele at each GWAS-SNP is presented in bold and is underlined (Allele in minor frequency / Allele in major frequency).									

A total of six LD blocks, each including one to four significant GWAS-SNPs detected using MLM, were identified in the AGILE lentil panel (Supplementary Table 2.E). The number of SNPs composing the LD blocks varied from 15 to 101, and the interval size of the blocks ranged from 19 Kb to 328 Kb. Among the six LD blocks, two blocks on chromosomes 1 and 2, named *Ae-Lc1.1* and *Ae-Lc2.1*, respectively, carried favorable haplotypes significantly associated with lower DS values than those associated with other haplotypes, explaining 16.6% and 11.7% of the variation, respectively. No favorable haplotype was significantly detected from the four remaining LD blocks (*Ae-Lc4.1*, *Ae-Lc5.1*, *Ae-Lc7.1*, and *Ae-Lc7.2*; $R^2 \leq 3.2\%$). Accessions carrying at least one favorable haplotype at *Ae-Lc1.1* and *Ae-Lc2.1*, showed slightly higher levels of partial resistance to RB84 ($n = 129$; EMMeans = 0.85 ± 0.83), compared to the accessions displaying no favorable haplotype ($n = 173$; EMMeans = 1.40 ± 0.93). Lentil accessions ($n = 18$) carrying favorable haplotypes at the two resistance LD blocks were classified in genetic subgroups 1, 2, and 3. Most of these lines originated from North America and Europe (Supplementary Table 2.F).

The total of 15 significant SNPs detected were included in the intervals of 10 genes, and the two resistance LD blocks showing significant favorable haplotypes, encompassed a total of eight genes (Supplementary Tables 2.D and 2.G). Among the most significant SNPs detected at each LD block for partial resistance to the RB84 strain of *A. euteiches*: (i) one GWAS-SNP, Lcu.2RBY.Chr7_515914271, encoded a WRKY protein domain and was the only variant predicted to cause a non-synonymous change in the protein sequence, (ii) three GWAS-SNPs were predicted to encode a protein phosphatase 2C family, substrate-binding subunit of tRNA, and carbohydrate kinase PfkB, on chromosomes 1, 5 and 4, respectively, and (iii) two GWAS-SNPs on chromosomes 2 and 7 were located in intergenic regions (Fig. 3).

3.4. Improved QTL mapping for resistance to *A. euteiches* in *M. truncatula*

The genetic map developed from the F83005.5 x DZA045.5 RIL population of *M. truncatula* included 758 markers distributed over eight LGs, covering 522.5 cM Haldane (Supplementary Table 3.C). The average marker density per LG ranged from 0.8 to 2.2 markers/cM. Non-Mendelian allelic segregation ($\alpha = 0.001$) was observed for 7.8% of the total number of markers which were distributed over the eight LGs. Most of the additional and filtered SNP markers ($n = 603$), implemented in this study to supplement the genotyping dataset previously published by Hamon et al. (2010), displayed a conserved order of genomic positions with the *M. truncatula* physical map (Supplementary Table 3.D).

A total of eight additive-effect genetic regions, each comprising one to two QTL, were associated with resistance to the four strains of *A. euteiches* studied in Hamon et al. (2010) (Table 3; Fig. 2.B). The *AER1* major broad-spectrum genetic region, previously identified on LGIII from the *LR3* RIL population (Pilet-Nayel et al. 2009; Hamon et al. 2010), was confirmed for resistance to the two pea-isolated strains. Indeed, *AER1* included overlapping major-effect ($R^2 = 80.3\%$) and minor-effect ($R^2 = 29.2\%$) QTL for resistance to RB84 and Ae109 strains, respectively, between 13.1 and 14.7 cM at the top of LGIII. Seven additional genetic regions detected with minor effects ($R^2 \leq 13.9\%$) were identified specifically for resistance to the Ae109, MF-1 or NC-1 strain on five LGs. All but one (*i.e. Ae-Mt8.3*) of the genetic regions identified in this updated study were previously detected by Hamon et al. (2010), and thus were renamed as previously. Allelic contribution to the resistance was brought by the resistant parent, DZA045.5, at all genetic regions but one on LGII. In the RIL population, the three lines accumulating the highest number of favorable alleles at all QTL peaks ($n = 9$), exhibited favorable values for partial resistance ($1.56 \leq \text{LSMeans} \leq 2.63$) against all *A. euteiches* strains (Supplementary Table 3.E).

Table 3
Aphanomyces root rot resistance QTL detected from the F83005.5 x DZA045.5 *M. truncatula* RIL population (*LR3*).

Linkage group - Chromosome	QTL region ^a	QTL ^b	Peak marker ^c	Position (cM) ^d	Position (pb) ^e	Confidence interval markers ^f	Confidence interval of position (cM) ^d	Confidence interval of position (pb) ^e	LOD ^g	R ² (%) ^h	Additive effect ⁱ
LGII - Chr2	<i>Ae-Mt2.1</i>	LR3_DS_MF-1	MtB304	24.40	30409221	MTIC315 - EM2.758	22.40–26.30	17045274–30409282	4.2	8.2	0.1
LGIII - Chr3	<i>AER1</i>	LR3_DS_Ae109	MtB199	13.70	2646027	SNP3_55 - SNP3_67	13.10–14.70	2638070–3792737	16.9	29.2	-0.2
LGIII - Chr3	<i>AER1</i>	LR3_DS_RB84	MtB199	13.70	2646027	MTIC1179 - SNP3_57	13.40–14.00	2638070–2646088	61.8	80.3	-0.9
LGIII - Chr3	<i>Ae-Mt3.2</i>	LR3_DS_Ae109	SNP3_96	66.60	36025582	SNP3_90 - EM2.420	60.90–69.20	33187001–37491683	3.7	5.4	-0.1
LGV - Chr5	<i>Ae-Mt5.1</i>	LR3_DS_NC-1	MtB94	18.50	14653075	MtB23 - SNP5_38	15.90–22.60	12674675–16309131	5.9	13.9	-0.2
LGVII - Chr7	<i>Ae-Mt7.2</i>	LR3_DS_MF-1	SNP7_65	46.70	41207620	MTIC471 - SNP7_67	44.40–48.30	39240485–42976875	4.0	7.8	-0.2
LGVIII - Chr8	<i>Ae-Mt8.1</i>	LR3_DS_MF-1	SNP8_12	6.50	7139375	SNP8_7 - SNP8_19	3.00–8.60	4652488–9326127	4.8	9.5	-0.2
LGVIII - Chr8	<i>Ae-Mt8.2</i>	LR3_DS_NC-1	MTIC135	28.40	28347376	MTIC308 - SNP8_28	22.40–32.20	15523202–28347437	3.5	8.0	-0.1
LGVIII - Chr8	<i>Ae-Mt8.3</i>	LR3_DS_Ae109	SNP8_38	43.90	33266914	SNP8_34 - SNP8_47	38.50–46.40	31367874–35085056	6.7	10.1	-0.1
^a QTL region presented in Hamon et al. (2010), repositioned on the updated genetic map of the F83005.5 x DZA045.5 RIL population (<i>LR3</i>).											
^b QTL detected from the <i>LR3</i> RIL population, using the CIM model. DS: Disease Severity. RB84 and Ae109 are <i>A. euteiches</i> strains belonging to the pea path and NC-1 are <i>A. euteiches</i> strains belonging to the alfalfa races 1 and 2, respectively.											
^c Nearest marker from the LOD score peak of the QTL.											
^d Genetic position on the genetic map of the RIL population.											
^e Physical position on the A17 <i>M. truncatula</i> genome v.5. For each marker that is not an SNP, the physical position of the nearest SNP on the genetic map is											
^f Markers at the LOD-1 drop-off from the QTL peak.											
^g LOD (Log of likelihood ratio) value at the QTL score peak. LOD threshold values were estimated at 2.8, 3.8, 3.7 and 3.4 to detect significant QTL associated LR3_DS_Ae109, LR3_DS_MF-1, and LR3_DS_NC-1 variables, respectively.											
^h Proportion of phenotypic variance (R ²) explained by the QTL.											
ⁱ Effect of substituting F83005.5 alleles for DZA045.5 alleles at the QTL. A positive sign indicates that the QTL allele increasing the resistance is contributed whereas a negative sign means that the resistant allele is brought by the resistant parent DZA045.5.											
^j Mean effects associated with F83005.5 and DZA045.5 alleles at each QTL, computed from LSMeans values assessed for each variable.											
^k Reference parent carrying the favorable resistance allele at each QTL.											

A considerable number of genes ($125 \leq n \leq 872$) were annotated within resistance QTL intervals detected from the F83005.5 x DZA045.5 *M. truncatula* RIL population, except for the major-effect QTL detected in the *AER1* genetic region for the RB84 strain, which included only three genes covering a short interval of 8 Kb. Two of these genes encoded tetracycline resistance and F-box kelch-repeat proteins and the third one displayed unknown functional annotation (Supplementary Table 3.F).

3.5. Synteny relationships between QTL identified for resistance to *A. euteiches* in legume species

Metadata for a total of 405 QTL detected for resistance to *A. euteiches* were integrated into the OrthoLegKB database developed by Imbert et al. (2023) (Supplementary Table 4.A). After placing the QTL on the respective genome sequences, 20 QTL with minor to moderate effects ($4.2\% \leq R^2 \leq 26.3\%$), considered low-informative due to their large confidence intervals (length ≥ 100 Mb) and high number of underlying annotated genes ($n \geq 1,000$), were excluded from the analyses. Among the 385 remaining QTL, five QTL were identified in this study from faba bean, 56 from lentil (six from this study and 50 from Ma et al. (2020)), 242 from pea (205 from Leprévost et al. (2023) and 37 from Wu et al. (2021)), and 82 from *M. truncatula* (nine from this study and 73 from Bonhomme et al. (2019)).

QTL positions were examined regarding syntenic blocks defined in OrthoLegKB as genomic regions comprising sets of orthologous genes ($n \geq 10$) exhibiting a conserved order between two different legume species. QTL were called syntenic when their confidence intervals were partially or fully comprised in

syntenic blocks defined between genomes and thus comprised orthologous genes. A total of 65 syntenic resistance QTL to *A. euteiches*, overlapping 24 syntenic blocks (named AeSBx), were identified (Fig. 4). The total length of the syntenic blocks ranged from 286 Kb to 168,420 Kb, depending on the legume genome. Among the 24 syntenic blocks, nine blocks were identified as inverted between legume species, indicating the presence of chromosomal rearrangements in QTL regions (Supplementary Table 4.B). Two to nine resistance QTL to *A. euteiches* were assigned to a same syntenic block, depending on the legume genome.

In pea, a total of 39 minor to moderate effect resistance QTL ($2.9\% \leq R^2 \leq 47.7\%$) identified overlapped with at least one of the 24 syntenic blocks (except for AeSB1). Most of these QTL were located within four consistent genetic regions (*Ae-Ps1.2*, *Ae-Ps4.3*, *Ae-Ps5.2*, and *Ae-Ps7.6b*) previously reported by Leprévost et al. (2023) or within 13 genetic regions (*AeMRCD1Ps* and *Fwt-Ps*) previously reported by Wu et al. (2021). In faba bean, two resistance QTL regions detected in this study overlapped with two blocks (AeSB1 and AeSB2), including the major-effect QTL *Ae-Vf3.1* ($R^2 = 66.5\%$) and the minor effect QTL *Ae-Vf6.2* ($R^2 = 8.7\%$). In lentil, (i) two minor-effect QTL regions identified in this study (*Ae-Lc1.1* and *Ae-Lc4.1*, $p\text{-value} = 2.75\text{E-}06$ and $1.58\text{E-}06$, respectively) overlapped with two blocks (AeSB3 and AeSB4), and (ii) four minor-effect QTL ($R^2 = 5.7\%$, $2.40\text{E-}06 \leq p\text{-value} \leq 3.80\text{E-}04$) previously reported by Ma et al. (2020) overlapped with six blocks (AeSB4 to AeSB9). Finally, in *M. truncatula*, (i) four QTL ($5.4\% \leq R^2 \leq 9.5\%$) (*Ae-Mt2.1*, *Ae-Mt3.2*, *Ae-Mt8.1*, and *Ae-Mt8.2*) identified in this study overlapped with seven blocks (AeSB10 to AeSB16), and (ii) 14 QTL previously reported by Bonhomme et al. (2019) overlapped with nine blocks (AeSB1, AeSB17 to AeSB24), with three of them closely located to the *AER1* region and showing major effects on resistance ($5.25\text{E-}35 \leq p\text{-value} \leq 4.78\text{E-}17$).

Among the 24 syntenic blocks, no synteny was identified in this study between the two major-effect QTL previously reported in pea, i.e. *Ae-Ps4.5* and *Ae-Ps7.6*, and resistance QTL detected in faba bean, lentil, and *M. truncatula*. However, AeSB1 hosted the major-effect QTL *Ae-Vf3.1* identified in this study on faba bean chromosome 3 for resistance to the Ae109 strain, and a minor-effect QTL ($p\text{-value} = 5.17\text{E-}06$) previously reported on *M. truncatula* chromosome 1 for resistance to the RB84 strain. In addition, AeSB21 hosted three major-effect QTL for resistance to both ATCC201684 and RB84 strains, located close to the major-effect *AER1/prAe1* region on *M. truncatula* chromosome 3, and two minor-effect QTL ($5.0\% \leq R^2 \leq 9.5\%$) previously detected on pea chromosome 5 from aerial decline index evaluated in France and in the USA, from two different AB and RIL populations.

A total of 1,336 annotated homologous genes were included within the conserved QTL regions overlapping the 24 syntenic blocks, with two to 344 genes per syntenic block (Supplementary Table 4.C). Annotations of these genes showed various protein functions, comprising receptor-like protein kinase and disease resistance NB-LRR proteins, which are known to be involved in plant responses to biotic stress. The *Ae-Vf3.1*-carrying syntenic block AeSB1, shared between *M. truncatula* and faba bean, comprised one group of four orthologous gene commonly annotated as α/β hydrolase fold, fungal lipase-like C2 domain. The *AER1/prAe1*-carrying syntenic block AeSB21, shared between *M. truncatula* and pea, displayed three groups of two orthologous genes, annotated as genes encoding homeobox, kinase domain-like, and NB-LRR proteins.

4. Discussion

4.1. Genetic basis of resistance to *A. euteiches* in faba bean

The present study is the first published QTL mapping report for quantitative resistance to *A. euteiches* in faba bean. We identified a total of five QTL associated with resistance to Ae109 and/or RB84 pea-infecting strains under growth chamber conditions. Notably, (i) a major-effect region, *Ae-Vf3.1*, was detected for resistance to Ae109 on chromosome 3. *Ae-Vf3.1* explained 66.5% of the phenotypic variation and resistance alleles at this QTL were derived from Silian, which was initially selected as a parent for the RILs due to its susceptibility to RB84 (Moussart et al., 2008). Additionally, three minor-effect regions (*Ae-Vf2.1*, *Ae-Vf6.1*, and *Ae-Vf6.2*) were identified for resistance to RB84, with *Ae-Vf2.1* conferring a resistance spectrum extended to Ae109. Resistance alleles at these three minor-effect regions were derived from the resistant parent Hiverna.

A distinct genetic architecture underlying quantitative resistance to Ae109 and RB84 was identified in faba bean. Narrow-spectrum QTL against *A. euteiches* pathotypes were also previously reported in pea (Leprévost et al. 2023) and *M. truncatula* (Hamon et al. 2010). Hiverna is a faba bean cultivar that has been demonstrated to be resistant to the pea pathotype I of *A. euteiches* (Moussart et al. 2008), the only pathotype reported in France to date (Moussart et al. 2024). Additionally, Hiverna is well adapted to freezing temperatures associated with continental European climates (Link et al. 2010), suggesting that it contains favorable alleles effective against both *Aphanomyces* root rot and freezing stresses. A total of 13 QTL associated with frost tolerance, with favorable alleles derived from Hiverna, were previously reported by Carrillo-Perdomo et al. (2022). However, no QTL detected in our study co-localized with frost tolerance QTL previously identified, suggesting that both stresses are controlled by different genetic determinants in faba bean. In this study, the normal distribution of estimated adjusted means associated with the "Pop2_DS_RB84" variable suggested that quantitative resistance to RB84 is under polygenic rather than monogenic inheritance. In addition, the combination of the three detected minor-effect QTL ($R^2 \leq 20.6\%$) was associated with an improved resistance to pathotype I in RILs. However, potential additional minor-effect resistance QTL may not have been detected regarding the high heritability of DS trait measured for RB84 in our study ($H^2=0.95$). The moderate size of the RIL population ($n = 147$) and the lack of marker density in some LG regions may have resulted in missing some minor-effect QTL (Holland 2007). Nevertheless, combining the three minor-effect QTL in faba bean NILs would be an interesting strategy to fully validate their resistance effect on *A. euteiches*.

Silian exhibited a comparable level of resistance to Ae109 as Hiverna in our study, despite being susceptible to RB84. The Ae109 American strain belongs to pea pathotype III, a pathotype that has never been detected in France to date (Moussart et al. 2024). Silian is a Sudanese spring *V. faba* spp. *minor* landrace from which resistance alleles at the *Ae-Vf3.1* major-effect QTL were detected. Crop wild relatives have historically faced challenges from diseases in natural environments and exhibit a rich reservoir of resistance genes acquired through a long history of co-evolutionary relationships with pathogens (Zhang et al.

2017). However, the geographic origin of *A. euteiches* is currently unknown, making it difficult to infer specific ancestral co-evolutionary relationships between *V. faba*, one of whose main centers of diversity is the Fertile Crescent (O'Sullivan and Angra 2016), and pathotypes of *A. euteiches* populations.

4.2. Quantitative resistance to *A. euteiches* in cultivated lentil *L. culinaris*

This study identified minor-effect genetic loci associated with partial resistance to the RB84 pea-infecting strain of *A. euteiches* under greenhouse conditions in the cultivated lentil species *L. culinaris*. Two LD blocks (*Ae-Lc1.1* and *Ae-Lc2.1*, $R^2 = 16.6\%$ and 11.7% , respectively) were found to carry significant favorable haplotypes for the disease resistance. Four additional LD blocks (*Ae-Lc4.1*, *Ae-Lc5.1*, *Ae-Lc7.1*, and *Ae-Lc7.2*, $R^2 \leq 3.2\%$) were identified but not confirmed through marker-haplotype analysis.

These results confirm the complex polygenic nature of partial resistance to *A. euteiches* in *L. culinaris* previously reported by Ma et al. (2020). The authors observed heritability values ranging from very low to high ($0.10 \leq H^2 \leq 0.73$) in two mapping populations, based on RRI (root rot index; similar to DS), RDL, and SDL traits evaluated using the same protocol as in this study but with a different strain from pathotype I. In our study, more efficient and accurate evaluation of the AGILE panel for resistance to *A. euteiches* may have been achieved using (i) a more controlled environment, such as a growth chamber, and (ii) high-throughput phenotyping methods like imaging-derived color features (Marzougui et al. 2019). Additionally, assessing the AGILE panel with different *A. euteiches* strains, such as those from pea-pathotype III or isolated from lentils, might have identified specific genetic determinants.

The AGILE diversity panel comprises 158 common lentil accessions previously characterized for resistance to Ae-16-04D pea-infecting strain by Ma et al. (2020) and a significant positive correlation ($r = 0.44$; $p\text{-value} < 0.001$) was observed between disease severity scores obtained in our experiment and those previously reported. The moderate correlation level suggest the presence of both common and strain-specific genetic resistance factors between the two diversity panels. In this study, *Ae-Lc1.1* (chromosome 1) and *Ae-Lc5.1* (chromosome 5) were respectively located near the minor-effect QTL *G.SAT-Lc1.1* ($R^2 = 4.2\%$) and the *QTL_cluster5* comprising two QTL ($1.7\% \leq R^2 \leq 6.9\%$) previously reported for *A. euteiches* resistance in lentil (Ma et al. 2020). Additionally, *Ae-Lc7.1* and *Ae-Lc7.2* were closely located to *QTL_cluster7*, including four QTL ($6.0\% \leq R^2 \leq 21.4\%$) on chromosome 7. Nevertheless, *Ae-Lc2.1* and *Ae-Lc4.1* were located in genomic regions not previously reported for resistance to *A. euteiches* on chromosomes 2 and 4, respectively. Specific genetic resistance regions identified in the AGILE panel may be associated with (i) differences in aggressivity levels between RB84 and Ae-16-04D, as shown by Sivachandra Kumar et al. (2021), (ii) new resistance alleles in the AGILE panel accessions, and (iii) the use of a high-density genotyping dataset (140,007 SNPs), which offers higher resolution in capturing genetic loci compared to the genotyping by sequencing (GBS) datasets ($n \leq 4,558$ SNPs) used in previous studies by Ma et al. (2020).

Among the 158 accessions common between our study and that of Ma et al. (2020), three landraces, i.e. PI 289073 LSP AGL (Hungary), PI 251248 LSP AGL (Egypt), and PI 193548 LSP AGL (Ethiopia), showed the most consistent levels of partial resistance ($2.15 \leq \text{raw mean DS scores} \leq 2.50$) to pathotype I in both experiments. Additionally, one accession, CN 105789 AGL (Ethiopia, unknown cultivation status), exclusive to the AGILE panel, exhibited the highest level of partial resistance (raw mean DS score = 1.70) to the RB84 strain in our study. These four sources of resistance belonged to genetic subgroups 1 or 3 and comprised zero to one favorable haplotype at resistance LD blocks identified in our study, suggesting that other alleles contributing to resistance in these accessions may have remained undetected. Unfortunately, the lentil landrace PI 432001 LSP AGL (Iran), which showed the highest level of partial resistance to the Ae-16-04D strain (raw mean DS score = 0.61) in the study of Ma et al. (2020), had a very low germination rate (number of germinated plants = 2) in our experiment and was not retained for further genetic analyses.

Evaluating the AGILE panel for phenological (Neupane et al. 2022), morphological, biotic, and abiotic stress response traits will be a valuable strategy to identify potential genetic linkages between Aphanomyces root rot resistance and other favorable traits in breeding programs. In addition, developing interspecific mapping populations between wild and cultivated lentil parents (*L. culinaris* $\times$ *L. orientalis*, *L. culinaris* $\times$ *L. ervoides*, and *L. culinaris* $\times$ *L. odemensis*) holds promise for identifying new resistance QTL from wild *L. culinaris* relatives, which can serve as suitable reservoirs of favorable alleles for breeding programs (Guerra-García et al. 2021).

4.3. Identification of three candidate genes underlying a major-effect QTL in *M. truncatula*

In our study, the improved QTL mapping analyses conducted for resistance to *A. euteiches* in *M. truncatula* (i) confirmed eight additive-effect QTL localized in seven genetic regions previously reported (*Ae-Mt2.1*, *AER1*, *Ae-Mt3.2*, *Ae-Mt5.1*, *Ae-Mt7.2*, *Ae-Mt8.1*, and *Ae-Mt8.2*), and (ii) identified a new genetic region (*Ae-Mt8.3*) on LGVIII. In this study, supplementing the F83005.5 $\times$ DZA045.5 genetic map with 603 new SNPs, and applying higher LOD thresholds in CIM analyses, unconfirmed seven QTL previously reported by Hamon et al. (2010) as displaying minor resistance effects ($4.0\% \leq R^2 \leq 8.9\%$). In pea, Leprévost et al. (2023) also disproved 15 to 42 minor-effect Aphanomyces root rot resistance QTL, depending on the RIL population, which was attributed to both a significant increase in genetic map resolution due to new molecular markers and a more stringent QTL detection method, identical to the one used in this study.

More particularly, our results (i) confirmed the broad-spectrum effect of *AER1* against pea pathotypes I and III, and (ii) re-identified a common peak marker (MtB304) associated with partial resistance to RB84 and Ae109 strains ($R^2 = 80.3$ and 29.2% , respectively). Notably, the physical position of the confidence interval of *AER1*, previously reported as spanning 2.6 cM (Hamon et al. 2010), was refined to only 0.6 cM, corresponding to about 8 Kb, on chromosome 3, and comprised only three genes. Among these three genes, two were functionally annotated ("MtrunA17_Ch3g0080431" and "MtrunA17_Ch3g0080451"), and encoded for tetracycline resistance and F-box associated domain proteins, respectively. Tetracycline is a broad-spectrum antibiotic that can eliminate a wide range of gram-positive and gram-negative bacteria (Verhaegen et al. 2023). However, the direct role of tetracycline resistance proteins in plant disease resistance remains to be fully elucidated. F-box proteins are part of the ubiquitin ligase complex tagging specific proteins for degradation via the ubiquitin-proteasome pathway, thereby regulating various cellular processes including plant immunity (Lechner et al. 2006). Using GWAS, Bonhomme et al. (2014) reported the identification of a gene named "MtrunA17_Ch3g0080491" (previously named "Medtr3g011020" on the A17 *M. truncatula* genome v.3.5) also

encoding an F-box domain protein, which was considered a major candidate for resistance to *A. euteiches* (Djébali et al. 2009). In this study, “MtrunA17_Chr3g0080491” was identified as located only 47 Kb outside the 8 Kb refined confidence interval of *AER1*. However, the functional validation of the three candidate genes identified within *AER1* will be necessary to fully confirm their roles in partial resistance to *A. euteiches*. Further cloning of quantitative resistance genes in *M. truncatula* represents a promising strategy to enhance our understanding of the genetic determinants of resistance in other legumes through translational genomics.

4.4. Synteny relationships between genomic regions harboring *Aphanomyces* root rot resistance QTL in legumes

The present study is the first report on synteny relationships between *Aphanomyces* root rot resistance QTL identified in legumes. We identified a total of 65 *Aphanomyces* root rot resistance QTL overlapping with 24 syntenic blocks defined between genomes of three grain legumes, pea, faba bean, lentil, and the model species *M. truncatula*. Among these, two blocks, AeSB1 and AeSB21, hosted major-effect QTL first reported in this study or previously, including *Ae-Vf3.1* on chr3 in faba bean and the region of *AER1/prAe1* on chr3 in *M. truncatula*.

Our results demonstrated that OrthoLegKB is a powerful tool for exploring orthology and synteny relationships across phylogenetically close legume genomes. To date, it is the only database that maps QTL data on the genomes of multiple legume species (Imbert et al. 2023). The high-quality genomes used in this study through OrthoLegKB were all assembled using long-read technology and represent the latest versions available in the literature. However, the reference pea and lentil genome assemblies integrated into the database were obtained from the *Aphanomyces* root rot-susceptible Cameor and Redberry lines, respectively. Therefore, integrating legume pangenomes into OrthoLegKB is a promising strategy to enhance the exploration of the complete gene repertoire of each species and their wild relatives, beyond the genetic diversity offered by a single linear reference genome, which may lack suitable resistance alleles. Recent pangenome assemblies have been reported in chickpea (Khan et al. 2024), pea (Yang et al. 2022), soybean (Liu et al. 2020; Bayer et al. 2022), and the model species *M. truncatula* (Zhou et al. 2017). OrthoLegKB also integrates published and unpublished expression data from transcriptomic resources, allowing for the investigation of variations in candidate gene expression under diverse experimental conditions. Additional evidence regarding the function of genes underlying *Aphanomyces* resistance QTL and their regulation at the post-transcriptional level may be supported by integrating other omics data in legumes, such as proteomics or epigenomics (Imbert et al. 2023).

Exploring synteny relationships between QTL mitigates the effects of genome duplication and fractionation, likely pinpointing true orthologs (Tang et al. 2008). Despite the significant conservation between pea, faba bean, lentil, and *M. truncatula* genomes (Lee et al. 2017), only 65 out of the 385 *Aphanomyces* root rot resistance QTL implemented in OrthoLegKB were identified as syntenic. This suggests either small syntenic regions with fewer genes than the detection threshold used in OrthoLegKB due to genomic rearrangements, and/or the presence of specific genetic determinants of resistance within each legume species. The genetic basis of resistance can depend on the co-evolutionary history between the host plant and its pathogen, leading to diversified molecular mechanisms of resistance between species (Thompson and Burdon 1992). Thus, genetic determinants for disease resistance traits are expected to be less conserved compared to physiological traits, such as flowering (Hecht et al. 2005). However, exceptions exist, such as the *Mlo* gene, initially reported in barley for conferring durable broad-spectrum resistance to *Blumeria graminis* f. sp. *hordei* (Jørgensen 1992), and later found in other species like *Arabidopsis thaliana* (Consonni et al. 2006), tomato (Bai et al. 2008), and pea (Humphry et al. 2011; Pavan et al. 2011), also providing resistance to powdery mildew. Further investigation into the genetic basis of resistance to *A. euteiches* in legumes, particularly in species such as *Vicia faba* and *Lens culinaris*, for which few QTL analyses have been reported in the literature, will be crucial for providing a comprehensive overview of the diversity and conservation of QTL conferring resistance.

Most syntenic regions identified in this study comprised minor-effect QTL. However, two blocks hosted minor-effect/major-effect QTL, suggesting that resistance effects associated with orthologous genes may vary when expressed in different legume genomes. Synteny relationships between QTL displaying varying effects of freezing tolerance have also been reported between faba bean, pea, and *M. truncatula* (Imbert et al. 2024). Our study also highlighted synteny relationships between QTL detected for resistance to different *A. euteiches* strains, suggesting that orthologous genes may exhibit different effects on various pathotypes depending on the legume genome. The variability of the QTL confidence intervals ($75 \text{ bp} \leq \text{CI} \leq 116 \text{ Mb}$) on genomes led to identify varying numbers of candidate orthologous genes depending on the legume species ($1 \leq \text{number of orthologous genes} \leq 175$). High-density genotyping and fine-mapping techniques will be important approaches to be considered in future QTL analyses to enhance mapping resolution and quantitative resistance gene cloning (Jaganathan et al. 2020).

Implementing rotations of quantitative resistance genes at the field scale is an effective strategy to limit and diversify selection pressure on pathogen populations, therefore preserve durability of resistance (Mundt 2014). The risk of resistance QTL erosion by *A. euteiches* populations has been suggested by Quillévéré-Hamard et al. (2021). These authors reported variations in aggressiveness among 43 *A. euteiches* isolates on resistance QTL in pea NILs, suggesting that the most aggressive isolates may be selected by resistant cultivars carrying major-effect QTL. The cultivation of resistant faba bean varieties in rotation with susceptible grain legumes is often recommended to limit soil inoculum potential (Moussart et al. 2008). In this study, we found no synteny relationships between major-effect QTL between pea and faba bean, suggesting the absence of conserved major genetic determinants between these species, which supports rotation recommendations as a mean to deploy resistance QTL at the cropping system scale. In this study, we also did not reveal any syntenic relationships between resistance QTL identified in pea and lentil, two legume species commonly grown in the same geographical regions. Further genetic studies will be needed in lentil to confirm the diversity of genetic determinants of resistance to *A. euteiches* between pea and lentil, and therefore their potential for durable deployment in future resistant varieties grown in same cropping systems by diversifying selection pressures on *A. euteiches* populations.

5. Conclusion

This study aimed to enhance our understanding of the genetic architecture controlling quantitative resistance to *A. euteiches* in legumes. Linkage analysis and GWAS are powerful and complementary tools for detecting genes associated with phenotypic traits. Both major and minor-effect QTL for resistance to *A. euteiches* were identified or confirmed in RIL populations of both faba bean and *M. truncatula*. Only minor-effect QTL were identified in a lentil diversity panel. Comparative genomics analysis enabled the identification of syntenic QTL regions underlying quantitative resistance to *A. euteiches* among pea, faba bean, lentil, and *M. truncatula*. This study provides new SNP markers closely linked to Aphanomyces root rot resistance QTL, identifies candidate genes for further functional validation, and will support efficient and durable deployment of quantitative resistance to *A. euteiches* in legume-rich cropping systems.

Declarations

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Authors' contributions

- TL managed the study, performed all the statistical and genetic analyses, and drafted the manuscript.
- BI curated and integrated Aphanomyces resistance QTL into the OrthoLegKB database.
- GB managed genotypic data and updated linkage analyses in the *M. truncatula* RIL population.
- CL participated in discussing various parameters to be applied in statistical and genetic models.
- HM and AL evaluated the Silian x Hiverna RIL population of faba bean in growth chamber conditions for resistance to *A. euteiches*.
- GA, ECP, and JK contributed to prepare faba bean genotypic and genomic data for this study.
- TL, RU, HS, and BB evaluated the AGILE lentil panel in greenhouse conditions for resistance to *A. euteiches*.
- CTC participated in the critical analysis of genotyping data on the AGILE lentil panel for GWAS.
- NT contributed to the scientific management of the OrthoLegKB database.
- YM and CJC coordinated the AGILE panel phenotyping experiment.
- AS and MLPN coordinated the overall study, reviewed, and edited the manuscript.

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Availability of data and material

Genotyping, phenotyping, QTL, and gene information are available in the main manuscript or as supplementary data.

Ethical standards: Authors declare that the described experiments comply with the French laws.

Conflict of interest: The authors declare that they have no conflict of interest.

References

1. Agrios GN (2005) Plant pathology, 5th edn. Elsevier Academic, Amsterdam; Boston
2. Alexander DH, Lange K (2011) Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics 12:246. <https://doi.org/10.1186/1471-2105-12-246>
3. Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. Genome Res 19:1655–1664. <https://doi.org/10.1101/gr.094052.109>
4. Bai Y, Pavan S, Zheng Z et al (2008) Naturally occurring broad-spectrum powdery mildew resistance in a central American tomato accession is caused by loss of *Mlo* function. MPMI 21:30–39. <https://doi.org/10.1094/MPMI-21-1-0030>

5. Bartoń K (2024) MuMIn: Multi-Model Inference
6. Bates D, Maechler M, Bolker B et al (2019) lme4: linear mixed-effects models using Eigen and S4
7. Bayer PE, Valliyodan B, Hu H et al (2022) Sequencing the USDA core soybean collection reveals gene loss during domestication and breeding. *Plant Genome* 15:e20109. <https://doi.org/10.1002/tpg2.20109>
8. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Stat Soc Ser B (Methodological)* 57:289–300. <https://doi.org/10.2307/2346101>
9. Bonhomme M, André O, Badis Y et al (2014) High-density genome-wide association mapping implicates an F-box encoding gene in *Medicago truncatula* resistance to *Aphanomyces euteiches*. *New Phytol* 201:1328–1342. <https://doi.org/10.1111/nph.12611>
10. Bonhomme M, Fariello MI, Navier H et al (2019) A local score approach improves GWAS resolution and detects minor QTL: application to *Medicago truncatula* quantitative disease resistance to multiple *Aphanomyces euteiches* isolates. *Heredity* 123:517–531. <https://doi.org/10.1038/s41437-019-0235-x>
11. Boutet G, Alves Carvalho S, Falque M et al (2016) SNP discovery and genetic mapping using genotyping by sequencing of whole genome genomic DNA from a pea RIL population. *BMC Genomics* 17:121. <https://doi.org/10.1186/s12864-016-2447-2>
12. Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19:889–890. <https://doi.org/10.1093/bioinformatics/btg112>
13. Browning BL, Zhou Y, Browning SR (2018) A one-penny imputed genome from next-generation reference panels. *Am J Hum Genet* 103:338–348. <https://doi.org/10.1016/j.ajhg.2018.07.015>
14. Carrillo-Perdomo E, Magnin-Robert J-B, Raffiot B et al (2022) A QTL approach in faba bean highlights the conservation of genetic control of frost tolerance among legume species. *Front Plant Sci* 13:970865. <https://doi.org/10.3389/fpls.2022.970865>
15. Carrillo-Perdomo E, Vidal A, Kreplak J et al (2020) Development of new genetic resources for faba bean (*Vicia faba* L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map. *Sci Rep* 10:6790. <https://doi.org/10.1038/s41598-020-63664-7>
16. Chang CC, Chow CC, Tellier LC et al (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaSci* 4:7. <https://doi.org/10.1186/s13742-015-0047-8>
17. Chatterton S, Schwinghamer TD, Pagé A et al (2023) Inoculum dose–disease response relationships for the pea root rot pathogen, *Aphanomyces euteiches*, are dependent on soil type and other pathogens. *Front Plant Sci* 14:1115420. <https://doi.org/10.3389/fpls.2023.1115420>
18. Christensen RHB (2019) ordinal: regression models for ordinal data
19. Cingolani P, Platts A, Wang LL et al (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain *w¹¹¹⁸*; *iso-2*; *iso-3*. *Fly* 6:80–92. <https://doi.org/10.4161/fly.19695>
20. Consonni C, Humphry ME, Hartmann HA et al (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat Genet* 38:716–720. <https://doi.org/10.1038/ng1806>
21. Cooper L, Elser J, Laporte M-A et al (2024) Planteome 2024 update: reference ontologies and knowledgebase for plant biology. *Nucleic Acids Res* 52:D1548–D1555. <https://doi.org/10.1093/nar/gkad1028>
22. Cooper L, Meier A, Laporte M-A et al (2018) The Planteome database: an integrated resource for reference ontologies, plant genomics and phenomics. *Nucleic Acids Res* 46:D1168–D1180. <https://doi.org/10.1093/nar/gkx1152>
23. Danecek P, Auton A, Abecasis G et al (2011) The variant call format and VCFtools. *Bioinformatics* 27:2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>
24. de Givry S, Bouchez M, Chabrier P et al (2005) CAR(H)(T)AGene: multipopulation integrated genetic and radiation hybrid mapping. *Bioinformatics* 21:1703–1704. <https://doi.org/10.1093/bioinformatics/bti222>
25. Desgroux A, L'Anthoëne V, Roux-Duparque M et al (2016) Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. *BMC Genomics* 17:124. <https://doi.org/10.1186/s12864-016-2429-4>
26. Djébali N, Jauneau A, Ameline-Torregrosa C et al (2009) Partial resistance of *Medicago truncatula* to *Aphanomyces euteiches* is associated with protection of the root stele and is controlled by a major QTL rich in proteasome-related genes. *MPMI* 22:1043–1055. <https://doi.org/10.1094/MPMI-22-9-1043>
27. Emms DM, Kelly S (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol* 20:238. <https://doi.org/10.1186/s13059-019-1832-y>
28. Emms DM, Kelly S (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol* 16:157. <https://doi.org/10.1186/s13059-015-0721-2>
29. FAOSTAT (2024) Food and Agriculture Organization of the United Nations. <https://www.fao.org/faostat/en/#data/QCL>. Accessed 1 Jun 2024
30. Fox J, Weisberg W (2020) car: companion to applied regression
31. Francis RM (2017) pophelper: an R package and web app to analyse and visualize population structure. *Mol Ecol Resour* 17:27–32. <https://doi.org/10.1111/1755-0998.12509>
32. Guerra-García A, Gioia T, von Wettberg E et al (2021) Intelligent characterization of lentil genetic resources: evolutionary history, genetic diversity of germplasm, and the need for well-represented collections. *Curr Protocols* 1:e134. <https://doi.org/10.1002/cpz1.134>
33. Haile TA, Heidecker T, Wright D et al (2020) Genomic selection for lentil breeding: empirical evidence. *Plant Genome* 13. <https://doi.org/10.1002/tpg2.20002>

34. Hamon C, Baranger A, Coyne CJ et al (2011) New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. *Theor Appl Genet* 123:261–281. <https://doi.org/10.1007/s00122-011-1582-z>
35. Hamon C, Baranger A, Miteul H et al (2010) A complex genetic network involving a broad-spectrum locus and strain-specific loci controls resistance to different pathotypes of *Aphanomyces euteiches* in *Medicago truncatula*. *Theor Appl Genet* 120:955–970. <https://doi.org/10.1007/s00122-009-1224-x>
36. Hamon C, Coyne CJ, McGee RJ et al (2013) QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. *BMC Plant Biol* 13:45. <https://doi.org/10.1186/1471-2229-13-45>
37. Harrell FE, Dupont C (2020) Hmisc: Harrell miscellaneous
38. Hecht V, Foucher F, Ferrándiz C et al (2005) Conservation of Arabidopsis flowering genes in model legumes. *Plant Physiol* 137:1420–1434. <https://doi.org/10.1104/pp.104.057018>
39. Hervé M (2020) RVAideMemoire: testing and plotting procedures for biostatistics
40. Holland J (2007) Genetic architecture of complex traits in plants. *Curr Opin Plant Biol* 10:156–161. <https://doi.org/10.1016/j.pbi.2007.01.003>
41. Hughes TJ, Grau CR (2013) Aphanomyces root rot or common root rot of legumes. *Plant Health Instructor*. <https://doi.org/10.1094/PHI-I-2007-0418-01>
42. Humphry M, Reinstädler A, Ivanov S et al (2011) Durable broad-spectrum powdery mildew resistance in pea *er1* plants is conferred by natural loss-of-function mutations in *PsMLO1*. *Mol Plant Pathol* 12:866–878. <https://doi.org/10.1111/j.1364-3703.2011.00718.x>
43. Imbert B, Kreplak J, Flores R-G et al (2023) Development of a knowledge graph framework to ease and empower translational approaches in plant research: a use-case on grain legumes. *Front Artif Intell* 6:1191122. <https://doi.org/10.3389/frai.2023.1191122>
44. Imbert B, Kreplak J, Lejeune-Hénaut I et al (2024) Genome-wide association study of frost tolerance in *Vicia faba* reveals syntenic loci in cool-season legumes and highlights relevant candidate genes. *bioRxiv preprint*. <https://doi.org/10.1101/2024.11.27.624268>
45. Jaganathan D, Bohra A, Thudi M, Varshney RK (2020) Fine mapping and gene cloning in the post-NGS era: advances and prospects. *Theor Appl Genet* 133:1791–1810. <https://doi.org/10.1007/s00122-020-03560-w>
46. Jayakodi M, Golicz AA, Kreplak J et al (2023) The giant diploid faba genome unlocks variation in a global protein crop. *Nature* 615:652–659. <https://doi.org/10.1038/s41586-023-05791-5>
47. Jørgensen JH (1992) Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. *Euphytica* 63:141–152. <https://doi.org/10.1007/BF00023919>
48. Khan AW, Garg V, Sun S et al (2024) *Cicer* super-pangenome provides insights into species evolution and agronomic trait loci for crop improvement in chickpea. *Nat Genet*. <https://doi.org/10.1038/s41588-024-01760-4>
49. Kim SA, Cho C-S, Kim S-R et al (2018) A new haplotype block detection method for dense genome sequencing data based on interval graph modeling of clusters of highly correlated SNPs. *Bioinformatics* 34:388–397. <https://doi.org/10.1093/bioinformatics/btx609>
50. Kreplak J, Madoui M-A, Cápál P et al (2019) A reference genome for pea provides insight into legume genome evolution. *Nat Genet* 51:1411–1422. <https://doi.org/10.1038/s41588-019-0480-1>
51. Lechner E, Achard P, Vansiri A et al (2006) F-box proteins everywhere. *Curr Opin Plant Biol* 9:631–638. <https://doi.org/10.1016/j.pbi.2006.09.003>
52. Lee C, Yu D, Choi H-K, Kim RW (2017) Reconstruction of a composite comparative map composed of ten legume genomes. *Genes Genom* 39:111–119. <https://doi.org/10.1007/s13258-016-0481-8>
53. Lenth R (2020) emmeans: Estimated Marginal Means. aka Least-Squares Means
54. Leprévost T, Boutet G, Lesné A et al (2023) Advanced backcross QTL analysis and comparative mapping with RIL QTL studies and GWAS provide an overview of QTL and marker haplotype diversity for resistance to *Aphanomyces* root rot in pea (*Pisum sativum*). *Front Plant Sci* 14:1189289. <https://doi.org/10.3389/fpls.2023.1189289>
55. Link W, Balko C, Stoddard FL (2010) Winter hardiness in faba bean: physiology and breeding. *Field Crops Res* 115:287–296. <https://doi.org/10.1016/j.fcr.2008.08.004>
56. Lippert C, Listgarten J, Liu Y et al (2011) FaST linear mixed models for genome-wide association studies. *Nat Methods* 8:833–835. <https://doi.org/10.1038/nmeth.1681>
57. Liu Y, Du H, Li P et al (2020) Pan-genome of wild and cultivated soybeans. *Cell* 182:162–176. <https://doi.org/10.1016/j.cell.2020.05.023>
58. Loridon K, Burgarella C, Chantret N et al (2013) Single-nucleotide polymorphism discovery and diversity in the model legume *Medicago truncatula*. *Mol Ecol Resour* 13:84–95. <https://doi.org/10.1111/1755-0998.12021>
59. Ma Y, Marzougui A, Coyne CJ et al (2020) Dissecting the genetic architecture of *Aphanomyces* root rot resistance in lentil by QTL mapping and genome-wide association study. *IJMS* 21:2129. <https://doi.org/10.3390/ijms21062129>
60. Malvick DK, Grau CR (2001) Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. *Plant Dis* 85:740–744. <https://doi.org/10.1094/PDIS.2001.85.7.740>
61. Malvick DK, Grau CR, Percich JA (1998) Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and random amplified polymorphic DNA analyses. *Mycol Res* 102:465–475. <https://doi.org/10.1017/S0953756297005029>
62. Malvick DK, Grünwald NJ, Dyer AT (2009) Population structure, races, and host range of *Aphanomyces euteiches* from alfalfa production fields in the central USA. *Eur J Plant Pathol* 123:171–182. <https://doi.org/10.1007/s10658-008-9354-6>
63. Marees AT, de Kluiver H, Stringer S et al (2018) A tutorial on conducting genome-wide association studies: quality control and statistical analysis. *Int J Methods Psychiatr Res* 27:e1608. <https://doi.org/10.1002/mpr.1608>

64. Marzougui A, Ma Y, Zhang C Advanced imaging for quantitative evaluation of *Aphanomyces* root rot resistance in lentil. *Front Plant Sci* 10:383., Even A, Tivoli MN et al (2019) B (2008) Reaction of genotypes from several species of grain and forage legumes to infection with a French pea isolate of the oomycete *Aphanomyces euteiches*. *Eur J Plant Pathol* 122:321–333. <https://doi.org/10.1007/s10658-008-9297-y>
65. Moussart A, Lavaud C, Onfroy C et al (2024) Pathotype characterization of *Aphanomyces euteiches* isolates collected from pea breeding nurseries. *Front Plant Sci* 15:1332976. <https://doi.org/10.3389/fpls.2024.1332976>
66. Moussart A, Onfroy C, Lesne A et al (2007) Host status and reaction of *Medicago truncatula* accessions to infection by three major pathogens of pea (*Pisum sativum*) and alfalfa (*Medicago sativa*). *Eur J Plant Pathol* 117:57–69. <https://doi.org/10.1007/s10658-006-9071-y>
67. Mundt CC (2014) Durable resistance: a key to sustainable management of pathogens and pests. *Infection. Genet Evol* 27:446–455. <https://doi.org/10.1016/j.meegid.2014.01.011>
68. Neupane S, Wright DM, Martinez RO et al (2022) Focusing the GWAS *Lens* on days to flower using latent variable phenotypes derived from global multi-environment trials. *Plant Genome* 16:e20269. <https://doi.org/10.1002/tpg2.20269>
69. O'Sullivan DM, Angra D (2016) Advances in faba bean genetics and genomics. *Front Genet* 7. <https://doi.org/10.3389/fgene.2016.00150>
70. Papaix J, Goyeau H, Du Cheyron P et al (2011) Influence of cultivated landscape composition on variety resistance: an assessment based on wheat leaf rust epidemics. *New Phytol* 191:1095–1107. <https://doi.org/10.1111/j.1469-8137.2011.03764.x>
71. Pavan S, Schiavulli A, Appiano M et al (2011) Pea powdery mildew *er1* resistance is associated to loss-of-function mutations at a *MLO* homologous locus. *Theor Appl Genet* 123:1425–1431. <https://doi.org/10.1007/s00122-011-1677-6>
72. Pecirix Y, Staton SE, Sallet E et al (2018) Whole-genome landscape of *Medicago truncatula* symbiotic genes. *Nat Plants* 4:1017–1025. <https://doi.org/10.1038/s41477-018-0286-7>
73. Pilet-Nayel M-L, Muehlbauer FJ, McGee RJ et al (2005) Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. *Phytopathology* 95:1287–1293. <https://doi.org/10.1094/PHTO-95-1287>
74. Pilet-Nayel M-L, Muehlbauer FJ, McGee RJ et al (2002) Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. *Theor Appl Genet* 106:28–39. <https://doi.org/10.1007/s00122-002-0985-2>
75. Pilet-Nayel M-L, Prospéri J-M, Hamon C et al (2009) *AER1*, a major gene conferring resistance to *Aphanomyces euteiches* in *Medicago truncatula*. *Phytopathology* 99:203–208. <https://doi.org/10.1094/PHTO-99-2-0203>
76. Purcell S, Neale B, Todd-Brown K et al (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559–575. <https://doi.org/10.1086/519795>
77. Quillévéré-Hamard A, Le Roy G, Lesné A et al (2021) Aggressiveness of diverse French *Aphanomyces euteiches* isolates on pea near isogenic lines differing in resistance quantitative trait loci. *Phytopathology* 111:695–702. <https://doi.org/10.1094/PHTO-04-20-0147-R>
78. R Core Team (2023) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available online at: <http://www.R-project.org>
79. Ramsay L, Koh CS, Kagale S et al (2021) Genomic rearrangements have consequences for introgression breeding as revealed by genome assemblies of wild and cultivated lentil species. <https://doi.org/10.1101/2021.07.23.453237>. bioRxiv
80. Rincet R, Moreau L, Monod H et al (2014) Recovering power in association mapping panels with variable levels of linkage disequilibrium. *Genetics* 197:375–387. <https://doi.org/10.1534/genetics.113.159731>
81. Sanderson L-A, Caron CT, Tan R et al (2019) KnowPulse: a web-resource focused on diversity data for pulse crop improvement. *Front Plant Sci* 10:965. <https://doi.org/10.3389/fpls.2019.00965>
82. Sivachandra Kumar NT, Caudillo-Ruiz KB, Chatterton S, Banniza S (2021) Characterization of *Aphanomyces euteiches* pathotypes infecting peas in Western Canada. *Plant Dis* 105:4025–4030. <https://doi.org/10.1094/PDIS-04-21-0874-RE>
83. Tang H, Bowers JE, Wang X et al (2008) Synteny and collinearity in plant genomes. *Science* 320:486–488. <https://doi.org/10.1126/science.1153917>
84. Thompson JN, Burdon JJ (1992) Gene-for-gene coevolution between plants and parasites. *Nature* 360:121–125. <https://doi.org/10.1038/360121a0>
85. VanRaden PM (2008) Efficient methods to compute genomic predictions. *J Dairy Sci* 91:4414–4423. <https://doi.org/10.3168/jds.2007-0980>
86. Verhaegen M, Bergot T, Liebana E et al (2023) On the use of antibiotics to control plant pathogenic bacteria: a genetic and genomic perspective. *Front Microbiol* 14:1221478. <https://doi.org/10.3389/fmicb.2023.1221478>
87. Wang Y, Tang H, DeBarry JD et al (2012) MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res* 40:e49–e49. <https://doi.org/10.1093/nar/gkr1293>
88. Wicker E, Hullé M, Rouxel F (2001) Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. *Plant Pathol* 50:433–442. <https://doi.org/10.1046/j.1365-3059.2001.00590.x>
89. Wicker E, Rouxel F (2001) Specific behaviour of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. *Eur J Plant Pathol* 107:919–929. <https://doi.org/10.1023/A:1013171217610>
90. Wright DM, Neupane S, Heidecker T et al (2021) Understanding photothermal interactions will help expand production range and increase genetic diversity of lentil (*Lens culinaris* Medik). *Plants People Planet* 3:171–181. <https://doi.org/10.1002/ppp3.10158>
91. Wu L, Chang K-F, Conner RL et al (2018) *Aphanomyces euteiches*: a threat to Canadian field pea production. *Engineering* 4:542–551. <https://doi.org/10.1016/j.eng.2018.07.006>
92. Wu L, Fredua-Agyeman R, Hwang S-F et al (2021) Mapping QTL associated with partial resistance to *Aphanomyces* root rot in pea (*Pisum sativum* L.) using a 13.2 K SNP array and SSR markers. *Theor Appl Genet* 134:2965–2990. <https://doi.org/10.1007/s00122-021-03871-6>

93. Yang T, Liu R, Luo Y et al (2022) Improved pea reference genome and pan-genome highlight genomic features and evolutionary characteristics. *Nat Genet* 54:1553–1563. <https://doi.org/10.1038/s41588-022-01172-2>

94. Zhang H, Mittal N, Leamy LJ et al (2017) Back into the wild—Apply untapped genetic diversity of wild relatives for crop improvement. *Evol Appl* 10:5–24. <https://doi.org/10.1111/eva.12434>

95. Zhou P, Silverstein KAT, Ramaraj T et al (2017) Exploring structural variation and gene family architecture with *De Novo* assemblies of 15 *Medicago* genomes. *BMC Genomics* 18:261. <https://doi.org/10.1186/s12864-017-3654-1>

Figures

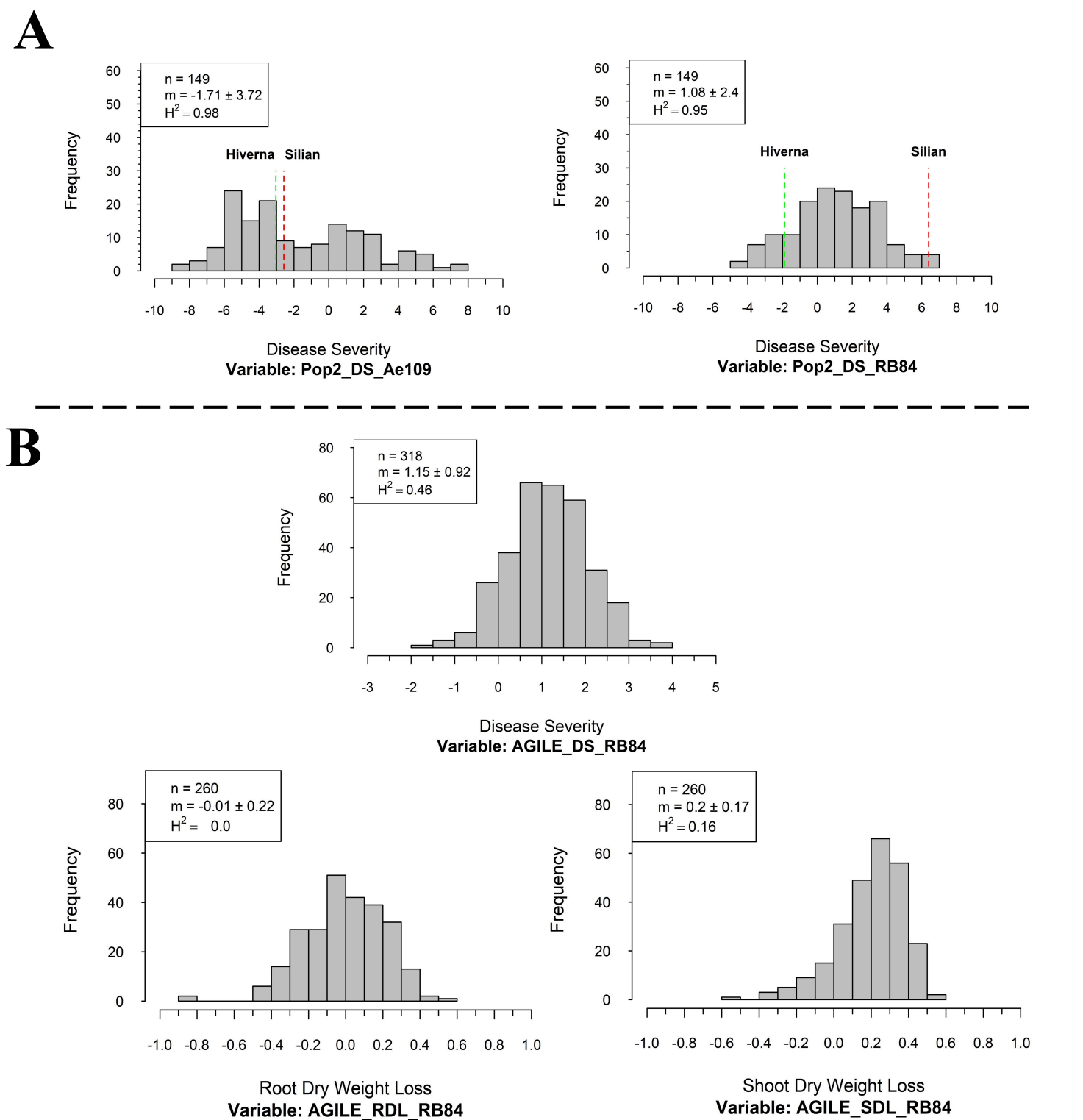


Figure 1

Frequency distribution of EMMean values obtained for *A. euteiches* resistance variables in the (A) Silian x Hiverna RIL population (*Pop2*), and (B) AGILE lentil panel. DS: disease severity; RDL: root dry weight loss; SDL: shoot dry weight loss. RB84 and Ae109 are *A. euteiches* strains belonging to the pea pathotypes I and III, respectively. Reference parental lines Silian and Hiverna are indicated in red and green, respectively. n: total number of evaluated genotypes; m: mean $\pm$ standard deviation; H²: broad-sense heritability.

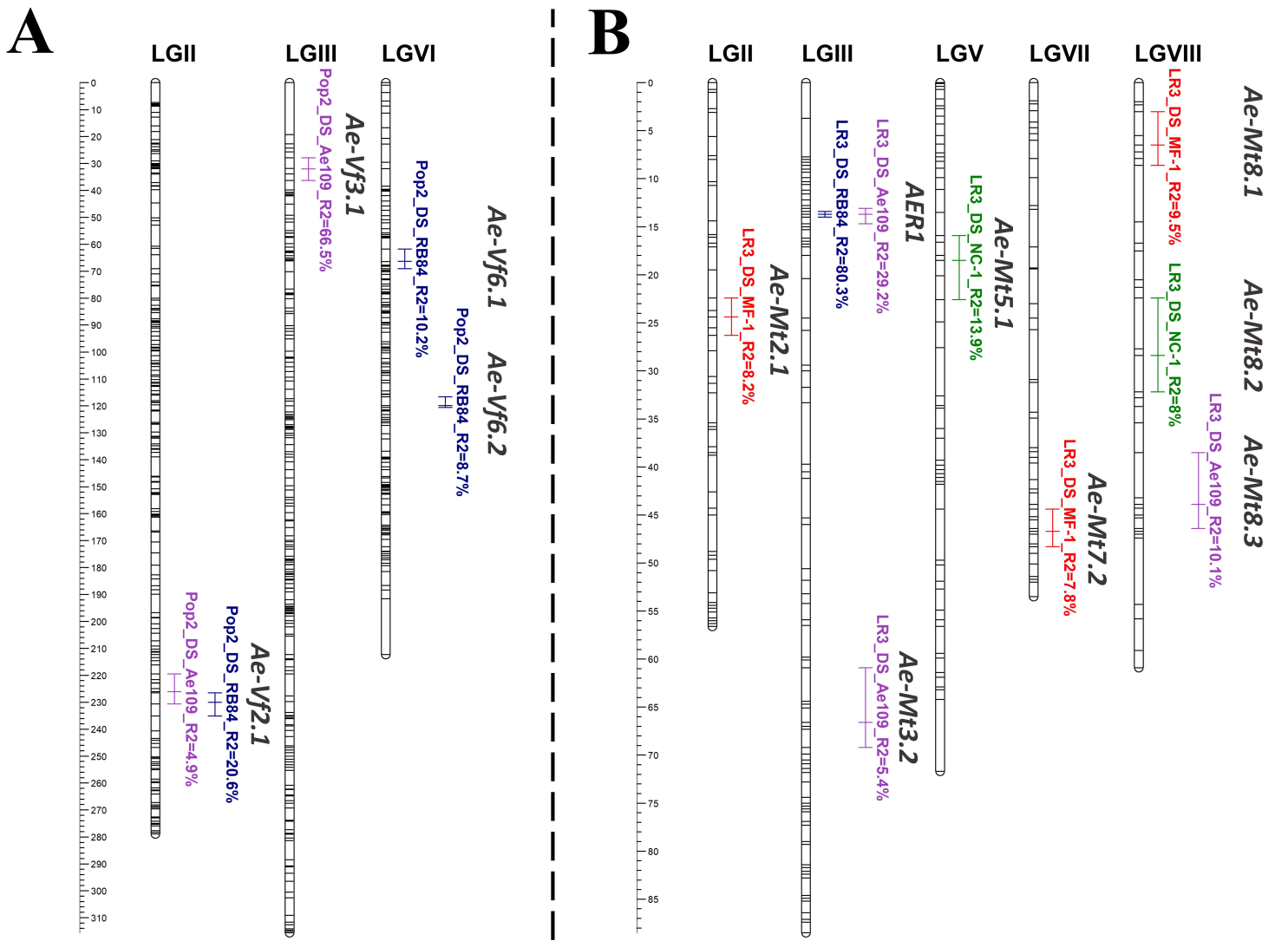


Figure 2

Genetic regions detected for partial resistance to *A. euteiches*. (A) LGII, LGIII, and LGVI are linkage groups (LG) associated with the genetic map developed from the Silian x Hiverna faba bean RIL population (*Pop2*), which was previously published in Carrillo-Perdomo et al. (2020). (B) LGII, LGIII, LGV, LGVII, and LGVIII are linkage groups associated with the genetic map generated in this study from the F83005.5 x DZA045.5 *M. truncatula* RIL population (*LR3*). Black bars located inside each LG represent the density of markers. Colored lines on the right of each LG indicate the locations of resistance QTL. The lengths of the lines are proportional to the lengths of the respective confidence intervals, and the bar inside each confidence interval represents the peak marker at the QTL. QTL associated with partial resistance to the (i) Ae109 and RB84 pea strains of *A. euteiches* are indicated in purple and blue, respectively, and (ii) MF-1 and NC-1 alfalfa strains of *A. euteiches* are indicated in red and green, respectively. R²: Proportion of phenotypic variance explained by the QTL.

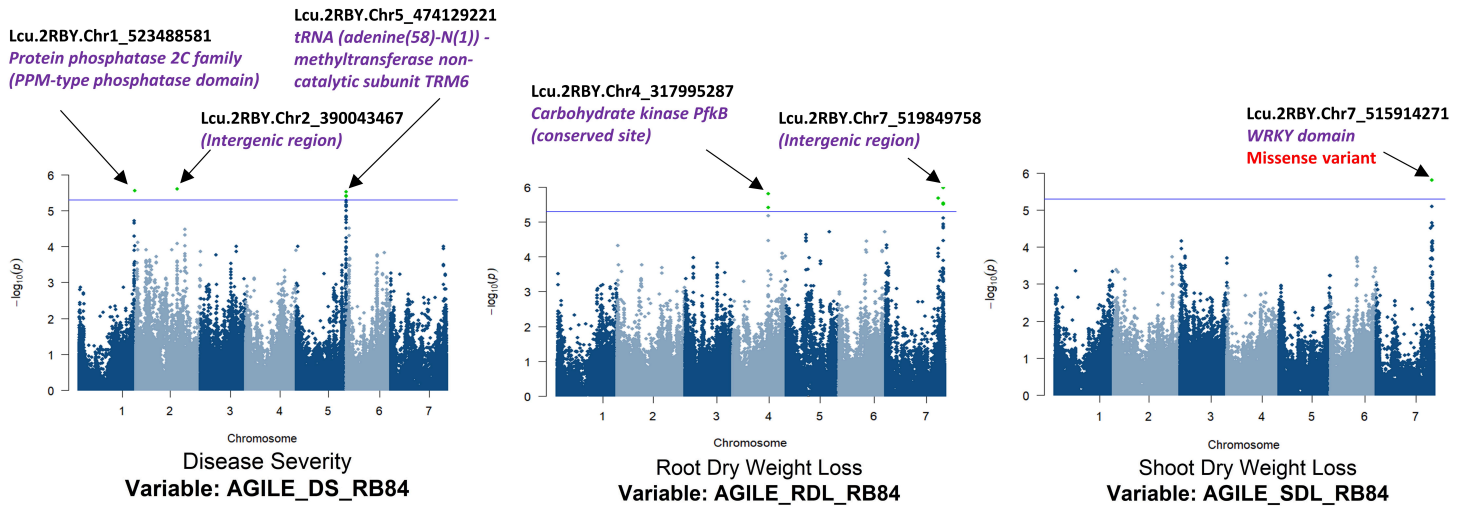


Figure 3

Manhattan plots indicating significant GWAS-SNPs ($p\text{-value} < 5.0 \times 10^{-6}$), detected for resistance to the RB84 strain of *A. euteiches* in the AGILE lentil panel. For each Manhattan plot, X-axis corresponds to the chromosome number and Y-axis represents the significance of marker-trait associations, computed using the formula $-\log_{10}(p\text{-value})$. Blue line indicates the genome-wide significant threshold. Significant GWAS-SNPs detected by MLM are presented in green. At each GWAS peak: the most significant SNP, the associated gene, and the effect of the variant if missense, are indicated with black, purple, and red font, respectively.

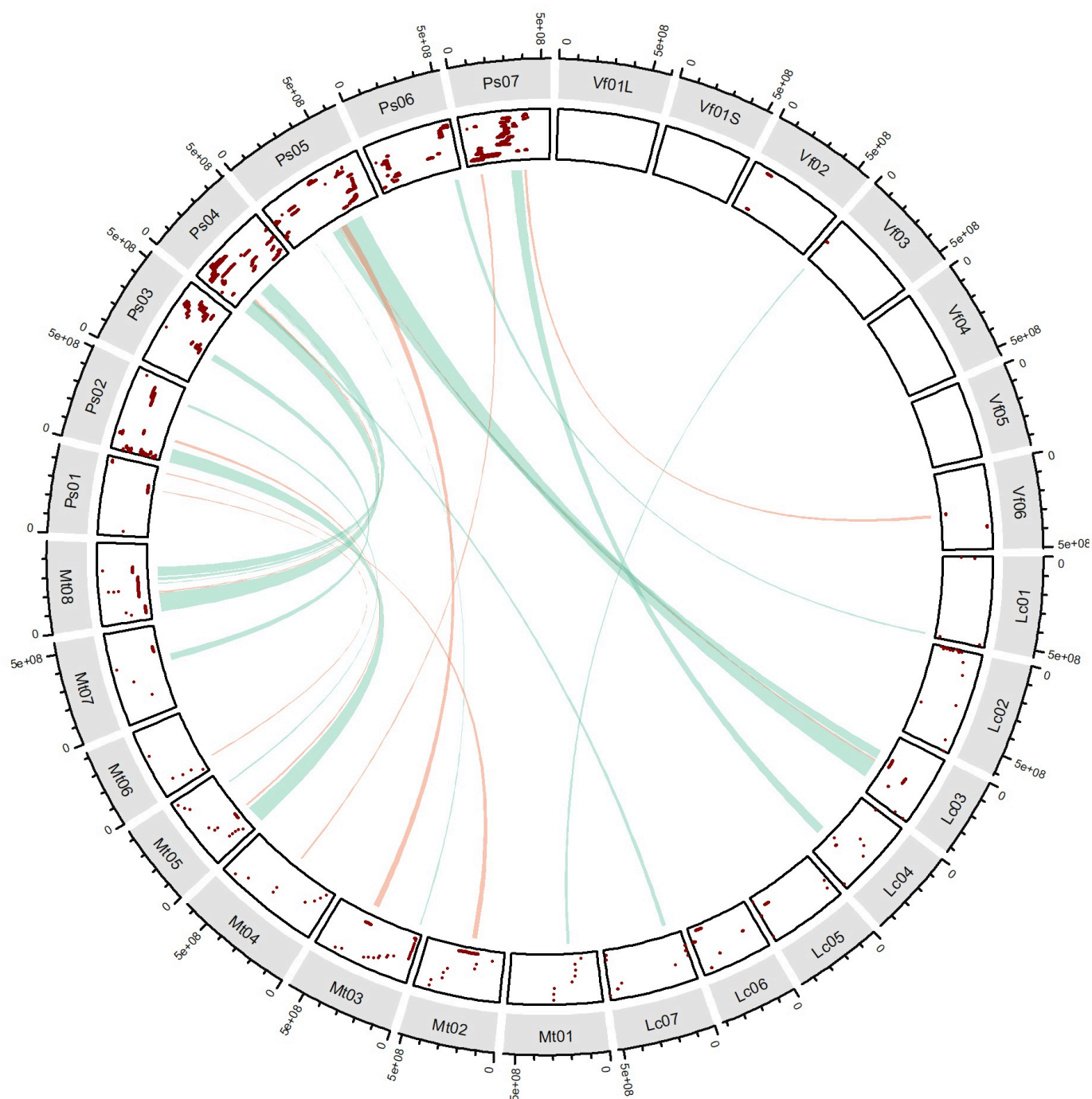


Figure 4

Circos plot showing *Aphanymyces* root rot resistance QTL overlapping with syntenic blocks among four legume genomes. Outermost circle: *L. culinaris*, *M. truncatula*, *P. sativum*, and *V. faba* chromosomes are represented with the abbreviations “Lc”, “Mt”, “Ps”, and “Vf”, respectively, followed by the chromosome number. For better visualization of the chromosome links, the sizes of the *M. truncatula* and *V. faba* chromosomes were multiplied by ten and divided by three, respectively. Second circle: the physical positions of the 385 QTL used in synteny and orthology studies are shown in dark red. Innermost circle: illustration of the 24 syntenic blocks hosting *Aphanymyces* root rot resistance QTL between the four legume genomes. Syntenic blocks are represented by green ribbons or, in the case of inverted syntenic blocks, light red ribbons, indicating that the order of genes within the chromosomal region is reversed compared to the corresponding region in the other species.

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